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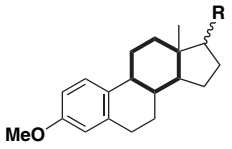
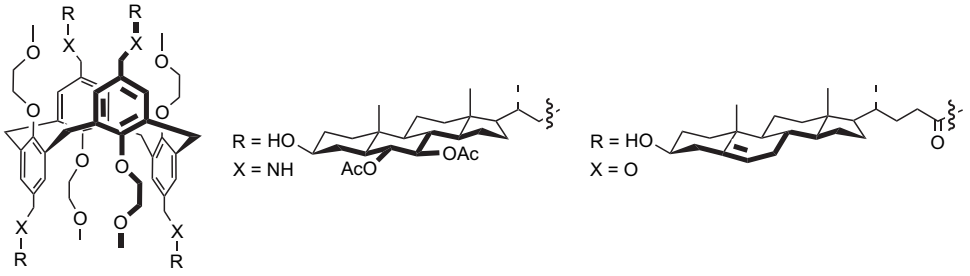
Contents

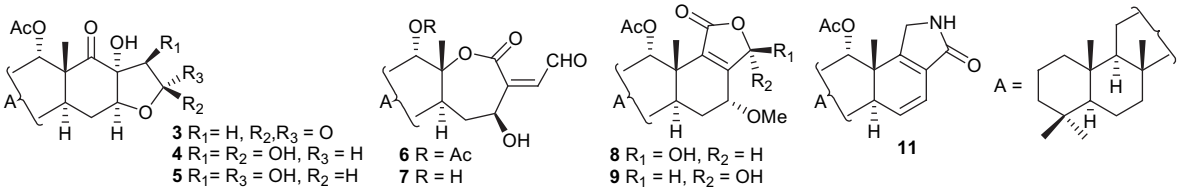
REPORT

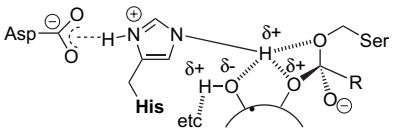


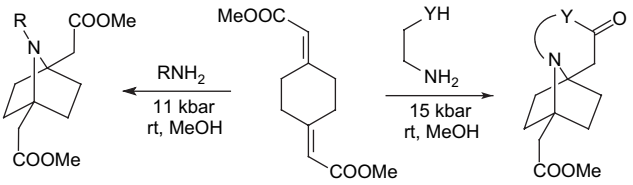
Steroid syntheses using Torgov syntheses, Michael additions and cyclisations, aldol cyclisation, polyene cyclisations, Friedel–Crafts cyclisations, Wittig reactions, McMurray reactions, Dieckmann cyclisation, Diels–Alder cyclisations, electrocyclisations, radical cyclisation and Mukaiyama chemistry for the construction of ring C are reviewed. The report contains 170 references.

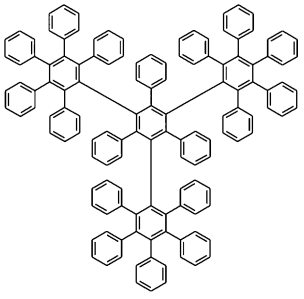
ARTICLES

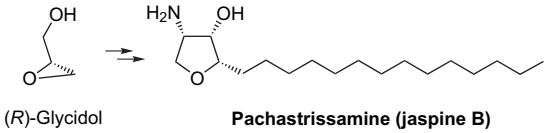


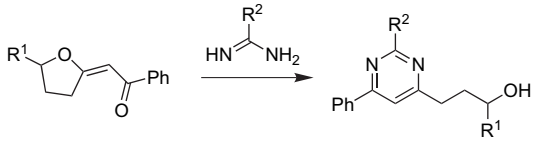


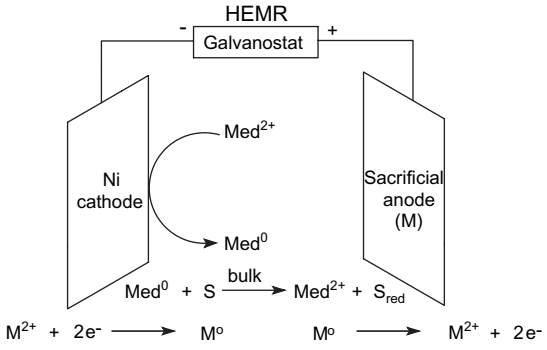


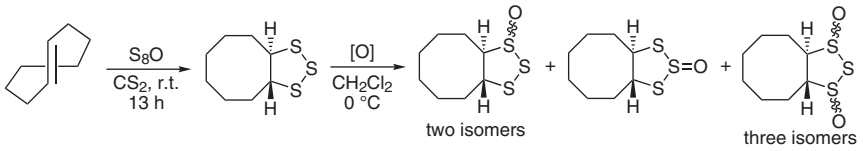


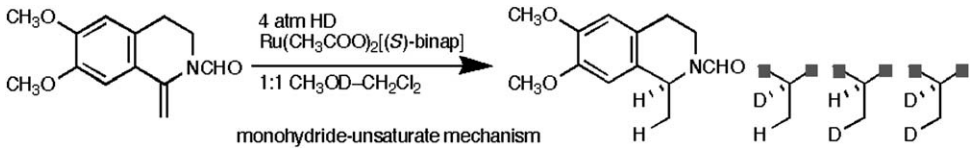


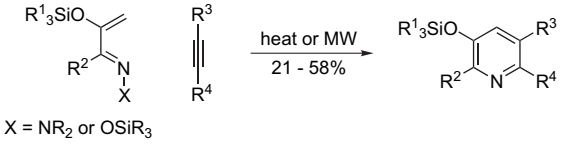


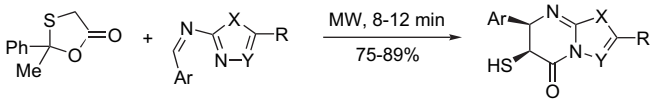


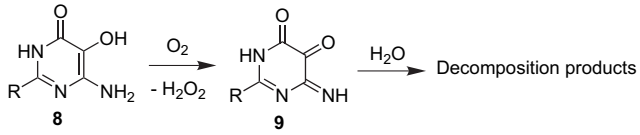








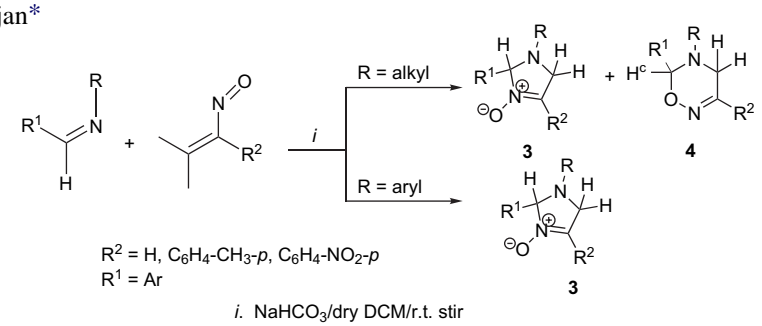




8a: R = CH_3
8b: R = H
8c: R = SCH_3
8d: R = OH (isouramil)
8e: R = NH_2 (divicine)

The rates of autoxidation of C2 substituted 5-hydroxy-6-aminopyrimidines were investigated and correlated with Hammett σ_p^+.

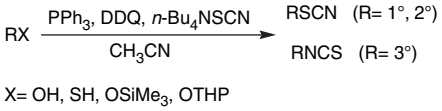
The effect of substituents on nitrogen of simple acyclic imines in their cycloaddition reactions with nitrosoalkenes supported by theoretical calculations is reported. The effect of substituents on carbon of imines has also been examined to broaden the scope of these reactions. The reactions of various simple as well as functionalized imines with nitroso-alkenes have been explored profoundly.

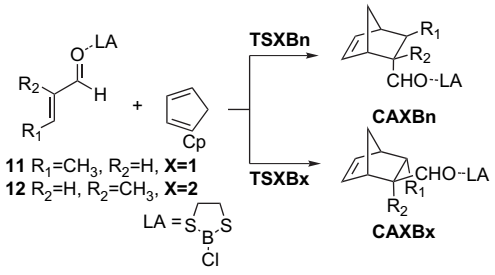


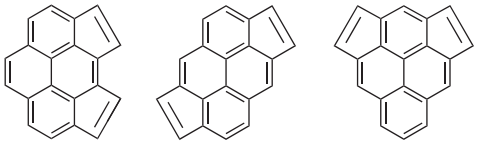
R^2 = H, C_6H_4-CH_3-*p*, C_6H_4-NO_2-*p*
R^1 = Ar

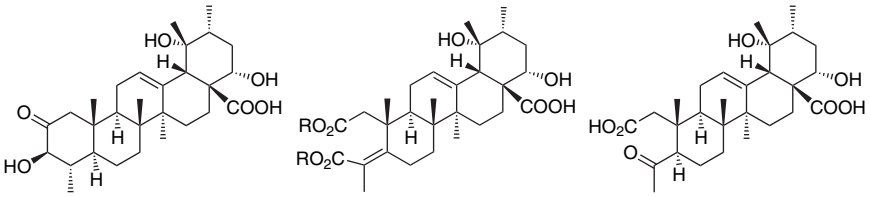
i. $NaHCO_3$/dry DCM/r.t. stir

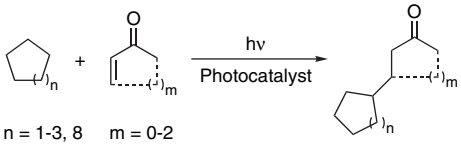
A series of multi-β-substituted cationic porphyrins have been synthesized. Their photooxidative abilities and interactions with DNA were investigated. Substituents at β-position of the porphyrins have significant effect on the interactions and binding modes of the porphyrins with DNA compared to the β-unsubstituted one. Increasing positive charges in porphyrins strengthen their interactions with DNA.

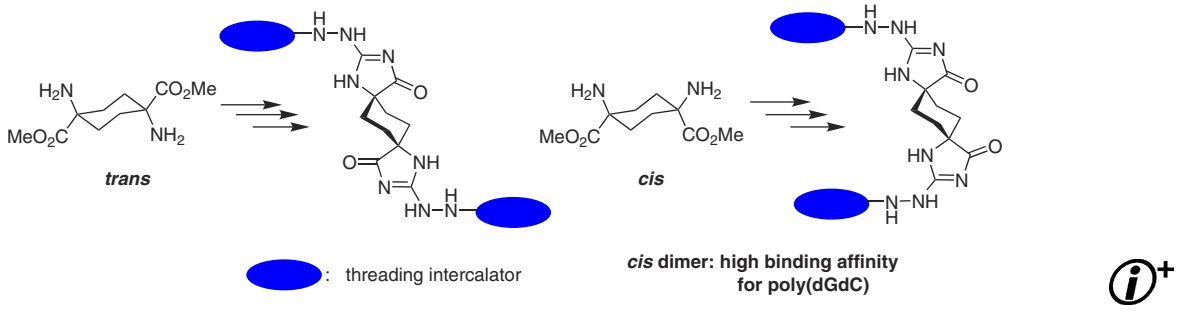












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*Corresponding author
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Ring C closure as key step in the synthesis of steroids

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Contents

1. Introduction

Syntheses of steroids can be achieved by partial synthesis, modification of readily available steroidal compounds or total synthesis. The latter is the most applied method and has been widely studied during the 20th century, the first total synthesis of a steroid being reported in 1939 by Bachmann, Cole and Wilds.[1] Steroid total synthesis remains to this day a topic of interest for synthetic organic chemists and the pharmaceutical industry, even though many elegant syntheses have been developed. The efficiency of routes towards adequately functionalised steroid skeletons, which preferably also should be chiral, short and versatile, can, however, be improved, and this explains why research in this area is still ongoing.

Several reviews have appeared over the years giving a good overview of the complete field of steroid total synthesis.[2–5] This review is focussed on approaches in which the formation of ring C is the crucial step. The review is further subdivided according to the reaction types used for the construction of ring C, thus giving an insight into closing methods used in this corner of steroid total synthesis.

2. Torgov syntheses

The Torgov synthesis has marked an important development in steroid total synthesis.[6–11] The method has proven to be highly versatile and has been extensively used and modified, leading to oestrone and its derivatives, homo steroids, hetero steroids, ring A non-aromatic steroids and also non-steroidal ring structures.[12–26] The method involves the condensation of the reactive alcohol **2**, easily obtained from methoxytetralone **1**, with its hydroxyl group at an allylic and benzylic position, with a 1,3-cyclodione, mostly 2-methylcyclopentane-1,3-dione **3** or 2-methylcyclohexane-1,3-dione. Subsequent cyclisation of the obtained intermediate **5** leads directly to steroid skeleton **6**, which can then be modified further, first by catalytic reduction to alkene **7** and finally

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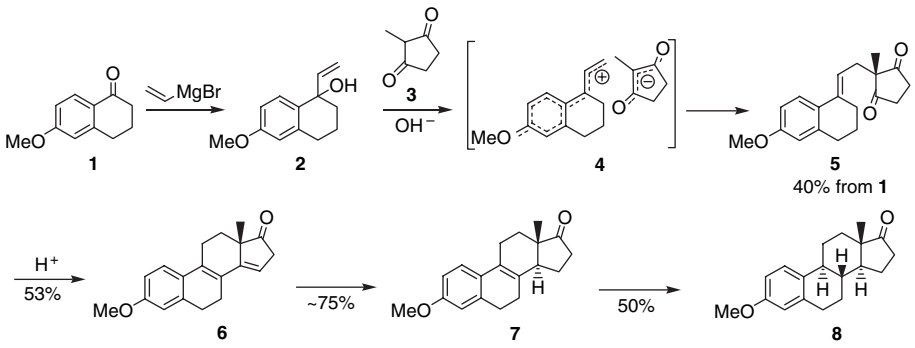


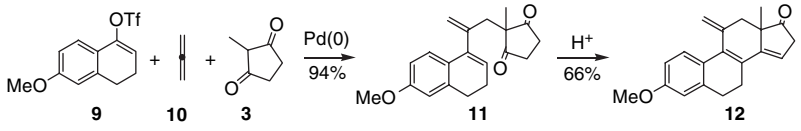
Scheme 1.

to the all *trans* steroid skeleton **8** (Scheme 1). Although, at first, the condensation step was believed to be base-catalysed, Kuo, Taub and Wendler proposed for this step an acid–base reaction mechanism proceeding through an ion-pair intermediate (**4**).[27] Simultaneously, they reported that the condensation and cyclisation steps could be performed in a one-pot reaction, yielding 60% of the tetracyclic steroid skeleton.[16]

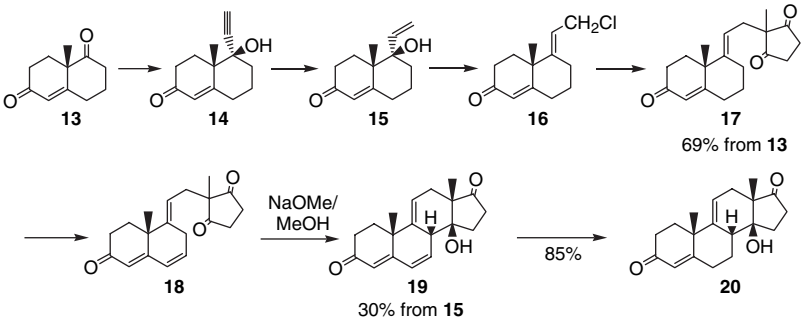
Goré and co-workers published in 1991 a method using carbopalladation of allenic compounds to obtain ring A-aromatic steroids in two steps, with interesting overall yields (40–70%), starting from α-tetralone triflate **9**, allene **10** and cyclopentanedione **3** (Scheme 2).[28,29] The intermediate **11** obtained after the first step resembles the intermediates from the Torgov synthesis, and cyclisation to skeleton **12** was performed accordingly.[21] Unfortunately, this method is limited to ring A-aromatic steroids and, as for the Torgov synthesis, it does not proceed with any stereoselectivity, yielding dienic compounds with Δ^8 and Δ^{14} double bonds.

A comparable approach using a Torgov-like addition step, but with an extended aldol condensation as the final cyclisation step, was used by the groups of Wiechert[30] and Daniewski.[31] This method gave access to non-aromatic ring A steroids bearing a C19 methyl group (Scheme 3). The addition of an acetylide to dione **13**, followed by selective reduction of the triple bond in **14** afforded the Torgov-type intermediate **15**. This intermediate had to transform into the allylic chloride **16** before coupling with cyclopentanedione to **17**. Before the extended aldol condensation can take place, an extra double bond has to be introduced at the Δ^6 position to activate C8 as in compound **18**. Now the aldol-type cyclisation to **19** can be performed in good yield, and reduction of the Δ^6 double bond then leads to **20**. As compound **13** was a racemate, this synthesis led again to a racemic steroidal product. Similar routes were published by Yates and co-workers in 1985, giving access to racemic steroids having a non-aromatic bridged A ring,[32] by Taguchi in 1991, yielding two diastereomers of a C10-trifluoromethyl-substituted steroid,[33] and, finally, by Zard in 1993, leading to 19-nor-steroids as single diastereomers in good yields.[14]

The standard Torgov reaction leads to racemic mixtures of the steroid skeletons, but several routes leading to enantiomerically pure compounds have been published over the years. These routes use either chemical[12,19] or enzymatic[15,17,18] resolution, a chiral ring D precursor[22] or a chiral catalyst.[23] The first chemical asymmetric synthesis was published by Bucourt and co-workers[12] and used L-tartaric

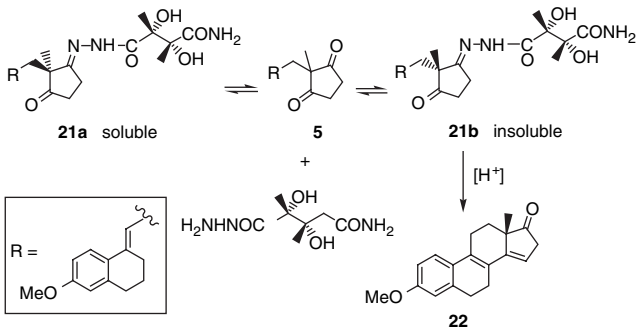


Scheme 2.



Scheme 3.

amide hydrazide to form the steroidal monohydrazones **21a** and **21b**, which could then be separated (Scheme 4).



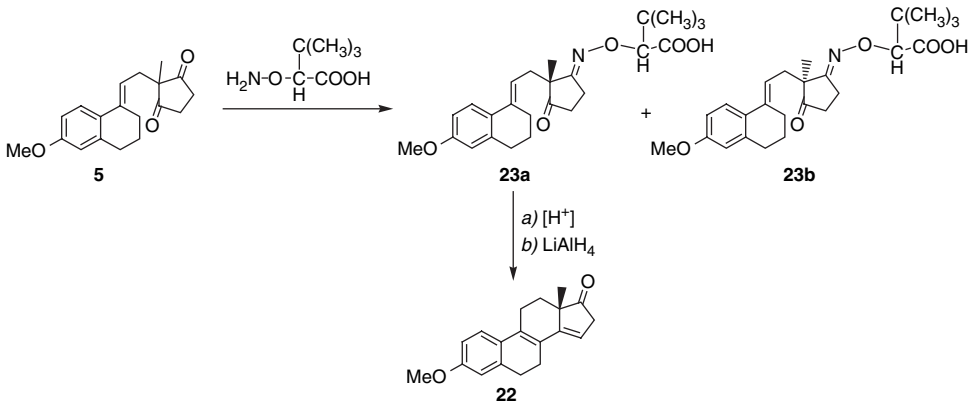
Scheme 4.

In a suitably chosen solvent system, selective crystallisation of the monohydrazone **21b** with a β-configuration on C13 could be achieved and over 75% yield of the pure product could be obtained. Consecutive treatment with acid gave the enantiomerically pure cyclised diene **22** in 80% yield.

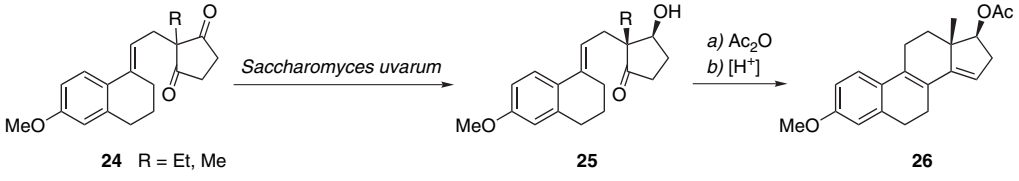
A few years later, a second method using chemical resolution was published by Pappo and co-workers.[19] They derivatised the intermediate seco steroids to the oxime ethers **23a** and **23b** using chiral amino oxycarboxylic acids and could selectively form mainly one of the two diastereomers on C13 via variation of the chemical environment (reaction conditions, used amine, etc.). In this way, compound **23a** was obtained in high optical purity with an overall yield of 36% (Scheme 5).

Gibian and co-workers[15,17,34] made use of enzymatic resolution of the seco steroidal intermediates from the Torgov route. Selective reduction of ketones **24** using *Saccharomyces uvarum* gave the compounds **25**, in 53 to 74% yield, having both a β-alcohol on C17 and a β-methyl or ethyl on C13. After acetylation, they could then cyclise these products in high yields to the acetate **26** and further transform them into known compounds (Scheme 6).

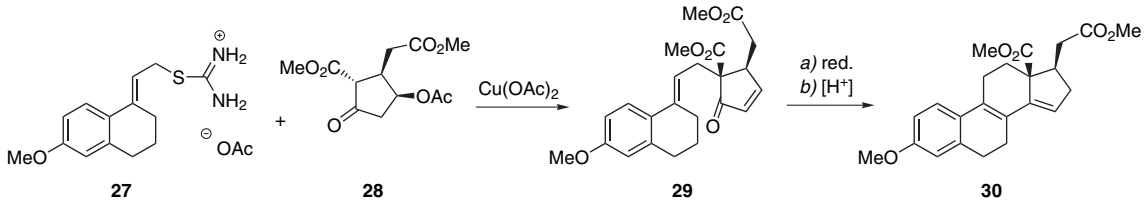
Johnson and Magriotis[22] used a synthesis in which the diketone that served as the ring D precursor in the traditional Torgov route was replaced by a chiral cyclopentanone **28**, derived from *S*-malic acid. This compound was coupled with a modified Torgov steroid precursor **27** to give the seco steroid **29** in an 8:1 diastereomeric mixture on C13, out of which the C13-β-isomer could be isolated in 95% optical purity and with a yield of about 40%. Subsequent cyclisation to compound **30** and further transformation enabled the synthesis of the optically active (+)-18-hydroxyestrone (Scheme 7).



Scheme 5.

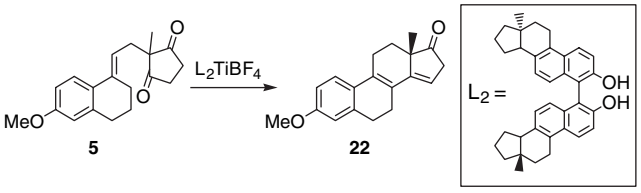


Scheme 6.



Scheme 7.

Finally, Enev and co-workers[23] used a chiral Lewis acid to perform the cyclisation step of ring C. Using a chirally modified Ti complex in combination with a bis-steroidal ligand, they were able to achieve a 72% yield of **22** in combination with an ee of 70% (Scheme 8).



Scheme 8.

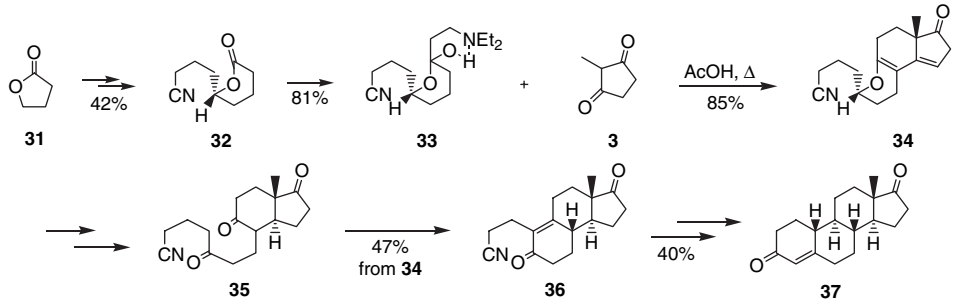
3. Michael additions and cyclisations

A second group of ring C closing strategies relies on Michael-type additions, mostly using 2-methyl-1,3-cyclopentadione **5**. One of the first was developed by Saucy and co-workers and has been referred to as the Hoffmann–LaRoche synthesis (Scheme 9).[35–42] It was used to produce steroid hormones via condensation of a Mannich base with 2-methyl-1,3-cyclopentanedione. The synthesis started with the easy production of lactone **32**, starting from butyrolactone **31**. Ester condensation of **31** and decomposition of the dimer with HCl gave 1,7-dichloro-4-heptanone, which was protected and reacted with cyanide. Deprotection and reduction of the carbonyl group followed by lactonisation afforded **32** in 42% overall yield. A selective reaction of vinylmagnesium bromide with the lactone followed by addition of diethylamine to the adduct gave the required Mannich base **33**. The route could even be applied for the synthesis of optically pure steroids when an optically active variant of the Mannich base **33** was used, leading to intermediate **34** after reaction with 2-methylcyclopentadione **3**. The enantiomerically pure Mannich base **33** was obtained via resolution of its oxalic acid salt derivative.

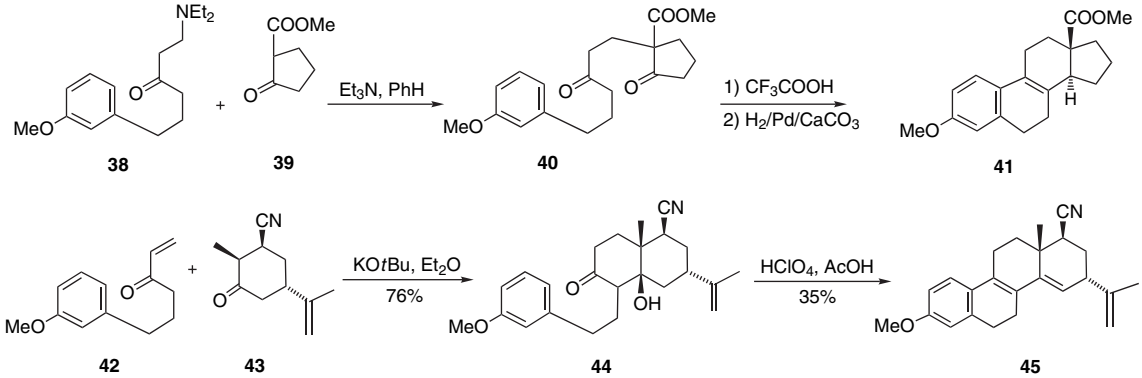
The condensation reaction and cyclisation of ring C took place consecutively in the same reaction vessel and occurred with high asymmetric induction, giving dienol ether **34** with the natural configuration around C13 as the major product. Hydrolysis of the dienol ether and oxidation of the hydroxyl group gave the triketone **35**, in which ring C was closed with an aldol condensation to **36**. Conversion of the nitrile to a methyl ketone and again an aldol condensation finally afforded the 19-nor-steroid skeleton **37**.

A variant, based on the approach of Smith and co-workers,[43] was developed for the synthesis of C18-functionalized steroids, using the reaction of Mannich base **38** with β-keto ester **39** to give the diketone **40**. Cyclisation of **40** and reduction of one double bond afforded the C18 ester **41**, which could be modified easily for the preparation of a variety of other C18-substituted compounds (Scheme 10).[44]

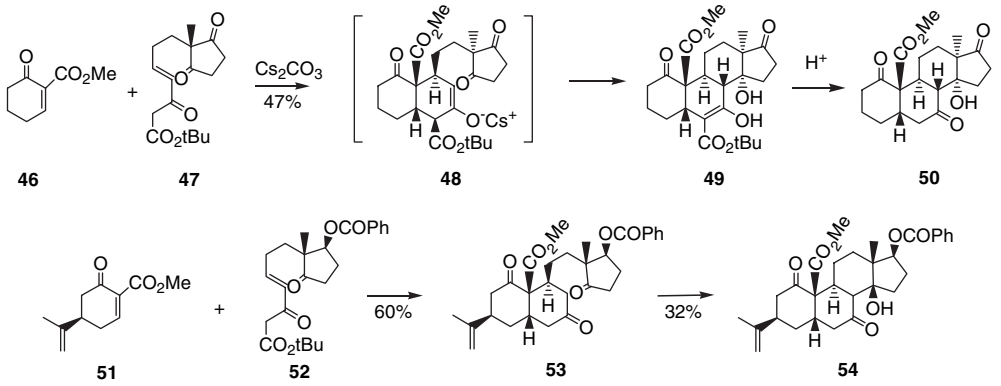
The high reactivity of β-cyanoketones in annelation reactions was demonstrated by the enantioselective synthesis of D-homo steroid skeletons from cyanocarvone **43** and 6-(3-methoxyphenyl)hex-1-en-3-one **42** (Scheme 10).[45] This Michael addition could be achieved under aprotic conditions with KO*t*-Bu in diethyl ether, giving one ketol **44** in



Scheme 9.



Scheme 10.



Scheme 11.

good (76%) yield. A 35% yield of the rather unstable dehydrated compound **45** could be obtained in a one-pot reaction using $HClO_4$ in AcOH.[46]

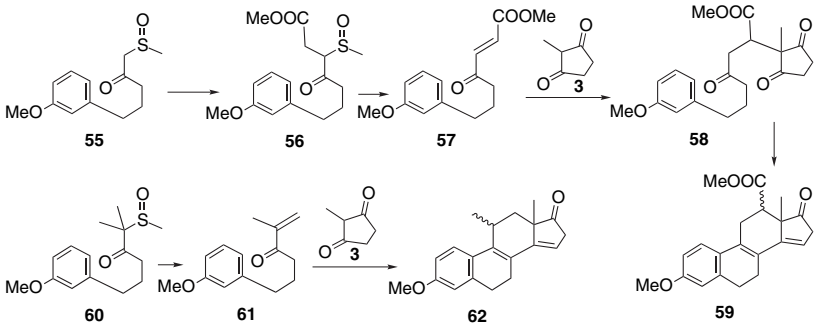
Deslongchamps and Lavallee have developed a one-step stereocontrolled method using an anionic cycloaddition catalysed by caesium carbonate as a polycyclisation reaction and yielding 13,14-*cis*-α-steroids (Scheme 11).[47,48] Addition of the Nazarov-type reagent **47** to 2-carbomethoxy-2-cyclohexenone **46**, intermediately followed by trapping of enolate **48** in situ, yielded the tetracyclic steroid skeleton **49**.

After selective decarboxylation, 13α-methyl,14α-hydroxy steroid **50** was obtained. Later syntheses, using a similar addition of an optically active Nazarov-type reagent **52** to an optically active 5-isopropylidene-2-carbomethoxy-2-cyclohexenone **51**, developed to obtain stereoselectively the more desired β-configuration on C13 and C14, did not yield directly a steroid skeleton in a one-step procedure, but led to open intermediates like **53**. Cyclisation using an aldol condensation then produced steroid **54** as the sole product, although in a low yield which could not be improved.[49–51]

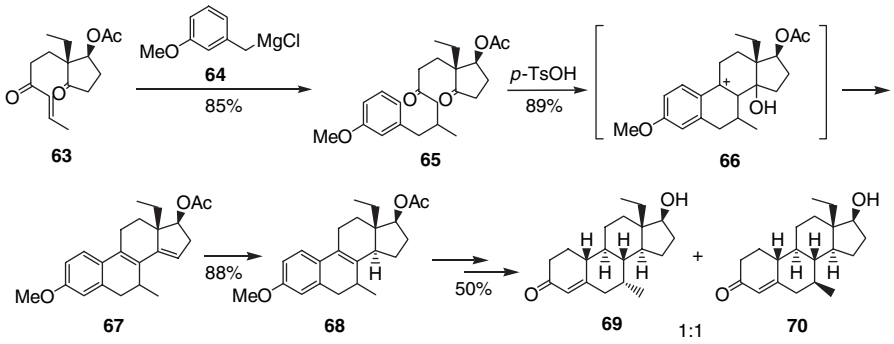
Thermal elimination in the C11 substituted sulfoxide **56**, obtained by alkylation with methyl bromoacetate of **55**, afforded the substituted α,β-unsaturated ketone **57**. After reaction of **57** with cyclopentanedione **3** to the open triketone **58**, aldol-type cyclisation gave the C12-substituted steroid skeleton **59**. In a similar way the C11-substituted enone **61** was obtained via thermal elimination of sulfoxide **60**. The reaction of cyclopentanedione **3** with enone **61**, followed by cyclisation, afforded the C11-substituted steroid skeleton **62**. (Scheme 12).[52,53]

4. Aldol cyclisation

A method in which ring C and ring B are closed simultaneously was reported by the groups of Kurosawa and Zhou.[54,55] This method was actually a modification of a route first reported by Smith in 1963[43,56] and, later, also used and adapted by several other groups.[46,57–67] These routes relied on the formation of a 2-methoxyphenylethyl-substituted indanone ring system, yielding rings A, C and D, followed by an acid-catalysed closure of ring B. Kurosawa and Zhou, however, cyclised both rings B and C in one step. The starting enone was obtained in 25% overall yield from acrolein in a sequence of steps involving the conversion of acrolein into a γ-keto-sulfoxide, which was then coupled to 2-ethyl-1, 3-cyclopentadione. A consecutive microbial asymmetric reduction and further conversion then led to compound **63**.[54] The diketone **65**, the substrate for cyclisation, was obtained by conjugate addition of **64** to the enone **63**. The acid-catalysed aldol reaction closing ring C to intermediate **66**, was followed immediately by a Friedel–Crafts-type reaction, closing ring B and then by dehydration to **67**. Reduction of the Δ^{14} double bond then gave **68**, with its trans-coupled C,D ring system. In this way, Zhou and Zhuang[54] synthesised 7α,18- and 7β,18-dimethyl-19-nor-testosterone (**69** and **70**, respectively) in six steps from intermediate **63** (Scheme 13).



Scheme 12.



Scheme 13.

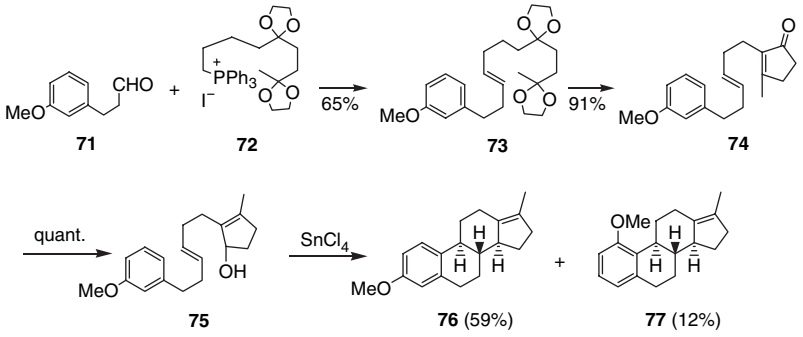
5. Polyene cyclisations

The traditional polyene cyclisations have been investigated and reviewed extensively by Johnson.[68] An example is the cyclisation developed by Bartlett and Johnson, where rings B and C were closed simultaneously (Scheme 14).[69] In contrast with the Smith cyclisations, *ortho* cyclisation can also take place, which leads to products like **77**, and can lower the yield considerably. The method consists of a Wittig–Schlosser condensation of aldehyde **71** with phosphonium iodide **72**, which gave the *trans*-alkene **73** with greater than 98% stereoselectivity. Hydrolysis of the diketal, aldol cyclisation and dehydration gave the enone **74** and after reduction the steroid precursor **75** was obtained. Cyclisation was catalysed by stannic chloride and gave compound **76** in 59% yield. Further conversion led to oestrone in 22% overall yield from **74**.

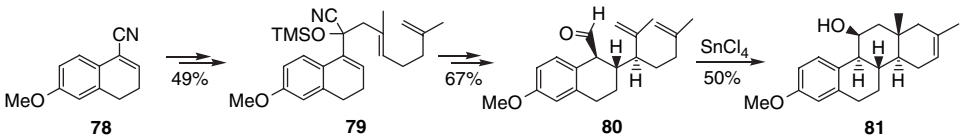
In an additional use of a polyene cyclisation, Ziegler and Wang reported the C/D-cyclisation of the oxo-diene **80**, which leads to the D-homo steroid **81** (Scheme 15).[70,71] The aryl triene **79** was prepared in 49% yield from nitrile **78**,[72,73] and converted into the oxo-diene through a (trimethylsilyl)-cyanohydrin Cope rearrangement, in which the stereochemistry at C8 and C9 is controlled. Acid-catalysed cyclisation then leads to the D-homo steroid skeleton **81**. Using a slightly modified approach, a compound with a five-membered D ring could also be obtained by the same group.[71] Ziegler and Lim additionally investigated a similar route involving a Cope–Claisen rearrangement, but this method showed a low stereoselectivity.[74]

6. Friedel–Crafts cyclisations

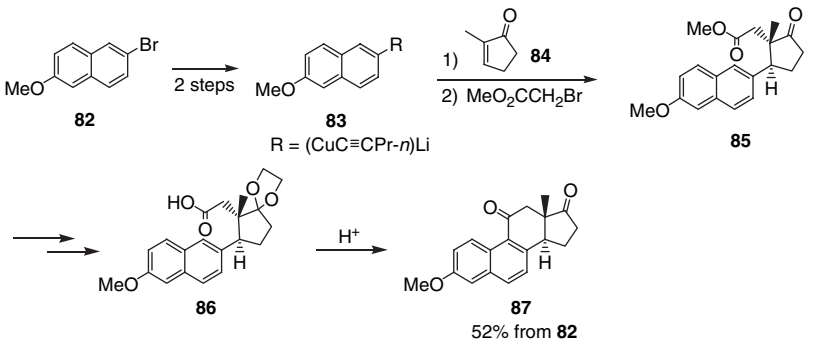
Posner et al. have developed a short and stereoselective synthesis using Friedel–Crafts chemistry where C ring formation is the key step (Scheme 16).[75,76] Using this method, 11-oxoequilenin methyl ether **87** was synthesised in only six steps from the readily available 6-methoxy-2-bromonaphthalene **82** (52% yield). Conjugate addition of the organometallic complex prepared from **82**, to 2-methyl-2-cyclopentenone **84** and trapping of the intermediate with bromoacetic acid led to the ester **85**. Hydrolysis of the ester and protection of the carbonyl group set the stage for the ring-closing Friedel–Crafts reaction in compound **86**. Later, Posner's group also devised a one-pot sequence following tandem Michael–Michael additions and using an intramolecular Wittig reaction for ring C closure (see Scheme 18).[77]



Scheme 14.



Scheme 15.

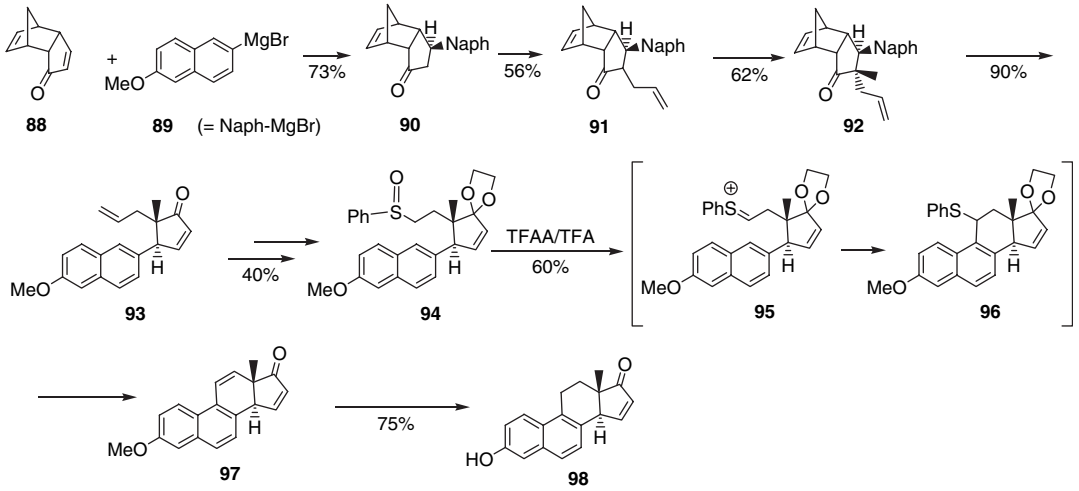


Scheme 16.

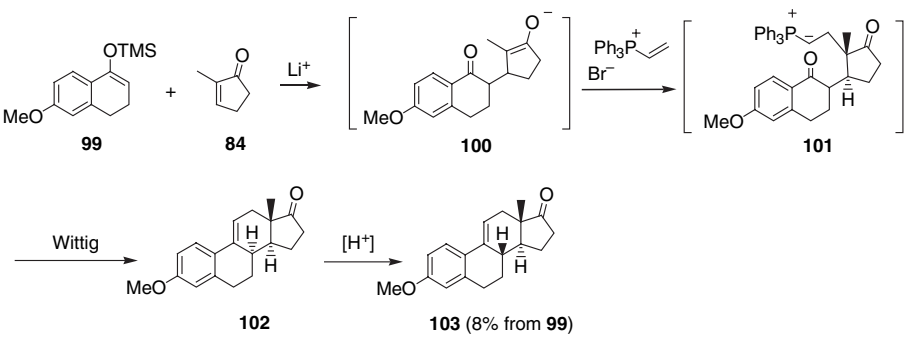
A method was developed by Takano and co-workers using stereoselective introduction of nucleophiles at the β-carbon of a tricyclic dienone **88**, which they had designed for the enantiocontrolled synthesis of natural compounds. They applied this method to the synthesis of (+)-equilenin (Scheme 17).[78] Compound **88** had been previously synthesised by the same group in an efficient conversion from dicyclopentadiene, using kinetic resolution by lipase in the key stage.[79]

The substrate for cyclisation was synthesised starting with the conjugate addition of the organometallic naphthalene derivative **89** to the enone in **88**. Stereoselective alkylation of first an allyl group to **91**, and a methyl group to **92**, followed by pyrolysis afforded **93**. The double bond in the allyl group was converted into the desired sulfoxide **94** in four steps. Closure of ring C was performed using a trifluoroacetic anhydride/trifluoroacetic acid (TFAA/TFA) catalysed cyclisation of this sulfoxide via the intermediate **95** and elimination of thiophenol in **96**, to afford the Δ^{11} unsaturated steroid skeleton **97** in 60% yield. Selective catalytic reduction of the Δ^{11} double bond gave (+)-equilenin methyl ether **98** in 90% yield. Racemic **93** has also been used for the synthesis of racemic equilenin in an earlier work by Horeau and co-workers.[80]

Similar approaches, also having the C9–C11 bond formation as the last step in a reaction sequence using the methoxytetralone moiety for the AB ring system, have been reported in the literature making use of a Wittig reaction for the ring



Scheme 17.



Scheme 18.

closure,[77,81] or a McMurry reductive cyclisation[82] as the final step.[74,83–85]

7. Wittig reaction for cyclisation

Posner et al. have published a one-pot synthesis for racemic 9,11-dehydroestrone methyl ether **103**[77] using an intramolecular Wittig reaction for the closure of ring C (Scheme 18). The reaction starts with the trimethylsilyl enol ether of methoxytetralone **99**, which is added to 2-methyl-2-cyclopentenone **84**. After formation of the intermediate adduct **100**, an unsaturated phosphonium salt is added, and in this way, the Wittig reagent **101** is created in situ. This now can react intramolecularly with the carbonyl group of the tetralone moiety to close ring C. The route yielded a steroid system **102** with trans-fusion around C13–C14 and cis-fusion around C8–C9, which could easily be isomerised to the trans-fused system of the oestrone skeletons. Although this reaction sequence to the racemic oestrone methyl ether **103** was short, the overall yield of the reaction sequence was only 8%.

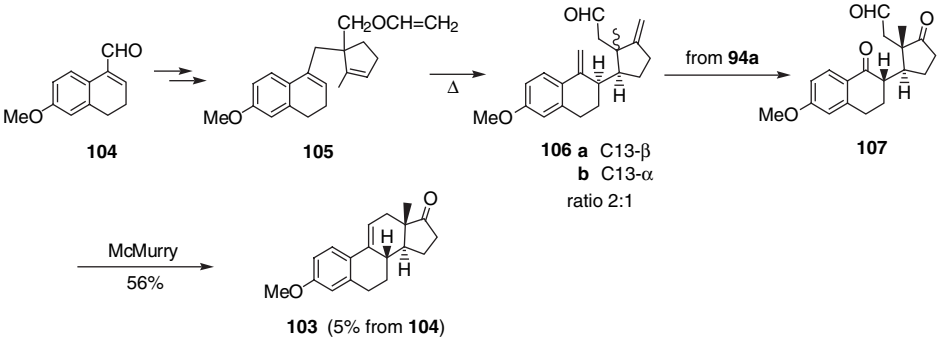
8. McMurry reaction for cyclisation

Shortly after Posner's work, Ziegler and Lim published a synthesis of the same compound, again as a racemic mixture, relying on a Cope–Claisen rearrangement and a McMurry reaction for the closure of ring C (Scheme 19).[74] After the Cope–Claisen rearrangement in compound **105**, obtained from aldehyde **104**, and ozonolysis of the exocyclic double bonds in **106**, the obtained keto aldehyde **107** was subjected to a McMurry radical reaction that gave **103** in 56% yield. Again, the overall yield of the reaction sequence was low (5%), now due to the low stereoselectivity of the thermal rearrangement, giving a mixture of cis- and trans-fusion around C13–C14.

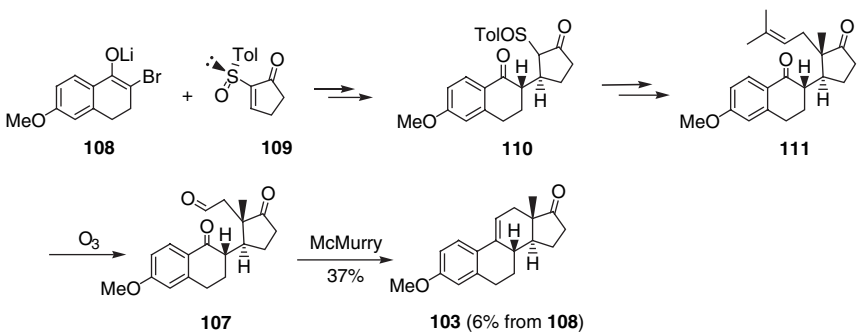
In his asymmetric synthesis of the natural (+)-oestrone methyl ether **103**, Posner also applied the McMurry cyclisation.[83] This sequence used a stereoselective Michael addition of enolate **108** with the enantiomerically pure cyclopentenone sulfoxide **109** to steer the reaction to the adduct **110** (Scheme 20). Introduction of an isopentenyl group in **110** gave **111** and ozonolysis of the double bond then led to Ziegler's triketone **107**. The cyclisation under McMurry conditions, in this case, gave a lower (37%) yield, but the reaction sequence was highly stereoselective, yielding optically pure (+)-oestrone methyl ether **103** in 6% overall yield.

The groups of Mikami[84,85] and Groen[81] both used a route based on a tandem Claisen-ene reaction to obtain the seco tricarbonyl steroid but different methods were used for the closure of ring C. These routes both led to optically pure compounds, starting from *R*-(+)-glyceraldehyde, which is first converted into an optically active and dioxolan protected derivative of 3*S*,4-dihydroxy-methylethylketone and then elongated using a *Z*-selective Still–Wittig olefination to **113a** (Scheme 21).

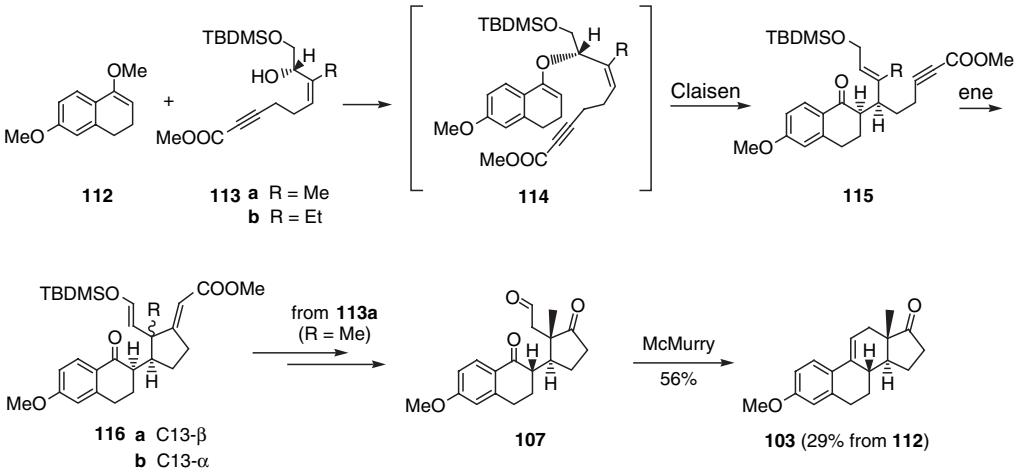
A Claisen-ene reaction of this C,D ring precursor with enol ether **112** as A,B ring precursor now led in a one-pot reaction first to intermediate **114**, which underwent the Claisen



Scheme 19.



Scheme 20.



Scheme 21.

rearrangement to **115** and finally the ene reaction to **116**. Hydrolysis of the *tert*-butyldimethylsilyl (TBDMS) enol ether and ozonolysis of the unsaturated ester then afforded Ziegler's triketone **107**.

While Mikami et al. reported the exclusive formation of the trans-stereochemistry around the C13–C14 bond in the tandem reaction to **116**, Groen et al. obtained an approximately 1:1 mixture of this isomer, together with the 13α-isomer. This difference can be attributed to the replacement of the methyl substituent on C13, in the route of Mikami, by an ethyl group in the compound synthesised by Groen. This ethyl group enhances the steric strain in the transition state of the ring D closure to such an extent that the conformation leading to the unwanted cis configuration (T_1) becomes as probable as that leading to the trans-product (T_2) (see Fig. 1).

After a few more steps, Mikami then cyclised the obtained aldehyde **107** via the McMurry reaction, previously published by Ziegler,[74] again in 56% yield. Groen reduced the obtained C13 ethyl substituted silyl enol ether **117** to the hydroxyl compound **118**, which was converted into the phosphonium salt **119** for the closure of ring C with a Wittig reaction (Scheme 22).

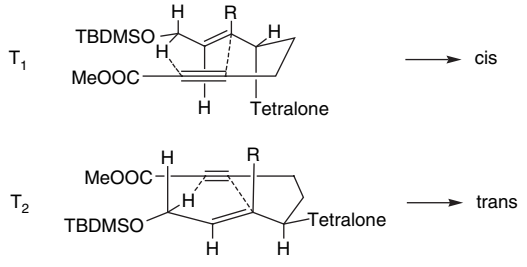


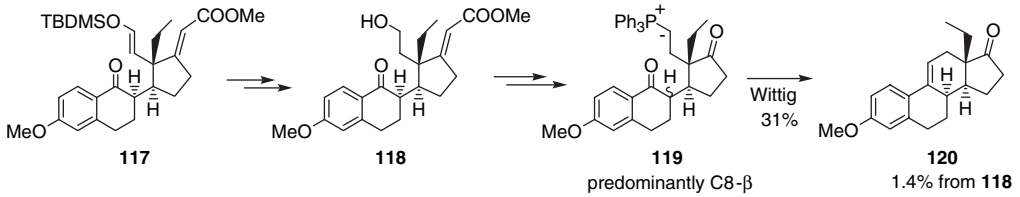
Figure 1.

The yield of the ring-closing reaction could not, however, be enhanced and was unfortunately even lower than the yield reported using the McMurry reaction. The only product **120** had the C8-α configuration, and this low yield could be due to the fact that the ring closure seems only to take place when the seco steroid **119** has this α-configuration around C8, while **119** is mainly present as the C8-β isomer. The exact reason for this behaviour was not explained,[81] but Posner reported similar results when performing his Wittig ring-closing reaction.[77] Surprisingly, the McMurry cyclisation gave significantly higher yields when performed on the 8β-isomer,[74] leading directly to the natural all trans configuration around the steroid ring system.

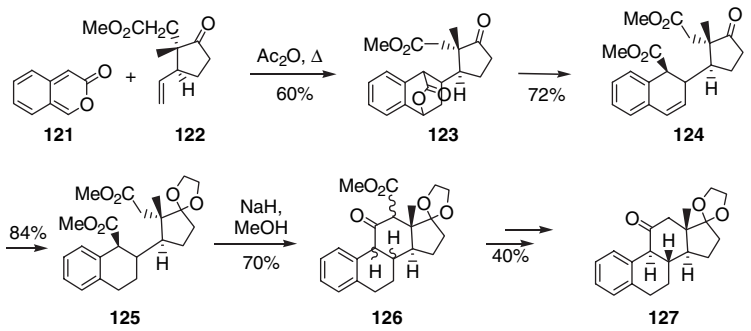
9. Dieckmann cyclisation

Bleasdale and Jones used an intermolecular Diels–Alder reaction followed by a Dieckmann cyclisation to synthesise estra-1,3,5(10)-triene-11,17-dione 17-ethylene ketal **127** (Scheme 23).[86,87]

The Diels–Alder reaction between 2-benzopyran-3-one **121** and the readily available Oppolzer olefin **122** appeared to be highly regioselective and gave the right regioisomer **123** for steroid synthesis in 60% yield. Protection of the carbonyl group in diester **124** gave **125**, and now a Dieckmann



Scheme 22.



Scheme 23.

cyclisation of this diester **125** was used for the closure of ring C. A fair yield (70%) of the C11-functionalized steroid skeleton **126** was obtained. When the methoxy-pyrone homologue of **121** was used as the starting material, the reactions gave similar results and the methoxy analogue of **127** was obtained.

10. Diels–Alder cyclisations

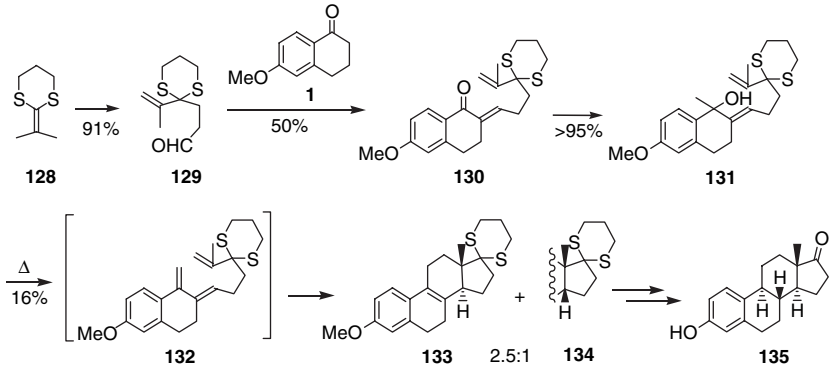
Jung and Halweg published a method using an intramolecular Diels–Alder reaction for ring closure to synthesise oestrone **135** (Scheme 24).[88] The alkene part of the substrate for the Diels–Alder reaction was prepared by alkylation of the ketene bisthioacetal **128** with bromopropionacetal followed by hydrolysis to aldehyde **129**. An aldol condensation of aldehyde **129** with methoxytetralone **1** was used to couple this aldehyde to the potential diene part of the substrate.

Unfortunately, the preparation of the diene from **130** to **132** proved to be extremely difficult. According to these workers, the presence of the 6-methoxy group in the tetralone ring decreased the ketone reactivity considerably, rendering the molecule inert to the normally successful methods such as (silyl-)Wittig reaction or Tebbe reagent. Finally they resorted to thermal dehydration of the tertiary carbinol **131**, giving a yield of 16% of the cyclised compounds **133** and **134**.

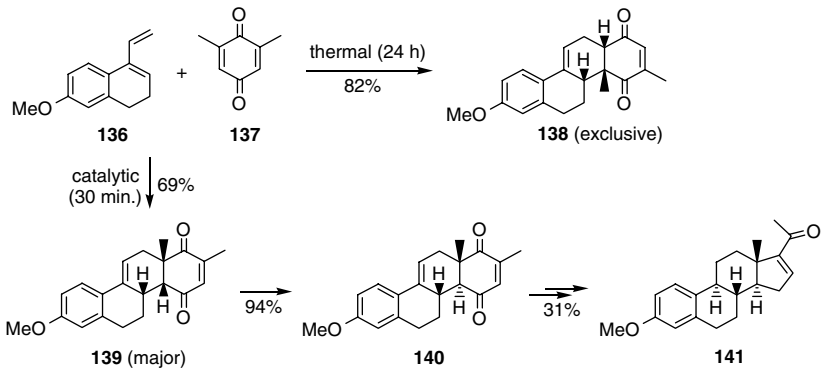
Intermolecular Diels–Alder reactions in steroid synthesis were investigated as early as 1939, but these were very quickly turned down because of failure.[89–91] Indeed, it appeared that the products like **138** did not only have the unwanted configuration, but also mostly had the wrong constitution, the reaction being entirely unselective.[92,93] When several groups tried the use of Lewis acids to catalyse the Diels–Alder reactions, however, these were shown not only to influence the reaction rate positively, but also to greatly improve the stereoselectivity of the addition.

Catalysed intermolecular Diels–Alder reactions of diene **136** and quinones **137** to **139** were used by Valenta and co-workers to obtain D-homo steroid skeletons (Scheme 25). After isomerisation of the configuration at C14, compound **140** was obtained and ring contraction then gave a precursor of oestrone **141** in 13 steps (starting from **136** and **137**) with an overall yield of 22%.[92] This intermediate was converted into oestrone in 31% yield according to a method described previously.[94–96] Similar work was carried out by Quinkert and co-workers, also leading to oestrone.[93,97]

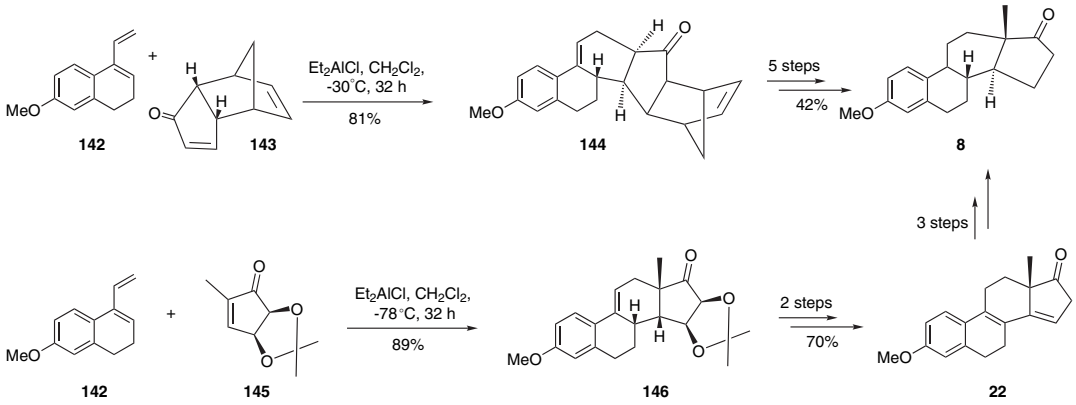
A variation on these syntheses came from Narasimhan and Bapat, who used a silyl ether substituent with intermolecular Diels–Alder chemistry to achieve regioselectivity in their synthesis of equilenin.[98] Two chiral variations on this chemistry have been published by the groups of Takano[99] and Ogasawara[100] (Scheme 26). The regioselectivity of the reaction was investigated by Taticchi and co-workers.[101] The syntheses started with the chiral enones **143** or **145**, which reacted stereo- and regioselectively with diene **142** using Lewis-acid catalysis, to the adducts **144** and **146**, respectively. The adduct **144** has been converted further in several steps using traditional chemistry involving C13



Scheme 24.



Scheme 25.



Scheme 26.

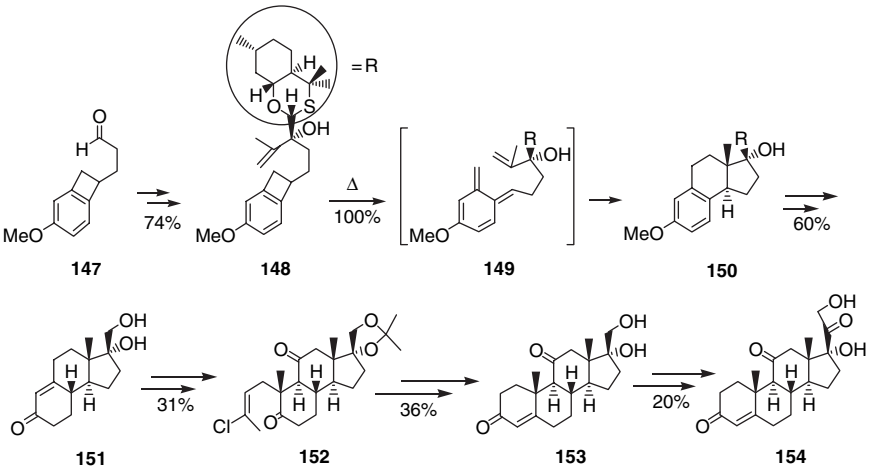
methylation, pyrolysis and reduction of the Δ^{15} double bond to afford (+)-oestrone **8**. In the adduct **146** the acetal first had to be removed using reductive elimination of the oxygen functionality from C16, followed by acid-catalysed elimination and isomerisation of the C15 hydroxyl group to the Δ^{14} double bond as in **22**. In this way also the configuration of C14 can be adjusted, as reduction of the two double bonds in **22** can be carried out in a traditional manner to afford also (+)-oestrone.

Other methods, also using Diels–Alder chemistry, but intramolecular versions, were, for example, developed and reviewed by Kametani, Nemoto and co-workers. This group used the thermolytic chemistry of benzocyclobutenes, giving orthoquinodimethanes, such as **132**, as intermediates,[102] to synthesise oestrone and estradiol,[103] ring-D aromatic steroids,[104,105] pregnanes,[106] (+)-chenodesoxycholic acid,[107] C17 spiro steroids,[108] (+)-11-deoxy-19-norcorticosterone,[109,110] des-A steroids[111,112] and (+)-cortisone.[113] In their synthesis of the last compound (Scheme 27), a chiral auxiliary, readily available in high yield from (+)-pulegon,[114] was coupled to the cyclobutane aldehyde **147**. Reoxidation of the resulting hydroxyl group to a carbonyl group and addition of isopropenylmagnesium bromide now afforded the chiral intermediate **148**.

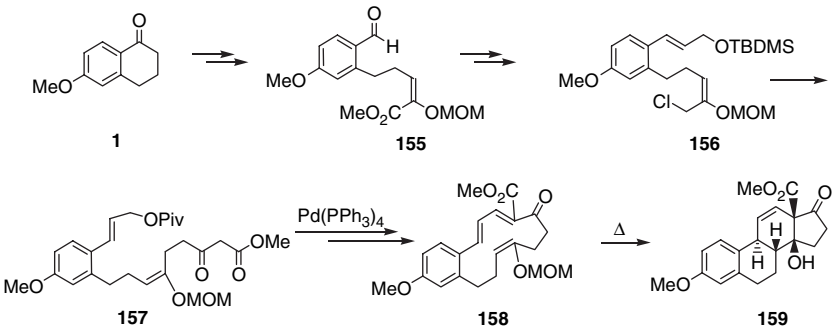
Next the orthoquinodimethane intermediate **149** was generated, in which the intramolecular Diels–Alder reaction led to **150**. The chiral auxiliary was removed, the aldehyde reduced and the aromatic ring converted into enone **151**. After protection of the diol and oxidation of C11, ring A was annelated via **152** and **153** using traditional chemistry. After deprotection of the diol, the side chain could be transformed into one with the C21-hydroxyl and the C20-carbonyl group, which is characteristic for cortisone **154**. In this way, enantiopure steroids have been produced.

At approximately the same time as Kametani and Nemoto, Oppolzer's group developed a synthesis route using similar chemistry, with orthoquinodimethane intermediates again being used, but these were produced via an alternate approach.[115,116] Thermal elimination of sulfur dioxide from sulfones yielded the dimethanes, after which further conversion into steroids could take place. Over the years, a few other groups have published similar methods involving orthoquinodimethane intermediates for the synthesis of steroids.[117–120]

The group of Deslongchamps has recently published a transannular Diels–Alder strategy (Scheme 28).[121,122] The sequence started from 6-methoxytetralone **1**, in which first ring B was reduced, dehydrated, ozonolysed and elongated to **155**. Further chain elongations first gave **156** and finally in overall 12 steps to the precursor **157**. A palladium-catalysed cyclisation and dehydration then led to the macrocyclic triene **158**, which was cyclised to the steroid skeleton **159**.



Scheme 27.

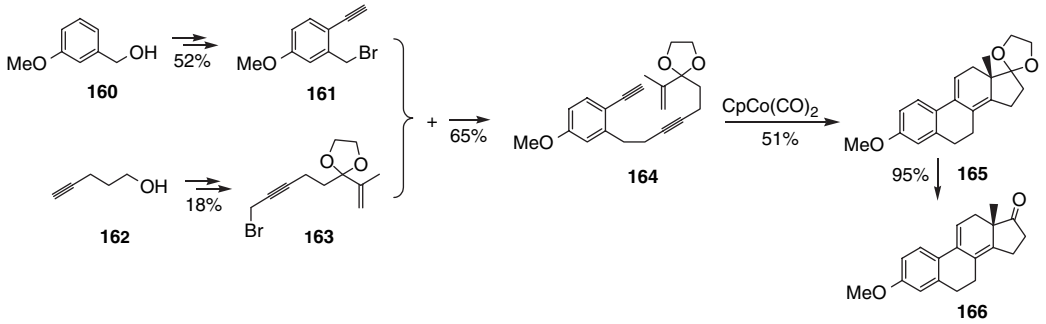


Scheme 28.

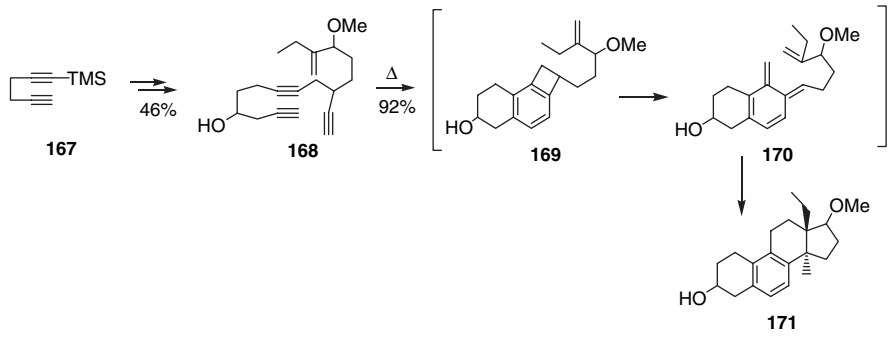
11. Electrocyclisations

The main drawback of the orthoquinodimethane chemistry was the fact that the stereochemical course of these reactions was highly dependent on the substituents used.[123–125] To circumvent this problem, Vollhardt and co-workers explored a new route using a cobalt-catalysed cyclisation of enediyne **164** to the diene intermediate **165** (Scheme 29).[126–129] The enediyne steroid precursor was prepared by coupling compounds **161** and **163**, which were prepared from **160** and **162**, respectively. Deprotection of **165** gave diene **166**, which is an intermediate in the Torgov synthesis of oestrone and can be converted into this compound in two steps.[9]

Developing this method further, the same workers used oligomerisation of the three acetylenic units present in compound **168**. This precursor was prepared in six steps from trimethylsilyl-1,5-hexadiyne **167** via alkylation of its 3,6-dilitio derivative followed by further transformations of the introduced chains. The trimerisation of the three acetylenic units in the presence of a cobalt carbonyl catalyst first led to the benzocyclobutene intermediate **169** and then via the orthoquinodimethane **170** to the B ring aromatic steroid **171**, (Scheme 30).[130] In this reaction sequence, the four rings of the steroid skeleton were assembled in only one step with good yields and with complete control of the trans-stereochemistry around the C,D ring junction.



Scheme 29.

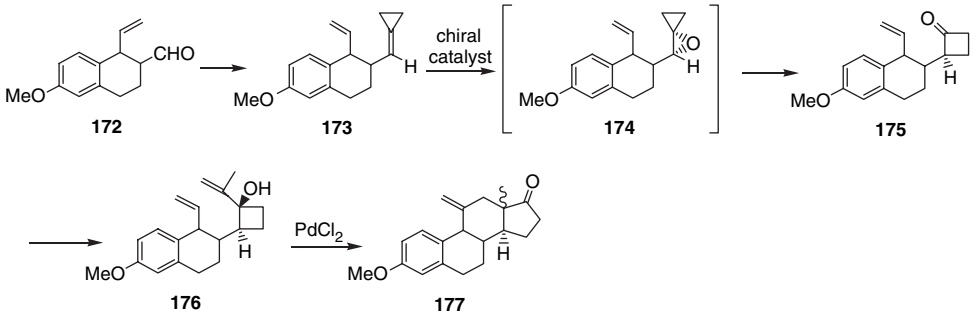


Scheme 30.

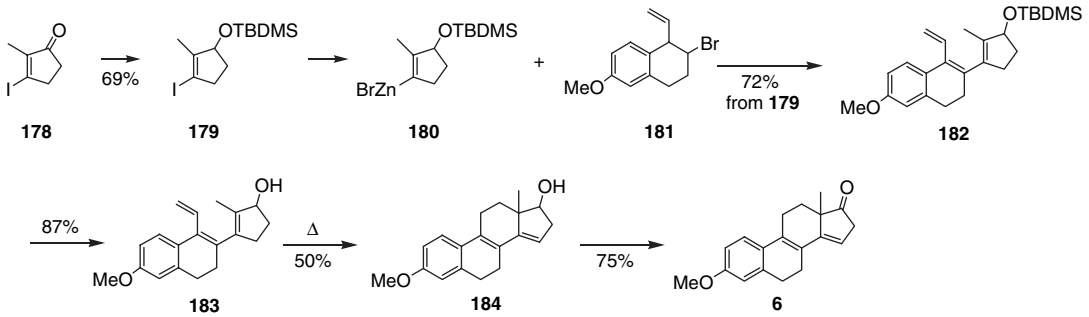
Another method published by the group of Nemoto, also utilising a cyclobutane intermediate, relies on a palladium-catalysed rearrangement leading to the formation of rings C and D (Scheme 31).[131,132] The substrate for the reaction was prepared starting from aldehyde **172**, which was reacted with cyclopropylidene-triphenylphosphorane to **173**. The epoxidation to the chiral intermediate **174** with 5 mol % of (*RR*)-(salen)Mn^{III} complex and sodium hypochlorite and the ring expansion to the cyclobutanone **175** followed by addition of isopropenylmagnesium bromide afforded the chiral substrate **176** for the cyclisation reaction. The palladium-catalysed cyclisation led to a mixture of diastereomers **177**, which was separated and further converted to enantiomerically pure (+)-equilenin.

Gilchrist and co-workers described an approach making use of an electrocyclic ring closure of trienic systems to close ring C (Scheme 32).[133] The trienes were prepared using a palladium(0)-catalysed cross-coupling reaction of vinylzinc bromides (**180**) and aryl halides (**181**). The protected vinyl iodide **179** was prepared starting from 3-iodo-2-methyl-2-cyclopentenone **178**. In this way, the starting triene **182** was obtained in good overall yield (72%). Deprotection of the hydroxyl group to **183** and thermal cyclisation then gave the steroid skeleton **184**. This group prepared several known steroidal compounds of the oestrone family (**6**), using this chemistry.[133–136]

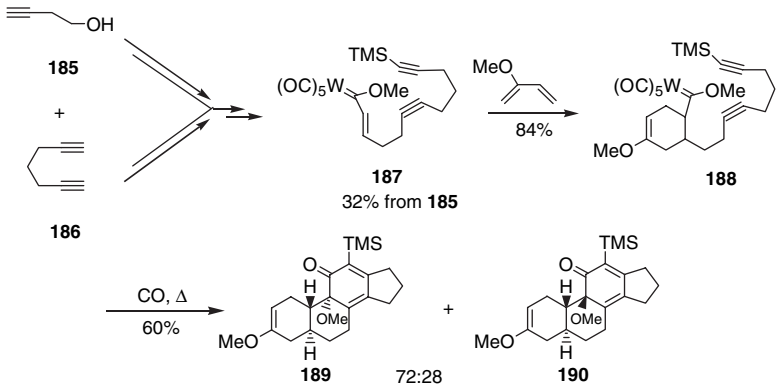
Wulff and co-workers used a tandem coupling of a Diels–Alder reaction of Fischer carbene complexes with a double intramolecular two-alkyne annelation to synthesise steroidal ring systems in which rings A and C are aromatic. In seven steps the linear precursor complex **187** was synthesised starting from the alkynes **185** and **186**. A Diels–Alder reaction of **187** with 2-methoxy-1,4-butadiene for the construction of ring A, then first leads to intermediate **188**, and next, in



Scheme 31.



Scheme 32.



Scheme 33.

a one-pot tandem reaction sequence, accessed the tetracyclic ring systems **189** and **190**, but the product bears no methyl on C13 (Scheme 33).[137]

12. Radical cyclisation

Finally, radical cyclisation was used by Malacria et al. to synthesise a steroid skeleton in a one-pot procedure, giving only two diastereomers **196** in 3:10 ratio, but unfortunately, both had the less desirable 13α-configuration and the usual methyl substituent on this position was missing.[138]

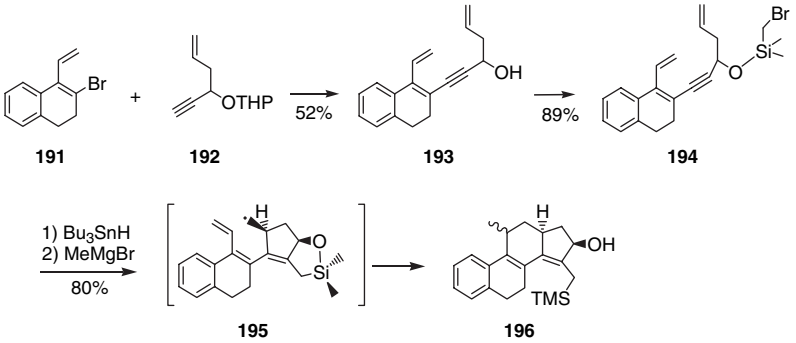
The key step of this reaction sequence consists of a tandem radical cyclisation of compound **194** (Scheme 34), which was obtained using a palladium-catalysed coupling reaction between the alkyne **192** and the aryl bromide **191**. The bromomethyldimethylsilyl group was introduced in compound **194** to initiate the radical cyclisation reaction. This first led to intermediate **195** with closure of ring D, and next to the closure of ring C to the two isomers of **196**.

13. Mukaiyama chemistry in ring C construction

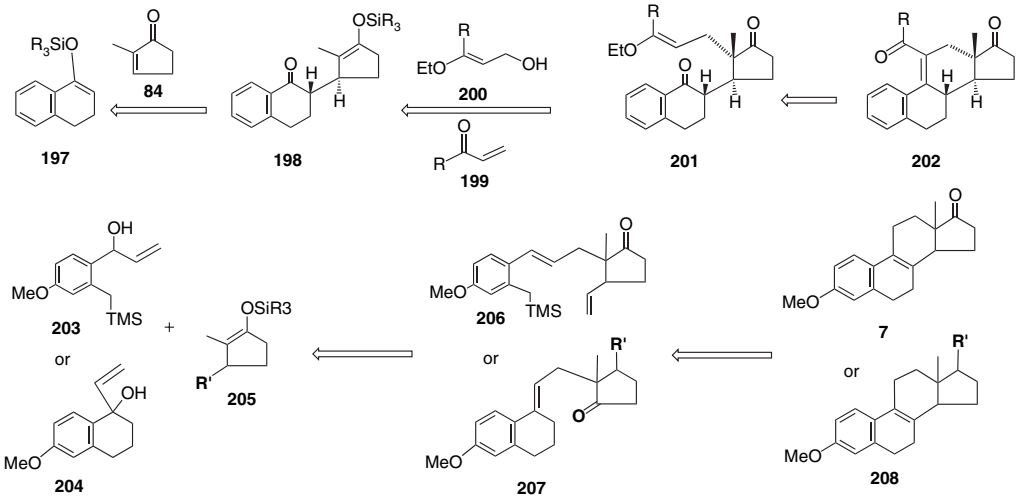
Two elements of the Mukaiyama reaction have been applied in the construction of ring C in steroid synthesis. The first element is the Mukaiyama–Michael reaction with transfer of the silyl group from the starting silyl enol ether, e.g., **197**, to the carbonyl group of the receiving enone, e.g., **84**.[139–147] In this way, a second silyl enol ether **198** is obtained, which enables either a selective reaction with the silyl enol ether in ring D, or a selective reaction with the unprotected carbonyl group in ring B. The second element is the reaction of a silyl enol ether in ring D, e.g., **198** or **205**, with a second enone, e.g., **199**, or with a reactive carbocation precursor, e.g., **200**, **203** or **204**, to construct the C12–C13 bond as in **201**, **206** or **207** (Scheme 35).[148–150] Different types of ring closure reactions then should lead to steroid skeletons like **202**, **7** or **208**.

A first example of the construction of the C12–C13 bond via the reaction of a carbocation precursor with a silyl enol ether was presented by the group of Magnus,[149] who reacted the carbocation precursor **209**, which was synthesised in three steps from *p*-methoxybenzoic acid, with silyl enol ether **210** to obtain the coupled product **211** in 88% yield (Scheme 36). Epoxidation of the $\Delta^{10,11}$ double bond to **212** set the stage for a caesium fluoride-catalysed elimination of the trimethylsilyl group combined with ring opening of the epoxide. In this way, the orthoquinodimethane intermediate **213** was formed for an intramolecular Diels–Alder reaction to **214**. Oxidation of the hydroxyl group then gave the C11-functionalized steroid skeleton **215**.

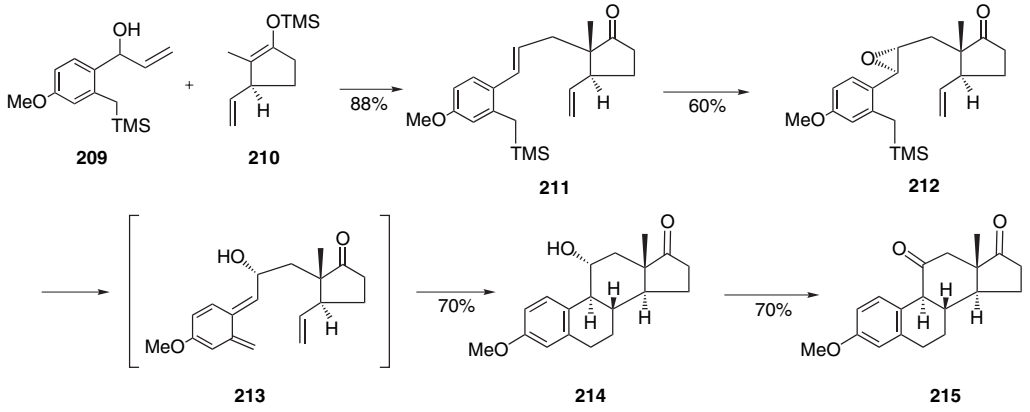
An original method for steroid synthesis was published in 1998 by Grieco and co-workers.[150] This method involved both of the above-mentioned elements of Mukaiyama chemistry. First, a Mukaiyama reaction with transfer of the silyl group from **216** to the second silyl enol ether **217**, followed by a reaction with the reactive carbocation precursor **218**, yielded compound **219** with the correct trans-stereochemistry at C13 and C14 (Scheme 37).



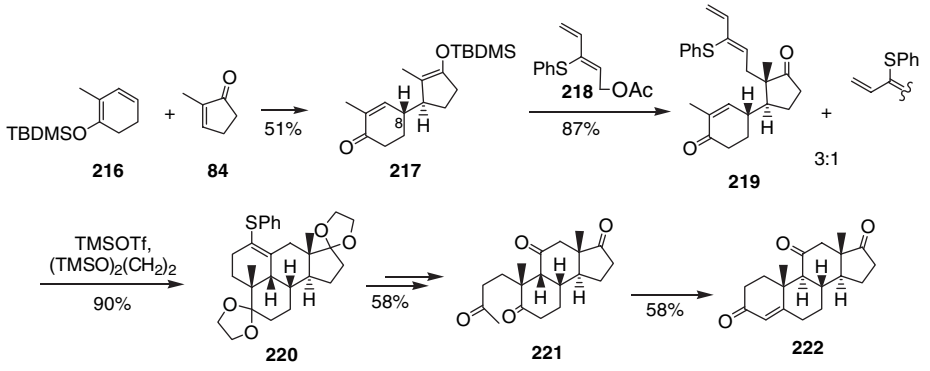
Scheme 34.



Scheme 35.



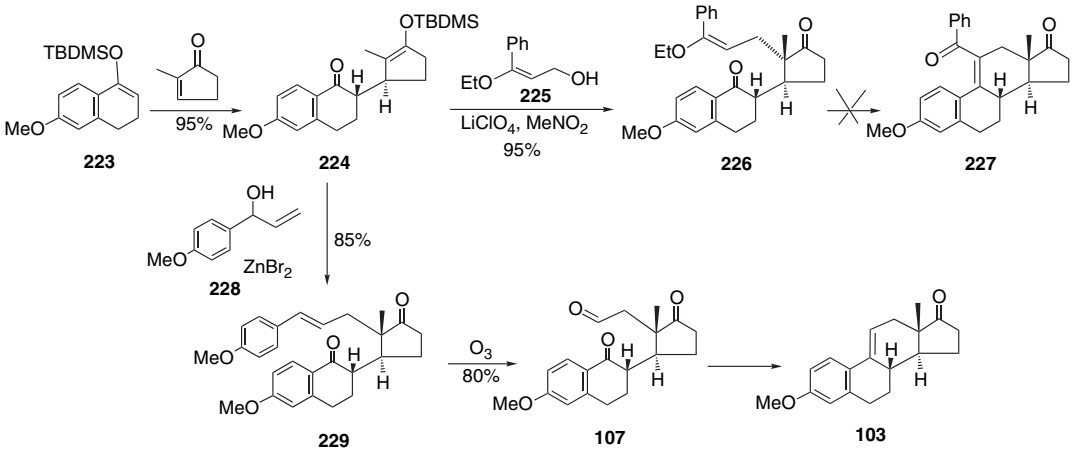
Scheme 36.



Scheme 37.

A tandem intramolecular Diels–Alder cycloaddition/olefin isomerisation then leads to the tetracyclic compound **220**. This intermediate could be converted into adrenosterone **222** via replacement of the thiophenyl group by a methyl, followed by ozonolysis to **221** and ring closure under basic conditions.

Several new routes for the syntheses of cis- and trans-C, D-coupled (D-homo) steroids have been developed by De Groot et al. that rely on a combination of Mukaiyama and Torgov chemistry as illustrated in Scheme 35. A Mukaiyama–Michael reaction with transfer of the silyl group from a silyl enol ether derived from 6-methoxy-1-tetralone **223** to 2-methyl-2-cyclopenten-1-one leads in high yield to the adduct **224**, which can take part in a second Mukaiyama addition. This second Mukaiyama reaction with methyl vinyl ketone did not, however, take place.[151] On the other hand, carbocations, obtained from reactive allylic



Scheme 38.

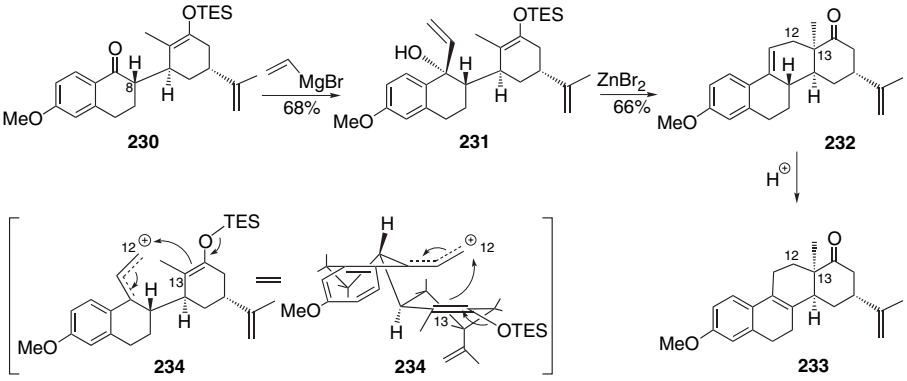
alcohols like **225**[148,152,153] or **228**,[154] did react with silyl enol ethers like **224** under mild Lewis acid conditions in good to excellent yields (Scheme 38).[155–157] An Aldol-type ringclosure of **226** to **227** could also not be accomplished, probably due to the low reactivity of the carbonyl group in the tetralone moiety of the molecule.[158]

A better alternative seemed to be offered by constructing the C9–C11 bond via cyclisation of Ziegler's triketone **107**, which has been reported previously to proceed in reasonable yields (see Schemes 19, 20 and 21).[149,150,159] A short synthesis of triketone **107** could be achieved via the addition of the carbocation precursor **228**, synthesised by the Grignard addition of vinylmagnesium bromide to anisaldehyde,[160] to silyl enol ether **224**, which gave an 85% yield of the adduct **229**. Ozonolysis of this adduct proceeded in good yield and, in this way, provided for a short and efficient synthesis of Ziegler's triketone **107** in 70% overall yield in four easy steps.[158]

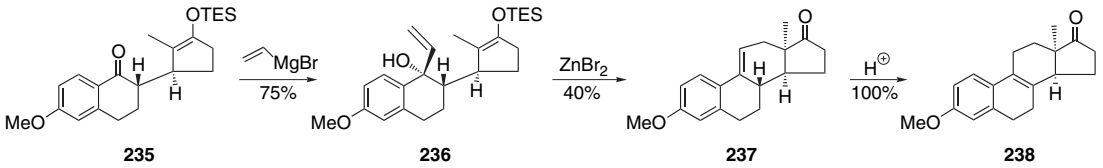
The Mukaiyama–Michael reaction with transfer of the silyl enol ether of methoxytetralone to the carbonyl group of the receiving enone is again an important element in a route leading to CD-cis-fused (D-homo) steroids. In the adduct **230**, a selective Grignard reaction of vinylmagnesium bromide with the unprotected carbonyl group of the methoxytetralone moiety leads to a Torgov-type intermediate **231** (Scheme 39). This can be converted easily into a carbocation, which then reacts intramolecularly with the silyl enol ether under formation of the C12–C13 bond to complete the synthesis of the (D-homo) steroid skeleton **232**.[159] With *R*-carvone as receptor, enantiomerically pure D-homo steroids with the natural steroid configuration at C14 are obtained as an enantiopure product in just five steps, starting from methoxytetralone, in 40% overall yield.

The oxime derived from **233** could be obtained in crystalline form and the X-ray structure[161] revealed the cis coupling of the C,D rings, as indicated in Scheme 39. This stereochemistry can be explained by a selective approach of the bulky silyl enol ether to R-(−)-carvone from the top face, opposite to the isopropenyl group. The carvone ring ends up in a favourable axial position next to the carbonyl group in the tetralone portion of the molecule and, consequently, the addition of vinylmagnesium bromide occurs from the top face. The closure of ring C via the intermediate carbocation **234** then leads to a cis-fused-CD ring system in the steroid skeleton. The TMS enol ethers and the TBDMS enol ethers reacted in a similar manner, ultimately giving the same final product **233** in 45% overall yield.

The Mukaiyama–Michael reaction of the TES enol ether of 3-methoxytetralone with 2-methylcyclopentenone gave diastereomeric mixtures of the new silyl enol ether **235** in



Scheme 39.



Scheme 40.

quantitative yield (Scheme 40; only one stereoisomer is indicated). The addition of vinylmagnesium bromide to **235** also went smoothly, but the cyclisation of the adducts **236** with $ZnBr_2$ gave a lower yield of the diastereomeric mixture of the steroid skeleton **237**, with dehydration as the major side reaction. Finally, acid-catalysed isomerisation of this mixture gave the known racemic **238**[162] in about 30% overall yield, starting from **235**.

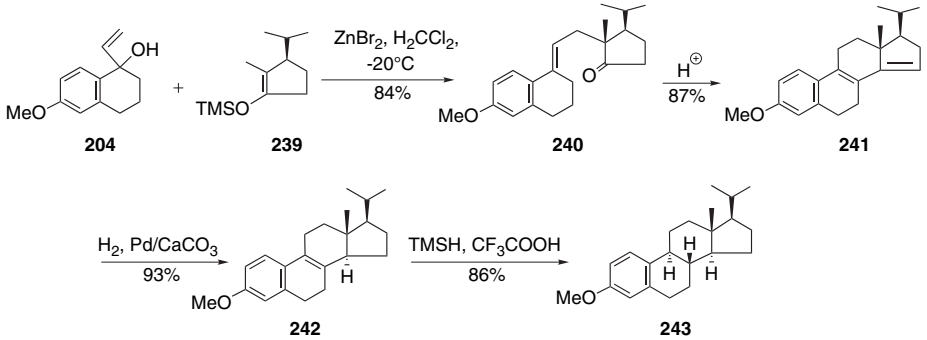
A second approach to CD-trans-fused (D-homo) steroid skeletons has been developed, in which the C12–C13 bond was formed in the second step of the sequence using an *intermolecular* Lewis acid-catalysed reaction of the Torgov-type reagent **204** with a silyl enol ether containing ring D precursor **205**, to give the seco steroid **207**.[163] A cyclisation reaction then gives the C8–C14 bond to close ring C, now as the last step (Scheme 35). This method gives a quick access to a wide variety of C17-substituted steroid skeletons with a similar set of double bonds in the C and D rings as in the products from the traditional Torgov reaction. Selective catalytic reduction then yields the CD-trans-fused (D-homo) steroid skeletons.

A wide variety of silyl enol ethers **205** has been obtained by conjugate addition followed by capture of the enolate with a silylating agent, via Mukaiyama–Michael reactions on enones with transfer of the silyl group from the starting enol ether to the enol of the adduct, or by direct silylation of ketones. The reaction of the Torgov reagent **224** with silyl enol ethers of cyclopentanones to seco steroids **207** proceeded in excellent yields, and also when the silyl enol ether had an ethyl group at C2. Similar reactions with silyl enol ethers derived from cyclohexanones, leading to D-homo steroid skeletons, gave more diverse results and steric hindrance quickly lowered the yields. The double bonds in the C and D rings in the steroid and D-homo steroid skeletons can be reduced catalytically to C,D-trans-fused steroid skeletons according to well-known literature procedures.[22,54,120,162,164] A specific example of the whole sequence is shown in Scheme 41, where the isopropyl substituted silyl enol ether **239** has been reacted with the Torgov reagent **204** to the ring C-open adduct **240**. Acid-catalysed ring closure to diene **241**, reduction of the Δ^{14} double bond to **242** and finally reduction of the Δ^{9} double bond gave the C17 substituted all *trans* steroid **243** in a high overall yield.

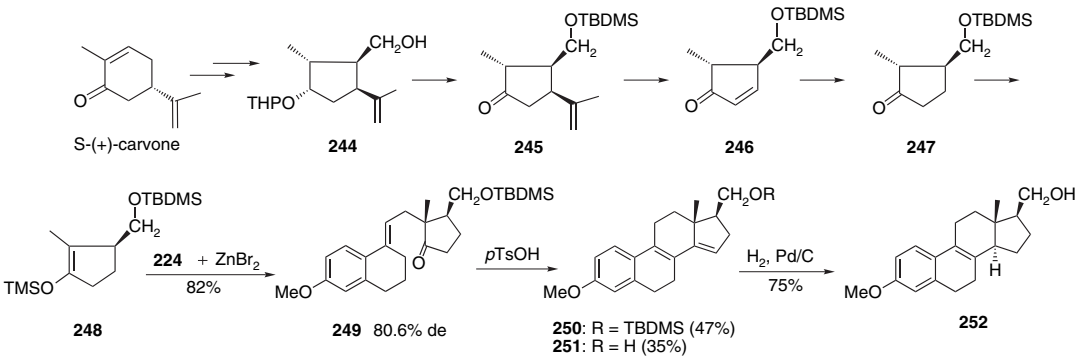
The approach mentioned in Schemes 35 and 41 is very suitable for the synthesis of *chiral* steroid skeletons, provided that chiral ring D precursors are available or easily accessible. Silyl enol ether ring D precursors have been used previously in other routes to steroids by the groups of Magnus[149] and Wicha,[141,165–167] and, on one occasion, a chiral ring D precursor has been used.[167] A synthesis of a suitable ring D precursor was developed starting from carvone, because a good procedure for the ring contraction of carvone, using a Favorskii rearrangement leading to compound **244**, was known from the literature (Scheme 42).[168]

Deprotection, selective reprotection of the primary alcohol and oxidation then gives the cyclopentanone **245**. Regioselective removal of the isopropenyl group to **246**, catalytic reduction of the double bond to **247**, and formation of the thermodynamic silyl enol ether finally affords the desired chiral ring D precursor **248**. When placed into a reaction with the Torgov reagent **204**, seco steroid **249** is obtained in 82% yield as a mixture of two C13 diastereomers, with a diastereomeric excess of 81%. Cyclisation of **249** gives the steroidal diene **250** in 47% yield, next to a 35% yield of the deprotected compound **251**.[169] Selective reduction of the diene **251** gave a 75% yield of **252**, the remainder being unreduced starting material. Thus complete deprotection of the alcohol moiety in the side chain directly after the cyclisation, followed by selective reduction, is the best procedure.

Although the overall yield of the optically active steroid ring D precursor **248** from (*S*)-(+)-carvone is only 10% and



Scheme 41.



Scheme 42.

requires 11 steps, the applicability of this approach for the preparation of enantiomerically pure steroid skeletons has been shown. The development of straightforward, easy and high-yielding syntheses for *chiral* steroid ring D precursors is, however, essential to make this route into a good method for the synthesis of optically active steroid skeletons.

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Biographical sketch

Dr. ir. Florence C. E. Sarabèr was born in 1977 in Rotterdam, The Netherlands. She graduated in 2000 with distinction in Molecular Sciences from Wageningen University, after a stay of 7 months at Nottingham University, in the group of Dr. N. R. Thomas. In 2005, she completed her Ph.D. thesis on the total synthesis of steroids, entitled 'Steroids from Carvone', at Wageningen University under the supervision of Prof. dr. Aede de Groot. Her field of interest remains the total synthesis of natural compounds, although she currently holds a position as Regulatory Affairs Scientist at Organon N.V., Oss, The Netherlands.

Professor Aede de Groot received his M.Sc. in Organic Chemistry and Technical Chemistry in 1964 from the University of Groningen in The Netherlands. He did his Ph.D. in Organic Chemistry in 1967 under the supervision of Prof. dr. Hans Wijnberg, also in Groningen, and he was a post doctoral fellow with Prof. dr. E. E. van Tamelen where he got his first training in synthesis of natural products. From 1969 to 1971 he gained industrial experience at the Dutch State Mines (DSM) on electro-organic synthesis, and after that he was an assistant professor at the Technical University of Eindhoven. In 1972 he was appointed as full professor in Bio-organic Chemistry at Wageningen University. He has retired from this position in 2004. His research interests concentrated on total syntheses of natural products, especially sesqui- and diterpenes with physiologically active properties in the field of crop protection (antifeedants, repellents, pheromones), flavour and fragrance and pharmaceuticals (steroids), and on syntheses starting from terpenes, which are abundantly available from Nature.

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On the importance of the pore inner cavity for the ionophoric activity of 1,3-*alternate* calix[4]arene/steroid conjugates

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Abstract—1,3-*Alternate* calix[4]arenes, decorated with four nonpolar 'all-trans' tetracyclic nuclei and cation-stabilizing β-methoxyethoxy appendages, were synthesized from commercially available starting materials and through straightforward functional groups transformations/couplings. Their Na^+-transport activities, when compared with those exerted by the known conformationally-rigidified 1,3-*alternate* calix[4]-arene AB/cis cholic acid conjugates, suggest that the cation conductance is related to the morphology of the pendant steroids.

1. Introduction

In living organisms, ion transport is mediated by integral membrane proteins that span from the internal to the external surface of the lipid bilayer. The intimate chemical details of the cation transport in biological systems still remain elusive,[1] but details of the conductance mechanisms are emerging thanks to the structure/function relationship analysis of natural and artificial transporters.[2]

In this context, we have recently demonstrated[3] that in conformationally-rigidified 1,3-*alternate* (i.e., **1**, Fig. 1) and *cone* calix[4]arene/hydroxycholanic derivatives, the observed unimolecular ion transport is dependent on the length of the channel.

In this paper we try to assess how the presence of a pre-organized pore cavity influences the cation transport process. This is a crucial issue for the design of artificial ion channels.

With the idea of clarifying the structural requisites useful for the ionophoric properties, we embarked on the synthesis of two conformationally immobilized 1,3-*alternate* calix[4] arene/all-trans steroid conjugates **2** and **3** (Fig. 1), and compared their ionophoric activity with that of the powerful Na^+-transporter **1**,[3] showing a 'folded' AB-cis-cholane framework.

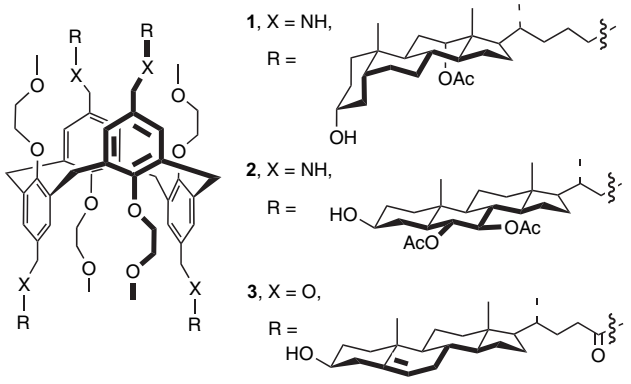

Figure 1. Calix[4]arenes conjugates **1**–**3**.

Compound **2** was designed in order to have approximately the same length as **1** (35±2 Å), an AB-trans ring junction and two equatorial acetoxy groups, useful for transient cation stabilization, lying in the same plane of the tetracyclic nucleus.

In conjugate **3**, the steroid moiety was simplified to the *flat* (20*R*)-3β-hydroxy-chol-5-enoate residue, showing a slightly longer side chain (estimated overall length of **3**: 39±2 Å)[4] and no oxygenated substituents, apart from the hydroxy group at C-3.

The absence of a pre-existing inner cavity in the N- and O-linked calix[4]arene/sterol **2** and **3** is suggested by molecular modeling studies.[5] Figure 2 displays the extended lowest energy conformations of conjugates **1**–**3**. In the case of the 'folded' AB-cis framework, as in **1**, the presence of an

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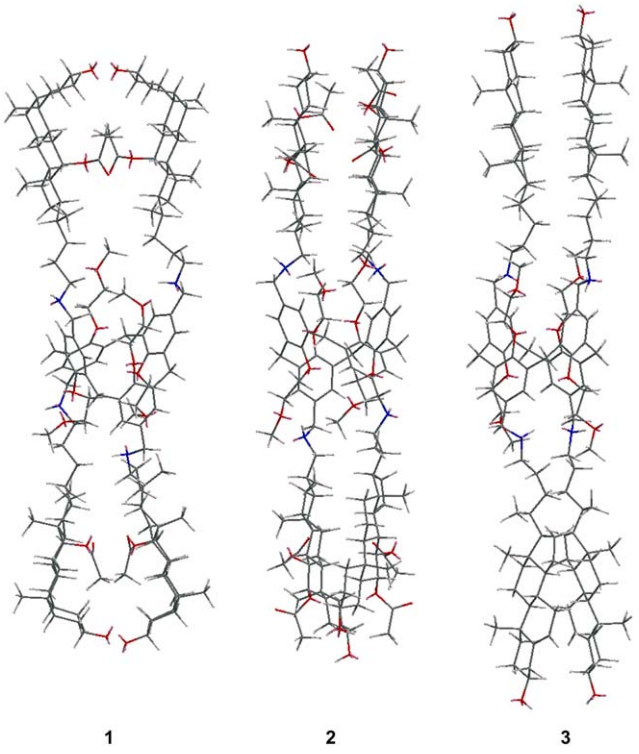


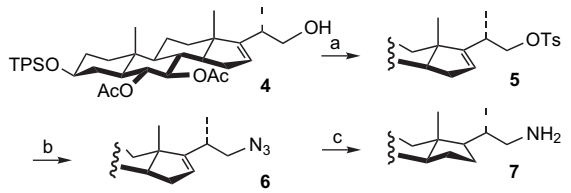
Figure 2. Energy-minimized structures obtained by molecular modeling of compounds **1–3** in their extended conformations.

intramolecular hollow is evident. In derivatives **2** and **3**, Van der Waals forces stabilize the interactions of the facing all-trans steroid pairs, packing the nonpolar appendages in a more 'compact' morphology.

2. Results and discussion

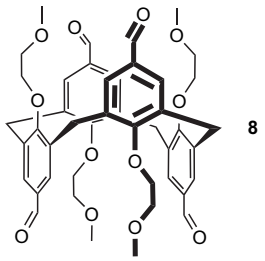
2.1. Synthesis of conjugate 2

The 1,3-*alternate* calix[4]arene/6α,7β-diacetoxy-5α-23,24-bisnorcholan-3β-ol-22-amino derivative was synthesized starting from the known[6] (20*S*)-6α,7β-diacetoxy-3β-[(*tert*-butyldiphenylsilyl)oxy]-5α-23,24-bisnorchol-16-en-22-ol (**4**, Scheme 1). This was first tosylated[7] at the primary hydroxy group and then substituted in the presence of sodium azide[8] to yield **6**. The C-22 azide was subsequently reduced,[9] with molecular hydrogen and Adam's catalyst, to give, in good yield and high stereoselectivity,[10] the saturated (20*S*)-6α,7β-diacetoxy-3β-[(*tert*-butyldiphenylsilyl)oxy]-22-amino-5α-23,24-bisnorcholane (**7**).

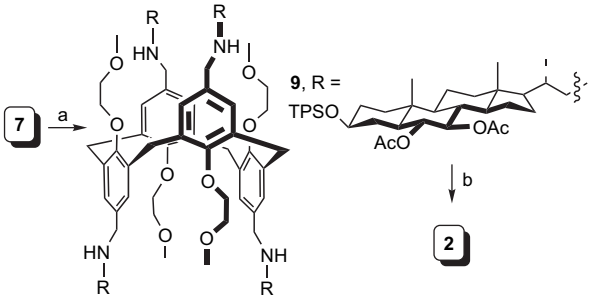


Scheme 1. Reagents and conditions: (a) TsCl, Py, CH_2Cl_2, 91%; (b) NaN_3, DMF, 60 °C, 96% and (c) H_2, PtO_2, EtOH, 79%.

With the aminosteroid **7** in our hands we performed the well described $NaBH(OAc)_3$-mediated reductive amination[11] in the presence of the known conformationally immobilized



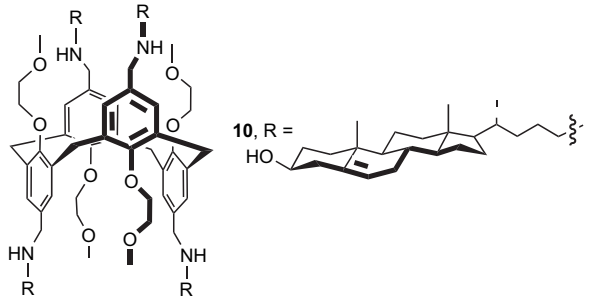
calix[4]arene-1,3-*alt* tetra-aldehyde **8**[12,3] and, to our delight, we isolated, after chromatographic purification, the tetra-adduct **9** in a satisfying 83% yield (Scheme 2). HF/pyridine-mediated *tert*-butyldiphenylsilyl deprotection, gave the expected target **2**.



Scheme 2. Reagents and conditions: (a) **8**, AcOH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 83% and (b) HF, Py, 48%.

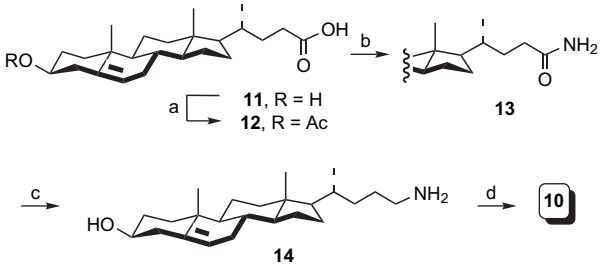
2.2. Attempted synthesis of conjugate 10

Having secured the first 1,3-*alternate* calix[4]arene/all-trans steroid derivative, we turned our attention to the synthesis of the Δ^5-conjugate **10**.



Its supposedly easier convergent synthesis (Scheme 3), started from a C-3 acetylation of the commercially available cholenic acid (**11**) to give the (20*R*)-3β-acetoxy-chol-5-enic acid (**12**). This was transformed into the carboxamide **13** (99% yield), using, as condensing agent, the diphenylphosphorylazide (DPPA)[13] and then reduced to the required (20*R*)-24-amino-chol-5-ene-3β-ol (**14**) with $LiAlH_4$. This time we failed to synthesize the requested tetra-amino calix[4]arene **10** using the $NaBH(OAc)_3$-mediated reductive amination conditions obtaining, instead, a complex mixture containing the mono and the bis-adducts (ESI-MS analysis). In order to improve the efficiency of the coupling reaction we decided to change the reaction conditions. In particular, considering the low solubility of the amine **14** in halogenated solvents, we turned to the more polar solvent DMF and, in a subsequent experiment, we increased the number of

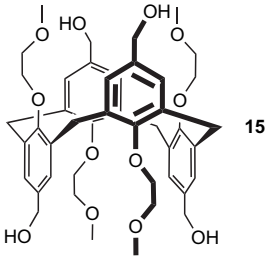
equivalents of the steroid primary amine (see Section 4). Unfortunately, none of the attempted reactions gave the desired tetra-aminated calix[4]arene **10**. We invariably observed the formation of a complex mixture of lower adducts (ESI-MS analysis, see Section 4) and no trace of the required **10**.



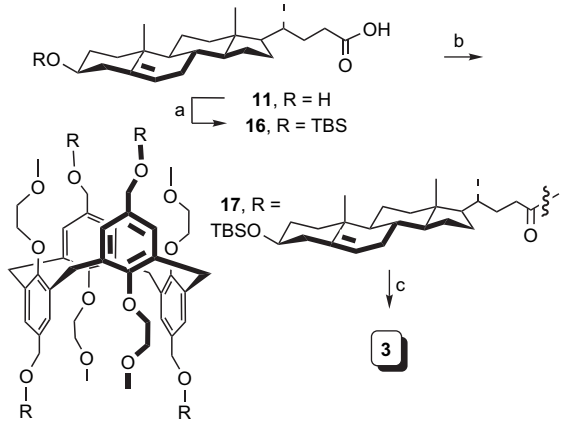
Scheme 3. Reagents and conditions: (a) Ac_2O, Py, CH_2Cl_2; (b) DPPA, NH_4Cl, NEt_3, DMF, 0 °C, 99%, for two steps; (c) $LiAlH_4$, THF, reflux, 54% and (d) **8**, AcOH, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$ or DMF (see Section 4).

2.3. Synthesis of conjugate 3

To overcome the difficulties posed by the reductive amination, we decided to link the two coupling partners (1,3-*alternate* calix[4]arene and the all-trans sterol) via an ester bond. Having the cholenic acid ready for use, we focused our attention to the reduction of the tetra-aldehyde **8**, in order to obtain the tetra-alcohol **15**. The conformationally restricted 1,3-*alternate* calix[4]arene tetraol **15** was thus easily formed through $NaBH_4$-induced carbonyl reduction of the tetra-aldehyde **8**.



The sterol partner used in the EDC-mediated condensation reaction was the 3β-[(*tert*-butyldimethylsilyl)oxy]-chol-5-enic acid (**16**), easily synthesized in two steps from the commercially available cholenic acid (**11**, Scheme 4).



Scheme 4. Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C then K_2CO_3, MeOH, THF, H_2O, 79%; (b) EDC, DMAP, CH_2Cl_2, 0 °C, 46% and (c) HF (48% in H_2O), THF, quant.

The esterification reaction went smoothly and gave, after chromatographic purification, the pure silylated tetra-adduct **17** (46% yield). Finally, HF-mediated desilylation, produced the C_4 symmetric target **3**.

2.4. Na^+-transporting activities of conjugates 2 and 3

To investigate the ionophoric properties of conjugates **2** and **3** we studied their ability to promote the transport of Na^+ across a lipid bilayer using a $^{23}Na^+$ NMR based methodology.[14] Figure 3 shows that conjugates **2** and **3** behave as poor ionophores: in the presence of 1% of each of them (percent of steroids with respect to the total concentration of lipid, curve ○ for **2** and ● for **3**), and after more than 13 h, the amount of Na^+ entering the internal vesicular compartment was only slightly higher than in the absence of any additive (curve ◆). On the contrary, as previously reported,[3] conjugate **1** efficiently induces the transport of Na^+ across the lipid bilayer and its transmembrane gradient is fully discharged in almost 12 h (curve ■).[15]

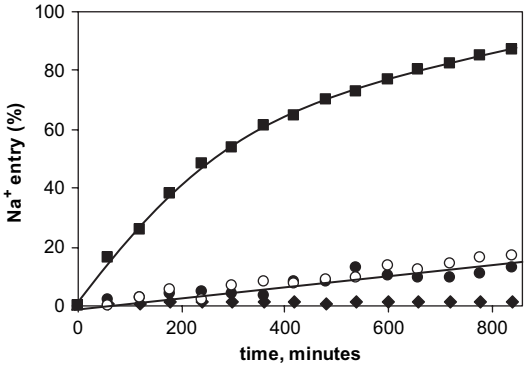


Figure 3. Kinetic profiles for the entry of Na^+ into 95:5 egg PC/PG vesicles containing **1** (1%, ■), **2** (1%, ○), and **3** (1%, ●), and without additives (◆) at 25 °C. The total concentration of lipid was 10 mM.

3. Conclusions

The results of this endeavor clarify the relevance of an extended cavity throughout the pore for the transport of Na^+ across a lipid bilayer. The two newly prepared 1,3-*alternate* calix[4]arene/steroid conjugates characterized by the presence of all-trans junctions behave as poor ionophores regardless to the presence of the acetoxy moieties. As suggested by the molecular modeling studies, in these conjugates the 'flat' steroid nucleus adopts a compact packing hampering the cation transport. On the other hand, the 'folded' AB-cis geometry of the steroid appendages present in **1** favors the formation of an inner cavity, which appears essential for the transport process.

4. Experimental

4.1. General methods

All reactions were carried out under a dry argon atmosphere using freshly distilled and dried solvents, unless otherwise

noted. Tetrahydrofuran (THF) was distilled from $LiAlH_4$. Toluene, methylene chloride, and diethyl ether were distilled from calcium hydride. Glassware was flame-dried (0.05 Torr) prior to use. When necessary, compounds were dried in vacuo over P_2O_5 or by azeotropic removal of water with toluene under reduced pressure. Starting materials and reagents purchased from commercial suppliers were generally used without purification. Reaction temperatures were measured externally. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm), visualized by UV light or with H_2SO_4–$Ce(SO_4)_2$ or ninhydrin solutions. Flash chromatography was performed on Merck silica gel (60, particle size: 0.040–0.063 mm). Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) pure materials. The NMR spectra were recorded at rt or, when indicated, at 80 °C or 100 °C on a Bruker DRX 400 (^1H at 400 MHz, ^{13}C at 100 MHz) and a Bruker DRX 300 (^1H at 300 MHz, ^{13}C at 75 MHz) spectrometers. Chemical shifts are reported relative to the residual solvent peak ($CHCl_3$: δ=7.26, $^{13}CDCl_3$: δ=77.0; $C_2H_2Cl_4$: δ=5.80, $^{13}C_2D_2Cl_4$ (TCDE): δ=72.1). HRES-MS was performed on a Q-Star Applied Biosystem mass spectrometer. Optical rotations were measured with a JASCO DIP-1000 polarimeter.

4.1.1. Compound 5. To a solution of the alcohol **4** (0.100 g, 0.145 mmol) in CH_2Cl_2 (1.5 ml), pyridine (0.035 ml, 0.435 mmol) and *p*-toluenesulfonylchloride (0.055 g, 0.290 mmol) were added and the reaction mixture was stirred overnight. Pyridine (0.023 ml, 0.29 mmol) and *p*-toluenesulfonylchloride (0.042 g, 0.217 mmol) were further added. After 24 h the reaction was treated with a saturated solution of NH_4Cl (4 ml), extracted with CH_2Cl_2 (3×5 ml), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was flash-chromatographed (10–40% ethyl acetate in petroleum ether) to give **5** (0.111 g, 91%) as a white amorphous solid.

5: $[\alpha]_D$ +17.3 (*c* 2.4, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.67 (3H, s, C*H*$_3$-18), 0.94 (3H, s, C*H*$_3$-19), 0.97 (3H, d, *J*=6.9 Hz, C*H*$_3$-21), 1.03 (9H, s, (*CH*$_3$)$_3$CSi–), 1.87 (3H, s, COC*H*$_3$), 1.96 (3H, s, COC*H*$_3$), 2.38 (1H, m, H-20), 2.44 (3H, s, C*H*$_3$(Ts)), 3.53 (1H, m, H-3), 3.83 (1H, br t, *J*=9.4 Hz, H-22), 3.98 (1H, dd, *J*=9.4, 5.9 Hz, H′-22), 4.65 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 4.79 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 5.20 (1H, br s, H-16), 7.36 (8H, m, Ar*H* and Ar*H*(Ts)), 7.63 (4H, m, Ar*H*), 7.65 (2H, d, *J*=7.2 Hz, Ar*H*(Ts)). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.3, 15.9, 18.3, 19.0, 20.6, 20.9, 21.3, 21.5, 26.9 (×3), 31.0, 31.5, 32.0, 32.1, 34.1, 35.9, 36.8, 37.8, 46.4, 47.8, 52.0, 54.6, 71.9, 73.8, 74.2, 77.5, 123.6, 127.4 (×4), 127.8 (×2), 129.5 (×2), 129.7 (×2), 133.2, 134.5, 134.6, 135.7 (×4), 144.5, 154.2, 170.5, 170.6. HRES-MS, *m*/*z*: 841.4135 (calcd 841.4169 for $C_{49}H_{65}O_8SSi$) [MH^+].

4.1.2. Compound 6. To a solution of the tosylate **5** (0.111 g, 0.132 mmol) in dry DMF (1 ml), sodium azide (0.043 g, 0.661 mmol) was added and the reaction mixture was stirred at 60 °C for 22 h. The reaction was quenched with brine (2 ml) and then extracted with Et_2O (3×4 ml). The combined organic phases were dried over K_2CO_3, filtered, and concentrated in vacuo. The residue was flash-chromatographed (10–30% ethyl acetate in petroleum ether) to give **6** (0.090 g, 96%) as a white amorphous solid.

6: $[\alpha]_D$ +9.2 (*c* 1.7, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.77 (3H, s, C*H*$_3$-18), 0.96 (3H, s, C*H*$_3$-19), 1.03 (12H, s, (*CH*$_3$)$_3$CSi– and C*H*$_3$-21), 1.87 (3H, s, COC*H*$_3$), 1.97 (3H, s, COC*H*$_3$), 2.32 (1H, m, H-20), 3.16 (1H, dd, *J*=12.0, 7.7 Hz, H-22), 3.35 (1H, dd, *J*=12.0, 5.9 Hz, H′-22), 3.54 (1H, m, H-3), 4.68 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 4.80 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 5.34 (1H, br s, H-16), 7.37 (6H, m, Ar*H*), 7.64 (4H, m, Ar*H*).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 13.3, 15.9, 19.0, 19.6, 20.6, 21.0, 21.4, 26.9 (×3), 31.1, 32.0, 32.1, 32.3, 34.4, 36.0, 36.8, 37.9, 46.4, 48.0, 52.1, 54.8, 56.8, 72.0, 74.2, 77.6, 123.1, 127.4 (×4), 129.5 (×2), 134.6 (×2), 135.7 (×4), 155.8, 170.5, 170.7. HRES-MS, *m*/*z*: 712.4119 (calcd 712.4146 for $C_{42}H_{58}N_3O_5Si$) [MH^+].

4.1.3. Compound 7. To a solution of the azide **6** (0.069 g, 0.097 mmol) in ethyl acetate (1.0 ml), PtO_2 (0.007 g) was added and the reaction mixture was stirred under hydrogen atmosphere for 48 h. The catalyst was then filtered off on a short pad of Celite™ washing with ethyl acetate. The solvent was removed under reduced pressure and the crude product was flash-chromatographed (10–20% methanol in chloroform) to give **7** (0.053 g, 79%) as a white amorphous solid.

7: $[\alpha]_D$ +11.8 (*c* 1.6, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ: 0.65 (3H, s, C*H*$_3$-18), 0.92 (3H, s, C*H*$_3$-19), 0.97 (3H, d, *J*=6.9 Hz, C*H*$_3$-21), 1.03 (9H, s, (*CH*$_3$)$_3$CSi–), 1.86 (3H, s, COC*H*$_3$), 1.93 (3H, s, COC*H*$_3$), 2.38 (1H, dd, *J*=11.9, 7.2 Hz, H-22 or H′-22), 2.71 (1H, br d, *J*=11.9 Hz, H-22 or H′-22), 3.51 (1H, m, H-3), 4.62 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 4.75 (1H, br t, *J*=9.5 Hz, H-6 or H-7), 7.37 (6H, m, Ar*H*), 7.63 (4H, m, Ar*H*).^{13}C NMR ($CDCl_3$, 100 MHz) δ: 12.0, 13.3, 16.9, 19.1, 20.7, 21.2, 21.5, 24.8, 26.9 (×3), 28.0, 31.1, 32.1, 35.7, 37.0, 39.0 (×2), 39.3, 43.6, 46.1, 46.9, 51.7, 52.4, 54.6, 72.0, 74.5, 77.9, 127.5 (×4), 129.5 (×2), 134.5 (×2), 135.7 (×4), 170.6, 170.9. HRES-MS, *m*/*z*: 688.4377 (calcd 688.4397 for $C_{42}H_{62}NO_5Si$) [MH^+].

4.1.4. Compound 9. To a solution of aldehyde **8** (0.051 g, 0.066 mmol) and **7** (0.454 g, 0.660 mmol) in 1,2-dichloroethane (2.5 ml) $NaBH(OAc)_3$ (0.084 g, 0.396 mmol) and AcOH (15 µl, 0.264 mmol) were added. The reaction mixture was stirred at rt overnight, quenched with 1 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The crude residue was flash-chromatographed (5–20% of methanol in dichloromethane) to give **9** (0.190 g, 83%) as a white amorphous solid.

9: $[\alpha]_D$ +12.2 (*c* 1.0, $CHCl_3$). ^1H NMR (TCDE, 100 °C, 400 MHz) δ: 0.52 (12H, s, C*H*$_3$-18), 0.77 (24H, m, C*H*$_3$-19 and C*H*$_3$-21 overlapped), 0.91 (36H, s, (*CH*$_3$)$_3$CSi–), 1.69 (12H, s, COC*H*$_3$), 1.75 (12H, s, COC*H*$_3$), 2.46 (8H, br m, C*H*$_2$NH), 3.16 (12H, br s, OC*H*$_3$), 3.16–3.67 (36H, m, ArOC*H*$_2$C*H*$_2$OCH$_3$, ArC*H*$_2$Ar, NHC*H*$_2$Ar and H-3 overlapped), 4.48 (4H, m, H-6 or H-7), 4.61 (4H, m, H-6 or H-7), 7.07 (8H, br s, Ar*H*), 7.17–7.24 (24H, m, Ar*H*(TPS)), 7.50 (16H, m, Ar*H*(TPS)). ^{13}C NMR (TCDE, 80 °C, 100 MHz) δ: 10.2 (×4), 11.5 (×4), 16.3 (×4), 17.2 (×4), 18.7 (×4), 19.5 (×4), 19.6 (×4), 22.9 (×4), 25.3 (×12), 25.8 (br, ×4), 27.8 (×4), 29.4 (×4), 30.5 (×4), 32.0 (br, ×4), 33.9 (×4), 35.4 (×4), 37.4 (×8), 42.0 (×4), 44.6 (×4), 48.0 (br, ×4), 50.1 (×4), 51.0 (×8), 52.8 (×4), 57.0 (×4), 68.9 (br,

×4), 69.8 (×4), 70.4 (×4), 72.7 (×4), 76.2 (×4), 121.0 (br, ×4), 125.7 (×16), 127.6 (×8), 130.2 (br, ×8), 132.4 (br, ×8), 133.0 (×4), 133.1 (×4), 133.9 (×16), 155.4 (br, ×4), 168.4 (×4), 168.6 (×4). ES-MS, *m/z*: 3455.0501 (calcd 3455.0703 for $C_{212}H_{293}N_4O_{28}Si_4$) [$MH^+$].

4.1.5. Compound 2. To a solution of **9** (0.064 g, 0.018 mmol) in pyridine (0.5 ml) at 0 °C, a solution of 70% hydrofluoric acid in pyridine (0.3 ml) was added. The reaction mixture was stirred overnight and concentrated under a stream of N_2. The residue was purified by flash chromatography (silica gel, 8–20% methanol in $CHCl_3$) to afford **2** (0.022 g, 48%) as a white amorphous solid.

2: R_f=0.4 (10% methanol in CH_2Cl_2). $[\alpha]_D$ +25.1 (*c* 1.0, MeOH). ^{1}H NMR (TCDE, 80 °C, 400 MHz) δ: 0.56 (12H, s, C*H*$_3$-18), 0.77 (24H, m, C*H*$_3$-19 and C*H*$_3$-21 overlapped), 1.79 (12H, s, COC*H*$_3$), 1.82 (12H, s, COC*H*$_3$), 2.67 (8H, br m, C*H*$_2$NH), 3.23 (12H, br s, OC*H*$_3$), 3.35–3.98 (36H, m, ArOC*H*$_2$C*H*$_2$OCH$_3$, ArC*H*$_2$Ar, NHC*H*$_2$Ar and H-3 overlapped), 4.57 (4H, m, H-6 or H-7), 4.68 (4H, m, H-6 or H-7), 7.07 (8H, br s, Ar*H*), 7.17–7.24 (24H, m, Ar*H*(TPS)), 7.50 (16H, m, Ar*H*(TPS)). ^{13}C NMR (TCDE, 80 °C, 100 MHz) δ: 10.2 (×4), 11.5 (×4), 15.2 (×4), 18.9 (×4), 19.6 (×8), 22.9 (×4), 25.7 (×4), 27.8 (×4), 29.3 (×4), 30.5 (×4), 31.9 (×4), 34.0 (×4), 35.3 (×4), 37.4 (×8), 42.2 (×4), 44.6 (×4), 48.0 (br, ×4), 50.1 (×4), 50.8 (×8), 52.7 (×4), 56.8 (×4), 68.8 (×4), 70.0 (×8), 72.8 (×4), 76.1 (×4), 121.0 (br, ×4), 129.8 (×8), 132.6 (×8), 155.4 (×4), 168.6 (×8). ES-MS, *m/z*: 2502.7001 (calcd 2502.5992 for $C_{148}H_{221}N_4O_{28}$) [MH^+].

4.1.6. Compound 13. To a solution of cholenic acid (0.288 g, 0.77 mmol) in dry CH_2Cl_2 (20 ml), pyridine (1.00 ml, 12.3 mmol), DMAP (0.005 g, 0.04 mmol), and Ac_2O (0.145 ml, 1.54 mmol) were added. The resulting mixture was stirred for 3 h, diluted with CH_2Cl_2 (10 ml), quenched with 1 M HCl (10 ml) and extracted with CH_2Cl_2 (2×10 ml). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product, which was used in the next step without further purification. To a solution of the crude compound in DMF (10 ml) at 0 °C, diphenylphosphorylazide (DPPA, 0.40, 1.85 mol), NH_4Cl (0.099 g, 1.85 mol), and Et_3N (0.054 ml, 3.85 mmol) were sequentially added. The mixture was stirred at −10 °C overnight, diluted with ethyl acetate (10 ml), treated with a saturated solution of NH_4Cl (10 ml) and extracted with ethyl acetate (2×15 ml). The combined organic phases were washed with a solution of $NaHCO_3$ (10% in water), brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was flash-chromatographed (2–10% ethyl acetate in petroleum ether) to give **13** (0.317 g, 99%, for two steps) as a white amorphous solid.

13: $[\alpha]_D$ −27.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.76 (3H, s, C*H*$_3$-18), 1.01 (3H, d, *J*=6.7 Hz, C*H*$_3$-21), 1.08 (3H, s, C*H*$_3$-19), 2.05 (3H, s, C*H*$_3$CO), 4.56 (1H, m, H-3), 5.42 (1H, br d, *J*=4.8 Hz, H-6). ^{13}C NMR (CD_3OD, 100 MHz) δ: 12.3, 18.9, 19.7, 21.2, 22.1, 25.3, 28.8, 29.2, 33.0, 33.2 (×2), 33.5, 36.9, 37.7, 38.2, 39.1, 41.0, 43.5, 51.5, 57.2, 58.0, 75.4, 123.6, 141.0, 172.4, 179.8. HRES-MS, *m/z*: 416.3209 (calcd 416.3165 for $C_{26}H_{42}NO_3$) [MH^+].

4.1.7. Compound 14. To a solution of **13** (0.387 g, 0.93 mmol) in THF, $LiAlH_4$ (1 M in THF, 2.3 mmol) was added. The reaction mixture was refluxed at 65 °C for 3 h, then water (0.1 ml) and NaOH (15% solution of in water, 0.1 ml) were added. The resulting mixture was vigorously stirred, filtered under reduced pressure washed with 30% CH_3OH in ethyl acetate. The solvent was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was flash-chromatographed (2–25% methanol in CH_2Cl_2/CH_3COOH 99:1) to give **13** (0.180 g, 54%) as a white amorphous solid.

14: $[\alpha]_D$ −12.8 (*c* 1.0, MeOH). ^{1}H NMR (CD_3OD, 300 MHz) δ: 0.76 (3H, s, C*H*$_3$-18), 1.01 (3H, d, *J*=6.7 Hz, C*H*$_3$-21), 1.05 (3H, s, C*H*$_3$-19), 2.90 (3H, s, C*H*$_2$NH$_2$), 3.43 (1H, m, H-3), 5.36 (1H, br d, *J*=4.8 Hz, H-6). ^{13}C NMR (CD_3OD, 100 MHz) δ: 12.3, 19.1, 19.9, 22.1, 25.3 (×2), 29.2, 32.3, 33.0, 33.2, 33.8, 36.8, 37.6, 38.5, 41.2 (×2), 43.0, 43.5, 51.6, 57.2, 58.1, 72.4, 122.4, 142.2. HRES-MS, *m/z*: 360.3285 (calcd 360.3266 for $C_{24}H_{42}NO$) [MH^+].

4.1.8. First attempted synthesis of 10. To a solution of amine **14** (0.105 g, 0.29 mmol) in 1,2-dichloroethane (1 ml), aldehyde **8** (0.038 g, 0.049 mmol), $NaBH(OAc)_3$ (0.062 g, 0.29 mmol), and AcOH (11 μl, 0.20 mmol), were added. The reaction mixture was stirred at rt overnight, quenched with 1 N NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The crude residue was flash-chromatographed (2–30% methanol in dichloromethane) to give a complex mixture containing the mono and the bis-adduct. ES-MS, *m/z*: 1111.6 (mono-adduct); *m/z*: 1455.0 (bis-adduct). Mass peaks ratio: 4:1.

4.1.9. Second attempted synthesis of 10. To a solution of amine **14** (0.105 g, 0.29 mmol) in 1,2-DMF (1 ml), aldehyde **8** (0.038 g, 0.049 mmol), $NaBH(OAc)_3$ (0.062 g, 0.29 mmol), and AcOH (11 μl, 0.20 mmol), were added. The reaction mixture was stirred at rt overnight, quenched with 1 M NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude residue was flash-chromatographed (2–30% methanol in dichloromethane) to give a complex mixture containing the mono and the bis-adduct. ES-MS, *m/z*: 1111.6 (mono-adduct); *m/z*: 1455.0 (bis-adduct). Mass peaks ratio: 3:1.

4.1.10. Third attempted synthesis of 10. To a solution of amine **14** (0.217 g, 0.60 mmol) in DMF (4 ml), aldehyde **8** (0.061 g, 0.079 mmol), $NaBH(OAc)_3$ (0.127 g, 0.60 mmol), and AcOH (22 μl, 0.39 mmol) were added. The reaction mixture was stirred at rt overnight, quenched with 1 N NaOH solution and extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude residue was flash-chromatographed (2–30% of methanol in dichloromethane) to give a complex mixture containing the mono and the bis-adduct. ES-MS, *m/z*: 1111.6 (mono-adduct); *m/z*: 1455.0 (bis-adduct). Mass peaks ratio: 3:1.

4.1.11. Compound 15. To a solution of aldehyde **8** (0.143 g, 0.19 mmol) in EtOH (2 ml) at 0 °C, $NaBH_4$ (0.032 g, 0.84 mmol), was added. The resulting mixture was stirred

at rt for 2 h, quenched with HCl (10% solution in water, 5 ml) and concentrated under reduced pressure to remove the excess of EtOH. The aqueous layer was extracted with ethyl acetate (3×10 ml) and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to give a residue, which was purified by flash chromatography (2–15% MeOH in EtOAc) to afford **15** (0.112 g, 76%) as a white amorphous solid.

15: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.50 (12H, s, CH_3O–), 3.67 (8H, s, Ar–CH_2–Ar), 3.71 (8H, m, –OCH$_2$CH_2O–), 3.91 (8H, m, –OCH_2CH$_2$O–), 4.45 (8H, s, –CH_2OH), 7.05 (8H, s, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 35.7 (×4), 58.7 (×4), 65.3 (×4), 71.5 (×4), 72.0 (×4), 130.3 (×8), 133.4 (×8), 135.0 (×4), 155.3 (×4). HRES-MS, *m*/*z*: 777.3811 (calcd 777.3850 for $C_{44}H_{57}O_{12}$) [MH$^+$].

4.1.12. Compound 16. To a solution of **11** (0.612 g, 1.63 mmol) in DMF (14 ml) at 0 °C, imidazole (0.888 g, 13.04 mmol), 4-dimethylaminopyridine (DMAP, 0.398 g, 3.26 mmol), and *tert*-butyldimethylsilyl chloride (TBSCl, 0.737 g, 4.89 mmol), were added. The resulting mixture was stirred at rt overnight, then quenched with HCl (1 M, 10 ml), extracted with ethyl acetate (3×30 ml), washed with brine, dried on Na_2SO_4, and concentrated in vacuo to give the crude, which was used in the next step without further purification. To a solution of the crude material in MeOH (14 ml) and THF (14 ml), a solution of K_2CO_3 (10% w/w, 7 ml) in water, was added. The resulting mixture was stirred at rt for 2 h then concentrated under reduced pressure to remove the excess of THF and MeOH. A saturated solution of NaCl was added and the aqueous layer was acidified with HCl (5%) to pH 3. The mixture was extracted with ethyl acetate (3×40 ml), dried on Na_2SO_4 and concentrated in vacuo to give pure **16** (0.630 g, 79%) as a white amorphous solid.

16: $[\alpha]_D$ −29.3 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.06 (6H, s, (CH_3)$_2$CSi–), 0.67 (3H, s, CH_3-18), 0.88 (9H, s, (CH_3)$_3$Si–), 0.93 (3H, d, *J*=6.7 Hz, CH_3-21), 0.99 (3H, s, CH_3-19), 3.48 (1H, m, H-3), 5.31 (1H, br s, H-6), 10.30 (1H, br s, COO*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: −4 (×2), 11.9, 18.3 (×2), 19.4, 21.0, 24.2, 25.9 (×3), 28.1, 30.8, 31.1, 31.9, 32.0, 35.3 (×2), 36.5, 37.4, 39.7, 42.4, 42.8, 50.1, 55.7, 56.7, 72.6, 121.1, 141.5, 180.6. HRES-MS, *m*/*z*: 489.3711 (calcd 489.3764 for $C_{30}H_{53}O_3Si$) [MH$^+$].

4.1.13. Compound 17. To a solution of **16** (0.41 g, 0.84 mmol) in dry CH_2Cl_2 (8 ml) at 0 °C, DMAP (0.048 g, 0.39 mmol), a solution of **15** (0.11 g, 0.14 mmol) in dry CH_2Cl_2 (2 ml) and EDC (0.17 g, 0.90 mmol) were sequentially added. The reaction mixture was stirred for 16 h, quenched with water (5 ml) and extracted with ethyl acetate (20 ml×3). The organic layer was washed with a saturated solution of $NaHCO_3$ and water, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude was purified by flash chromatography (1–25% of a solution of 1% AcOH in EtOAc in petroleum ether), to furnish **17** (0.172 g, 46%) as a white amorphous solid and 0.060 g of a mixture containing **16** and **17** in a 6:1 ratio.

17: $[\alpha]_D$ −20.2 (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.06 (24H, s, (CH_3)$_2$CSi–), 0.67 (12H, s, CH_3-18), 0.89 (36H, s, (CH_3)$_3$Si–), 0.92 (12H, d, *J*=6.7 Hz, CH_3-21), 0.99 (12H, s, CH_3-19), 3.44 (12H, s, CH_3O–), 3.45 (4H, m, H-3), 3.54 (8H, s, Ar–CH_2–Ar), 3.63 (8H, m, –OCH$_2$CH_2O–), 3.83 (8H, m, –OCH_2CH$_2$O–), 4.92 (8H, br s, –CH_2OCO), 5.31 (1H, br s, H-6), 7.08 (8 H, s, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: −4.6 (×8), 11.8 (×4), 18.2 (×4), 18.3 (×4), 19.4 (×4), 21.0 (×4), 24.2 (×4), 25.9 (×12), 28.1 (×4), 30.9 (×4), 31.3 (×4), 31.9 (×8), 32.0 (×4), 34.8 (×4), 35.3 (×4), 36.5 (×4), 37.3 (×4), 39.7 (×4), 42.3 (×4), 42.8 (×4), 50.1 (×4), 55.8 (×4), 56.7 (×4), 58.8 (×4), 66.2 (×4), 71.2 (×4), 71.9 (×4), 72.6 (×4), 121.1 (×4), 128.9 (×4), 130.5 (×8), 133.3 (×8), 141.5 (×4), 155.7 (×4), 174.0 (×4). ES-MS, *m*/*z*: 2680.6845 (2680.7990 calcd for $C_{164}H_{256}NaO_{20}Si_4$) [MNa$^+$].

4.1.14. Compound 3. To a solution of **17** (0.098 g, 0.0368 mmol) in THF (1.0 ml) at 0 °C, a solution of 48% hydrofluoric acid in water (40 µl, 2.21 mmol) was added. The reaction mixture was stirred overnight then concentrated under a stream of N_2. The residue was purified by flash chromatography (silica gel, 1–10% methanol in $CHCl_3$) to afford **3** (0.081 g, quant.) as a white amorphous solid.

3: $[\alpha]_D$ −23.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.65 (12H, s, CH_3-18), 0.91 (12H, d, *J*=6.7 Hz, CH_3-21), 0.99 (12H, s, CH_3-19), 3.45 (12H, s, CH_3O–), 3.50 (4H, m, H-3), 3.53 (8H, s, Ar–CH_2–Ar), 3.61 (8H, m, –OCH$_2$CH_2O–), 3.81 (8H, m, –OCH_2CH$_2$O–), 4.90 (4H, d, *J*=12.1 Hz, –CH*H*OCO), 4.93 (4H, d, *J*=12.1 Hz, –C*H*HOCO), 5.33 (1H, br d, *J*=4.8 Hz, H-6), 7.07 (8H, s, Ar). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 11.8 (×4), 18.3 (×4), 19.4 (×4), 21.0 (×4), 24.2 (×4), 28.1 (×4), 30.9 (×4), 31.6 (×4), 31.8 (×12), 34.9 (×4), 35.3 (×4), 36.4 (×4), 37.2 (×4), 39.7 (×4), 42.2, (×4), 42.3 (×4), 50.0 (×4), 55.8 (×4), 56.7 (×4), 58.8 (×4), 66.2 (×4), 71.2 (×4), 71.6 (×4), 71.8 (×4), 121.5 (×4), 128.9 (×4), 130.5 (×8), 133.3 (×8), 140.8 (×4), 155.7 (×4), 174.0 (×4). ES-MS, *m*/*z*: 2224.4679 (2224.4531 calcd for $C_{140}H_{200}NaO_{20}$) [MNa$^+$].

Acknowledgements

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Sesterterpene metabolites from the sponge *Hyatella intestinalis*

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Abstract—The sponge *Hyatella intestinalis* from the Gulf of California contains the new scalarane-related sesterterpenes hyatelones A–C and hyatolides A–B, together with the new scalaranes hyatolides C–E, hyatelactam, 12-*O*-deacetyl-19-*epi*-scalarin and the new norscalarane 12-*O*-deacetylnorscalaral B. The structures of the new metabolites have been established by spectroscopic analysis of the natural products and, in some instances, of their acetyl derivatives. The new compounds hyatelone A, 19,20-di-*O*-acetylhyatelone B, hyatolide A, 20-*O*-acetylhyatolide C, hyatelactam, and 12-*O*-deacetylnorscalaral B have shown activity as growth inhibitors of several tumor cell lines.

1. Introduction

Among the natural terpenoids, the C_{25} derivatives or sesterterpenes are the least common group, with most of the compounds described from marine organisms, in particular from sponges of the Order Dictyoceratida.[1] From a structural point of view, a significant group of marine-derived sesterterpenes displays a tetracarbocyclic framework formed by six-membered rings. Within this group, scalarin (**1**)[2] is the parent compound of the scalarane skeleton, which possess C-19 and C-20 linked to C-18 and C-17 of ring D, respectively. On the other hand, furoscalarol (**2**)[3] was the first member of a more unusual series of scalarane-related compounds whose framework is characterized by possessing a two-carbons unit (C-19 and C-20) linked to C-17 of ring D. From a biomedical point of view, a number of scalaranes and related sesterterpenes has shown interesting properties, recent examples of which include antibacterial[4] and cytotoxic activities.[5]

As a part of our project directed to the search for cytotoxic metabolites from sponges, we have studied specimens of the species *Hyatella intestinalis* whose extracts showed growth inhibitory activity of the tumor cell lines A-549 and HT-29. Previous studies of this sponge collected in Australia and the Indo-Pacific region have yielded spongiane diterpenes,[6] scalaranes,[7] and sesquiterpene-quinones.[8] In addition, macrolides,[9] likely of symbiotic origin, and ansa farnesyl quinols[10] have been obtained from unidentified species of the genus *Hyatella* collected from the Indonesian and Kenyan coasts, respectively. Herein we report the chemical study of *H. intestinalis* from the Gulf of California, that has yielded the new sesterterpenes hyatelones A–C (**3**–**5**), hyatolides A–E (**6**–**10**), hyatelactam (**11**), 12-*O*-deacetyl-19-*epi*-scalarin (**12**), and 12-*O*-deacetylnorscalaral B (**13**). The sponge also contained the known compounds furoscalarol (**2**),[3] norscalaral A,[11] norscalaral B (**14**),[11] deoxoscalarin,[12] 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide,[13] 12-*epi*-12-*O*-acetylscalarolide,[11] 12-*epi*-12-*O*-deacetyl-19-deoxyscalarin,[14] and scalarin (**1**).[2]

2. Results and discussion

Specimens of *H. intestinalis* were collected by hand using SCUBA diving, liophylized, and exhaustively extracted with acetone/MeOH (1:1). After evaporation of the solvent under reduced pressure, the residue was partitioned between H_2O and Et_2O and the resulting organic extract was subjected to column chromatography. Fractions eluted with hexane/Et_2O (60:40 to 30:70) and $CHCl_3$/MeOH (80:20) were subjected to repeated HPLC separations to yield the new compounds **3**–**13**. Compounds **4**, **5**, **8**, and **10** were characterized as their acetyl derivatives **4a**, **5a**, **8a**, and **10a**, respectively.

2.1. Hyatelones A–C (3–5)

Hyatelone A (**3**) was obtained as a solid whose molecular formula $C_{27}H_{40}O_6$ was determined by HRCIMS. The ^{13}C NMR spectrum displayed 27 signals (Table 1), two of them attributable to an acetoxyl group [δ 169.6 (CH_3COO–) and 21.3 (CH_3COO–)]. The remaining 25 carbon signals, together with the five singlets observed in the ^1H NMR spectrum at

Keywords: Natural products; Sponges; Sesterterpenes; Structure determination; Cytotoxicity.
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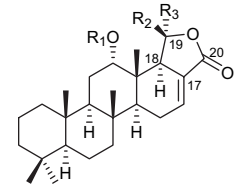
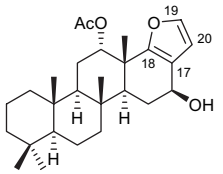


1 R_1 = Ac, R_2 = H, R_3 = OH
12 R_1 = R_3 = H, R_2 = OH



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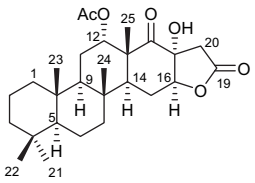

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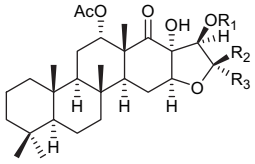
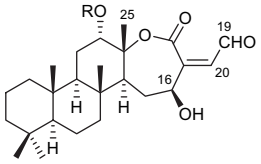
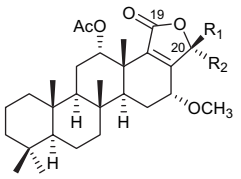


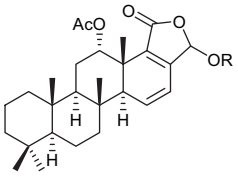
4 R_1 = R_3 = H, R_2 = OH
4a R_1 = Ac, R_2 = OAc, R_3 = H
5 R_1 = R_2 = H, R_3 = OH
5a R_1 = Ac, R_2 = H, R_3 = OAc



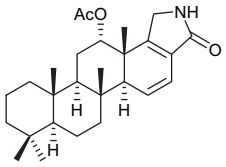
6 R = Ac
7 R = H



8 R_1 = OH, R_2 = H
8a R_1 = OAc, R_2 = H
9 R_1 = H, R_2 = OH



10 R = H
10a R = Ac



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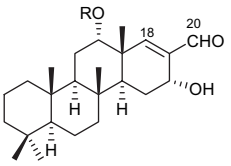


13 R = H
14 R = Ac

δ 1.27 (3H), 0.93 (3H), 0.86 (3H), 0.82 (3H), and 0.81 (3H), suggested that **3** was a sesterterpene. In particular, the presence of a scalarane or related tetracarbocyclic skeleton was deduced from the ^{13}C NMR signals due to three methines [δ 56.5 (C-5), 52.3 (C-9), and 44.0 (C-14)] and four quaternary sp^3 carbons [δ 51.1 (C-13), 38.2 (C-8), 36.9 (C-10), and 33.3 (C-4)]. The NMR signals at δ_C 72.8 (d)/δ_H 5.31 (1H, dd, J=3.5 and 2.2 Hz) were assigned to a methine bearing the acetoxyl group. This methine was located at C-12 based on the HMBC correlation between the carbon at δ 72.8 and Me-25 (δ 1.27). The presence of a ketone carbonyl at C-18 was deduced from the ^{13}C NMR signal at δ 210.1 that showed HMBC correlations with H-14 and Me-25 (Fig. 1). The NMR spectra also showed signals due to the carbonyl of an ester [δ_C 172.2 (s)] and two carbons bearing oxygenated functions [δ_C 85.1 (d)/δ_H 4.56 (dd, J=11.5 and 6.6 Hz) and δ_C 78.9 (s)]. These data together with an isolated methylene [δ_C 42.2 (t)/δ_H 2.71 (1H, d, J=17.8 Hz) and 2.51 (1H, d, J=17.8 Hz)] and the hydroxyl function observed in the IR spectrum (3463 cm^{-1}) were consistent with the presence of a β-hydroxy-γ-lactone unit involving carbons C-16, C-17, C-19, and C-20 of a scalarane-related framework. This proposal was confirmed by the HMBC correlations between the methylene proton at δ 2.71 (H-20) and the carbonyl at C-18 and between the oxymethine carbon at δ 85.1 (C-16) and H-14 (Fig. 1).

In the NOESY spectrum the Me-24 (δ 0.93) was correlated with Me-23 (δ 0.82) and Me-25 (δ 1.27), while the methine proton H-9 (δ 1.24) was correlated with the methines H-5 (δ 0.82) and H-14 (δ 1.69). These data were in agreement with an all *trans*-fusion of rings A–B–C–D of the tetracarbocyclic skeleton, being Me-23, Me-24, and Me-25 axially oriented to the β-face, whereas H-5, H-9, and H-14 are axially oriented to the α-face. On the other hand, the coupling constants of H-12 (dd, J=3.5 and 2.2 Hz) indicated an equatorial (β) orientation for H-12. This assignment was supported by the NOESY correlations of H-12 with H-11eq, H-11ax, and Me-25. The α-orientation of H-16 was deduced from the NOESY cross peaks of this proton with H-15α and H-14, while the α-orientation of the hydroxyl at C-17 was deduced from the correlations of the proton H-20 at δ 2.71 with H-15β and Me-25. All these data led to propose structure **3** for hyatelone A.

Hyatelones B (**4**) and C (**5**) were obtained as a mixture that could not be separated by HPLC under all the assayed conditions. Treatment of the mixture with Ac_2O/Py followed by HPLC yielded 19,20-di-*O*-acetylhyatelone B (**4a**) and 19,20-di-*O*-acetylhyatelone C (**5a**). The NMR spectra of **4a** were related to those of hyatelone A (**3**) except for the absence of the signals due to the lactone carbonyl at C-19 and the methylene at C-20, showing in turn the signals of two oxymethines [δ_C 94.7 (d)/δ_H 6.42 (1H, d, J=4.2 Hz) and δ_C 79.9 (d)/δ_H 5.20 (1H, d, J=4.2 Hz)] together with two additional acetyl groups. The HMBC cross peaks of C-16 (δ 84.1) with the protons at δ 6.42 and 5.20 confirmed the location of the oxymethines at C-19 and C-20, respectively, each one linked to one acetyl group. The α-orientation of H-19 and H-20 was proposed from the NOESY correlations of both protons with H-16, which in turn showed cross peaks with H-15α and H-14. All these data defined the structure **4a** for 19,20-di-*O*-acetylhyatelone B, and hence structure **4** for the natural compound hyatelone B. The NMR spectra of compound **5a** were almost identical to those of **4a** except for the signals around the five-membered ring. In particular, H-19 and H-20 appeared as two singlets at δ 6.07 (s) and 5.12 (s), suggesting that **5a** differed from **4a** in the stereochemistry at C-19 and/or C-20. In the NOESY spectrum no cross peak was observed between H-19 and H-16 while the methyl

Table 1. NMR data for compounds **3**, **4a**, and **6**[a,b]

	3		**4a**		**6**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.7	1.58 (m) β, 0.60 (ddd, 13.7, 13.7, 3.7) α	39.7	1.55 (m) β, 0.60 (ddd, 13.4, 13.4, 4.2) α	39.7	1.58 (m) β, 0.68 (ddd, 13.2, 13.2, 3.3) α
2	18.4	1.62 (m), 1.40 (m)	18.4	1.57 (m), 1.43 (m)	18.4	1.58 (m), 1.38 (m)
3	41.8	1.40 (m) β, 1.12 (ddd, 13.2, 13.2, 3.5) α	41.9	1.37 (m) β, 1.11 (ddd, 13.4, 13.4, 4.2) α	41.8	1.38 (m) β, 1.15 (m) α
4	33.3		33.3		33.2	
5	56.5	0.82 (m)	56.5	0.85 (m)	56.2	0.86 (m)
6	18.0	1.62 (m) α, 1.40 (m) β	18.0	1.62 (m) α, 1.43 (m) β	18.4	1.58 (m) α, 1.38 (m) β
7	41.0	1.87 (m) β, 1.10 (ddd, 12.6, 12.6, 4.0) α	40.7	1.85 (ddd, 12.5, 3.3, 3.0) β, 1.07 (m) α	41.3	1.90 (m) β, 1.15 (m) α
8	38.2		37.9		38.8	
9	52.3	1.24 (m)	52.2	1.24 (m)	51.2	1.43 (dd, 13.1, 2.0)
10	36.9		36.9		36.9	
11	21.1	1.87 (m) α, 1.67 (m) β	21.6	1.88 (ddd, 14.9, 3.3, 2.7) α, 1.67 (ddd, 14.9, 13.4, 2.4) β	23.1	1.86 (ddd, 14.8, 3.7, 2.0) α, 1.58 (m) β
12	72.8	5.31 (dd, 3.5, 2.2)	74.1	5.27 (dd, 3.3, 2.4)	74.7	5.11 (dd, 3.7, 2.2)
13	51.1		52.1		85.4	
14	44.0	1.69 (dd, 13.0, 1.2)	44.3	1.71 (d, 12.8)	50.9	2.06 (dd, 12.9, 3.2)
15	25.0	2.43 (dd, 12.8, 6.6) α, 1.55 (m) β	24.8	2.25 (dd, 12.8, 7.3) α, 1.77 (ddd, 12.8, 12.8, 10.7) β	33.8	2.37 (ddd, 13.4, 3.2, 3.2) α, 1.79 (ddd, 13.4, 12.9, 10.8) β
16	85.1	4.56 (dd, 11.5, 6.6)	84.1	4.20 (dd, 10.7, 7.3)	69.9	4.44 (br dd, 10.8, 2.7)
17	78.9		81.5		158.0	
18	210.1		212.1		165.2	
19	172.2		94.7	6.42 (d, 4.2)	191.1	9.96 (d, 7.7)
20	42.2	2.71 (d, 17.8) β, 2.51 (d, 17.8) α	79.9	5.20 (d, 4.2)	130.2	6.52 (dd, 7.7, 1.5)
21	33.2	0.86 (s)	33.2	0.86 (s)	33.2	0.87 (s)
22	21.3	0.81 (s)	21.3	0.81 (s)	21.4	0.81 (s)
23	16.1	0.82 (s)	16.0	0.82 (s)	16.3	0.80 (s)
24	17.5	0.93 (s)	17.7	0.92 (s)	15.9	0.85 (s)
25	20.2	1.27 (s)	19.2	1.25 (s)	21.9	1.47 (s)
***C*H$_3$COO-(12)**	21.3	2.00 (s)	21.1	1.97 (s)	21.2	2.14 (s)
CH$_3$COO-(12)	169.6		169.7		169.8	
***C*H$_3$COO-(19)**			20.5[c]	2.06 (s)		
CH$_3$COO-(19)			168.9			
***C*H$_3$COO-(20)**			20.9[c]	2.06 (s)		
CH$_3$COO-(20)			169.0			

[a] ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 MHz and 150 MHz, respectively.
[b] Assignments were aided by COSY, HSQC, HMBC, and NOESY experiments.
[c] Signals with the same superscript in the same column may be interchanged.

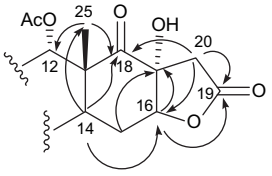


Figure 1. Selected HMBC correlations observed for hyatelone A (**3**).

group of the acetoxyl function at C-19 was correlated with H-20. All these data suggested a β-orientation of H-19 and an α-orientation of H-20, and therefore structure **5a** for 19,20-di-*O*-acetylhytelone C. To the best of our knowledge, hyatelones A–C (**3**–**5**) are the first scalarane-related sesterterpenes containing a carbonyl group at C-18 and a five-membered ring fused to C-16, C-17 of ring D. This novel positioning of the heterocyclic ring represents a significant structural departure from the sestertepenes so far reported, exemplified by furoscalarol (**2**)[3] and scalarolbutenolide.[15]

2.2. Hyatolides A (6) and B (7)

The molecular formula of hyatolide A (**6**), $C_{27}H_{40}O_6$, was determined by HRCIMS. The ^{13}C NMR spectrum displayed 27 signals, two of them corresponding to an acetoxyl group [δ 169.8 (CH_3COO–) and 21.2 (CH_3COO–)]. The remaining 25 signals together with the five methyl singlets in the ^{1}H NMR spectrum at δ 1.47, 0.87, 0.85, 0.81, and 0.80, indicated that **6** was also a sesterterpene. Furthermore, the analysis of the COSY, HSQC, HMBC, and NOESY spectra allowed us to assign the ^{1}H and ^{13}C NMR signals corresponding to rings A–B–C of a scalarane-related compound bearing oxygenated functions at C-12 and C-13. Thus, the presence of the secondary acetoxyl group at C-12 was inferred from the ^{13}C NMR signal at δ 74.7 (d) that was correlated in the HSQC spectrum with the signal at δ 5.11 (1H, dd, J=3.7 and 2.2 Hz) and in the HMBC spectrum with the signals of H-9, H-11eq, and Me-25. On the other hand, the ^{13}C NMR signal at δ 85.4 (s), corresponding to a fully substituted carbon linked to an oxygenated function, was assigned to C-13 upon observation of its HMBC correlations with the signals of H-12, H-14, and Me-25 (Fig. 2). The remaining signals of the ^{13}C NMR spectrum were assigned to an aldehyde [δ 191.1 (d)], the carbonyl of an ester [δ 165.2 (s)], a conjugated-trisubstituted double bond [δ 158.0 (s) and 130.2 (d)], an oxygenated methine [δ 69.9 (d)], and a methylene [δ 33.8 (t)]. These data together with the hydroxyl function observed in the IR spectrum (3375 cm^{-1}) were

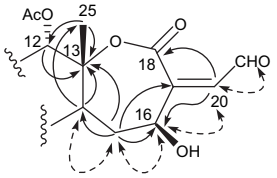


Figure 2. Selected HMBC (plain arrows) and COSY (dashed arrows) correlations observed for hyatolide A (**6**).

accommodated in a seven-membered lactone as follows: the aldehyde proton [δ 9.96 (1H, d, J=7.7 Hz)] was coupled with the olefinic proton [δ 6.52 (1H, dd, J=7.7 and 1.5 Hz)], which showed HMBC correlations with the carbonyl of the ester and with the oxymethine carbon (Fig. 2). These data indicated the presence of an α,β-unsaturated aldehyde whose carbon β was linked both to the carbonyl of the ester and to the oxygenated methine. This latter one was identified as C-16 since the oxymethine proton at δ 4.44 (1H, dd, J=10.8 and 2.7 Hz) showed in the COSY spectrum cross peaks with the methylene protons at δ 2.37 (H-15α) and 1.79 (H-15β), which in turn were coupled with H-14. Consequently, the ester carbonyl had to be located at C-18, the double bond at C-17, C-20, and the aldehyde at C-19. The correlations observed in the NOESY spectrum determined that the stereochemistry of the ring junctions and at C-12 was identical to that described for compound **3**. The NOESY cross peaks of H-16 with H-15α and H-14 indicated the α-orientation for H-16. Finally, the Z geometry of the double bond was supported by the correlation between the olefinic proton H-20 and H-15β. All these data led to propose structure **6** for hyatolide A.

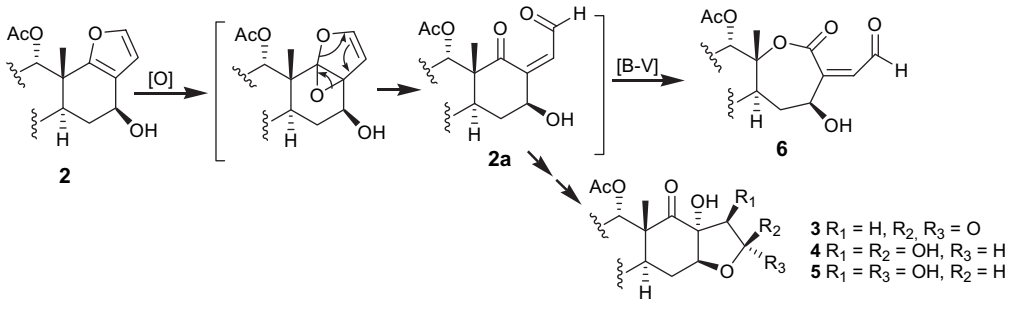
Hyatolide B (**7**) displayed NMR spectra similar to that of compound **6**, except for the absence of the signals due to the acetyl group and the ^{1}H and ^{13}C chemical shifts of the methine C-12 [δ_C 73.9/δ_H 3.88 (dd, J=3.4 and 2.4 Hz)]. These data implied that hyatolide B (**7**) was the 12-*O*-deacetyl derivative of **6**.

The structural features of the seven-membered lactone present in hyatolides A and B (**6** and **7**) are rather unusual. This ε-lactone moiety could be biosynthetically derived from a furan-containing precursor, as shown in Scheme 1. Thus, the transformation of furoscalarol (**2**) into the dicarbonyl intermediate **2a**, followed by a Baeyer–Villiger oxidation of the ketone carbonyl, could lead to hyatolide A (**6**). The involvement of a furan precursor is in agreement with the report on the obtention of a C_{15} compound containing a ε-lactone by reaction of the furanosesquiterpene furodysin with singlet oxygen.[16] Furthermore, hyatelones A–C (**3**–**5**) could also be derived from furoscalarol (**2**) via the dicarbonyl intermediate **2a**.

2.3. Hyatolides C–E (8–10)

Hyatolides C (**8**) and D (**9**) were obtained as a mixture (ca. 2.5:1). In the ^{1}H NMR spectrum, signals due to the major component **8** included six singlets, two of them at δ 1.95 (3H) and 3.44 (3H) were assigned to an acetoxyl and a methoxyl group, respectively, while the remaining singlets at δ 1.15 (3H), 0.91 (3H), 0.85 (3H), and 0.81 (6H) were appropriated for the methyl groups of a scalarane sesterterpene. In addition, the spectrum showed three deshielded methine protons at δ 6.11 (1H, s), 5.51 (1H, dd, J=3.2 and 2.7 Hz), and 4.05 (1H, dd, J=4.5 and 1.0 Hz). Treatment of the mixture with Ac_2O/Py followed by HPLC separation led to the derivative **8a**, whose ^{1}H NMR (Table 2) exhibited the singlet of an additional acetyl group and the methine singlet significantly downfield shifted [δ 6.89 (s) in **8a**, δ 6.11 (s) in **8**]. Similar to the compounds above described, the signal at δ 5.49 (dd, J=2.9 and 2.3 Hz) indicated the location of one axial acetoxyl group at C-12 of **8a**. The attachment of the methoxyl group at C-16 was deduced from the proton signal at δ 3.92 (1H, dd, J=4.6 and 1.2 Hz, H-16) that was correlated in the HMBC spectrum with C-14 (δ 45.8) and the *O*-methyl group (δ 57.4) (Fig. 3). Furthermore, the coupling constants of H-16 indicated an equatorial (β) orientation for this proton. The signals at δ_C 91.1 (d)/δ_H 6.89 (1H, s) were assigned to an acetylated hemiacetal methine. This methine together with a carbonyl [δ 167.9 (s)] and a tetrasubstituted double bond [δ 154.0 (s) and 140.1 (s)] defined the presence of a γ-acetoxy-α,β-unsaturated-γ-lactone involving carbons C-17 to C-20 of the scalarane framework. The location of the double bond at C-17 and C-18 was supported by the HMBC correlations of the olefinic carbons at 154.0 (s, C-17) and 140.1 (s, C-18) with H-15 and Me-25, respectively (Fig. 3). Furthermore, the ^{13}C chemical shifts of C-17 and C-18 indicated the location of the carbonyl at C-19 and defined the regiochemistry of the lactone.

The NOESY correlations were in agreement with the all *trans*-fused A–B–C–D rings system and the equatorial (β) orientation of H-12 (cross peaks with H-11eq, H-11ax, and Me-25) and H-16 (cross peaks with H-15ax and H-15eq). The α-orientation of H-20 was based on the NOESY correlations of H-20 with H-16 and the methoxyl group. All these data were consistent with the structure **8a** and therefore with



Scheme 1. Proposed biosynthetic pathway for the formation of hyatolide A (**6**) and hyatelones A–C (**3**–**5**) from furoscalarol (**2**).

Table 2. NMR data for compounds **8a**, **11**, and **13**[a,b]

	8a		**11**[c]		**13**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	39.6	1.59 (m) β, 0.57 (m) α	39.8	1.57 (m) β, 0.60 (ddd, 13.6, 13.6, 4.8) α	39.8	1.61 (m) β, 0.81 (m) α
2	18.4	1.59 (m), 1.42 (m)	18.4	1.59 (m), 1.43 (m)	18.5	1.58 (m), 1.43 (m)
3	41.9	1.36 (m) β, 1.10 (m) α	41.9	1.38 (m) β, 1.14 (ddd, 13.4, 13.2, 4.4) α	42.0	1.37 (m) β, 1.14 (m) α
4	33.3		33.3		33.2	
5	56.4	0.86 (dd, 12.4, 2.3)	56.7	0.86 (m)	56.6	0.91 (dd, 12.5, 2.4)
6	18.0	1.59 (m) α, 1.42 (m) β	17.8	1.61 (m) α, 1.45 (m) β	18.0	1.58 (m) α, 1.43 (m) β
7	41.1	1.82 (ddd, 12.5, 3.3, 3.3) β, 1.09 (m) α	40.7	1.96 (ddd, 13.0, 3.3, 3.3) β, 1.03 (m) α	40.9	1.82 (m) β, 1.12 (m) α
8	37.0		37.3		37.1	
9	52.6	1.24 (dd, 13.2, 2.3)	52.0	1.29 (dd, 13.2, 2.4)	52.3	1.39 (m)
10	36.8		36.8		37.0	
11	21.1	2.03 (ddd, 15.0, 2.9, 2.3) α, 1.66 (ddd, 15.0, 13.2, 2.3) β	21.8	1.91 (ddd, 14.7, 3.2, 2.4) α, 1.67 (ddd, 14.7, 13.2, 2.2) β	25.1	1.81 (m) β, 1.70 (ddd, 14.5, 3.1, 2.9) α
12	73.5	5.49 (dd, 2.9, 2.3)	73.5	5.06 (dd, 3.2, 2.2)	74.5	3.90 (dd, 2.9, 2.9)
13	39.2		42.2		42.8	
14	45.8	1.81 (dd, 13.1, 1.9)	52.5	2.68 (br s)	43.3	1.88 (dd, 13.4, 2.0)
15	21.7	2.13 (br d, 14.7) α, 1.59 (m) β	129.2	5.97 (dd, 9.7, 2.4)	25.2	1.89 (br d, 14.1) α, 1.63 (m) β
16	69.6	3.92 (dd, 4.6, 1.2)	117.9	6.33 (dd, 9.7, 3.1)	62.0	4.59 (dd, 4.9, 1.4)
17	154.0		129.2		139.6	
18	140.1		159.8		162.5	6.72 (s)
19	167.9		44.2	3.88 (d, 19.8) β, 3.83 (d, 19.8) α	—	
20	91.1	6.89 (s)	172.6		195.1	9.48 (s)
21	33.2	0.85 (s)	33.2	0.86 (s)	33.2	0.84 (s)
22	21.3	0.81 (s)	21.5	0.82 (s)	21.3	0.82 (s)
23	15.9	0.81 (s)	15.9	0.83 (s)	16.1	0.85 (s)
24	16.9	0.91 (s)	18.8	1.05 (s)	17.1	0.89 (s)
25	19.5	1.16 (s)	17.5	1.03 (s)	19.9	1.01 (s)
***C*H$_3$COO-(12)**	20.9	1.96 (s)	21.3	2.11 (s)		
CH$_3$*C*OO-(12)	169.9		170.2			
***C*H$_3$COO-(20)**	20.7	2.16 (s)				
CH$_3$*C*OO-(20)	169.1					
OMe	57.4	3.39 (s)				

[a] ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 MHz and 150 MHz, respectively.
[b] Assignments were aided by COSY, HSQC, HMBC, and NOESY experiments.
[c] The NH signal was clearly observed when the ^{1}H NMR spectrum was recorded in $(CD_3)_2CO$, see Section 3.

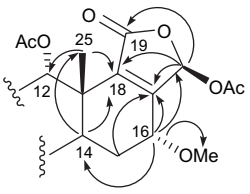


Figure 3. Selected HMBC correlations observed for 20-*O*-acetylhyatolide C (**8a**).

structure **8** for the natural metabolite hyatolide C. Although the acetyl derivative of **9** could not be recovered, the analysis of the NMR spectra of the natural mixture of **8** and **9**, allowed us to identify hyatolide D (**9**) as the C-20 epimer of **8**. Thus, in the NOESY spectrum the signal due to H-20 of **9** [δ 5.93 (s)] was only correlated with H-16 [δ 4.08 (br d, J=4.3 Hz)], in agreement with a β-orientation of H-20.

The ^{1}H NMR spectrum of hyatolide E (**10**) was related to those of compounds **8** and **9**, except for the absence of the signal due to the methoxyl group and the presence of the signals of a disubstituted double bond at δ 6.39 (1H, dd, J=9.7 and 2.7 Hz) and 6.31 (1H, dd, J=9.7 and 3.1 Hz). Complete characterization of this compound was achieved after acetylation and HPLC purification to yield compound **10a**. The comparison of the NMR spectra of **10a** with those of **8a** indicated that both compounds possessed an identical A–B–C rings system, and the same butenolide unit. Therefore, the disubstituted double bond of **10a** had to be located at C-15, C-16, conjugated with the butenolide ring. This assignment was fully confirmed by the HMBC correlations of the olefinic proton H-15 (δ 6.41) with C-13 and C-17 whereas the proton H-16 (δ 6.19) was correlated with C-14 and C-18. The stereochemistry at C-20 remains undetermined since no correlations were observed in the NOESY spectrum neither for H-20 nor for its geminal acetyl group. All these data allowed us to define structure **10a** and therefore structure **10** for the natural metabolite hyatolide E.

2.4. Hyatelactam (11)

Hyatelactam (**11**) possessed the molecular formula $C_{27}H_{39}NO_3$, determined from HRCIMS. A comparison of the NMR data of **11** with those of compound **10a** above described, indicated that **11** also displayed a scalarane framework containing an axial acetoxyl group at C-12 and a 15,17-diene moiety. The remaining signals of the spectra were assigned to a carbonyl [δ_C 172.6 (s)] and to a methylene

[δ_C 44.2 (t)/δ_H 3.88 (1H, d, J=19.8 Hz) and 3.83 (1H, d, J=19.8 Hz)], that together with the nitrogen atom of the molecular formula, were accommodated in a γ-lactam ring fused to C-17 and C-18 of ring D. Differing from **10a**, the most deshielded olefinic carbon signal [δ 159.8 (s)] was assigned to C-18 from the HMBC correlations of this signal with H-14 (δ 2.68) and Me-25 (δ 1.03). Therefore the carbonyl of the lactam ring had to be located at C-20. This assignment was further supported by the NOESY cross peaks of the methylene proton at δ 3.88 (H-19β) with H-12 and Me-25. All these data led to propose structure **11** for hyatelactam. After the isolation of molliorin A, possessing a *N*-(2-methylbutyl)pyrrole ring,[17] hyatelactam (**11**) represents the second example of a nitrogen-containing scalarane.

2.5. 12-*O*-Deacetyl-19-*epi*-scalarin (12)

The NMR spectra of 12-*O*-deacetyl-19-*epi*-scalarin (**12**) were quite similar to those of scalarin (**1**),[2] also isolated in this study. However, several diagnostic differences were observed. The most evident were the absence of the signals due to the acetyl group, the upfield shift of H-12 at δ 4.00 (br s) and the ^{13}C shift of C-19 at δ 106.6 (d) [δ 97.9 (d) in **1**]. Furthermore, the signal assigned to the hemiacetal proton H-19 [δ 5.57, d, J=6.7 Hz] was correlated in the NOESY spectrum with H-18, which in turn was correlated with H-14. These correlations indicated the α-orientation of H-18 and H-19, and therefore, compound **12** possessed at C-19 an opposite configuration to that of scalarin (**1**). All these data led to define the structure 12-*O*-deacetyl-19-*epi*-scalarin for compound **12**.

2.6. 12-*O*-Deacetylnorscalaral B (13)

The five singlets due to methyl groups in the ^{1}H NMR spectrum of compound **13** (δ 1.01, 0.89, 0.85, 0.84, and 0.82), together with the presence in the ^{13}C NMR spectrum of the signals corresponding to three methines [δ 56.6 (C-5), 52.3 (C-9), and 43.3 (C-14)] and four quaternary sp^3 carbons [δ 42.8 (C-13), 37.1 (C-8), 37.0 (C-10), and 33.2 (C-4)], indicated that **13** possessed a tetracarbocyclic skeleton related to the scalaranes above described. However, the molecular formula $C_{24}H_{38}O_3$, deduced from HRCIMS, together with the 24 carbons counted in the ^{13}C NMR spectrum were consistent with a norscalarane structure for **13**. In particular, the NMR spectra of **13** were closely similar to those of norscalaral B (**14**),[11] except for the absence of the signals due to the acetyl group at C-12 and the upfield shift of H-12 at δ 3.90. Therefore, compound **13** was 12-*O*-deacetylnorscalaral B.

2.7. Cytotoxic activity

The new compounds hyatelone A (**3**), 19,20-diacetylhyatelones B (**4a**) and C (**5a**), hyatolides A (**6**) and B (**7**), 20-*O*-acetylhyatolide C (**8a**), hyatelactam (**11**), 12-*O*-deacetyl-19-*epi*-scalarin (**12**), and 12-*O*-deacetylnorscalaral B (**13**) were tested in assays directed to detect cytotoxic activity against the tumor cell lines MDA-MB-231 (breast carcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Compounds **3**, **4a**, **6**, **8a**, **11**, and **13** showed mild activity with GI_{50} values of 4.0–9.3 μg/mL (Table 1), while compounds **5a**, **7**, and **12** were inactive against the three lines tested.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter. IR spectra were recorded with a Perkin–Elmer FTIR System Spectrum BX. UV spectra were registered on a Philips PU 8710 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz, respectively, on a Varian INOVA 600 spectrometer using $CDCl_3$ or $(CD_3)_2CO$ as solvent. ^{1}H and ^{13}C NMR chemical shifts were referenced using the corresponding solvent signals [δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 2.04 and δ_C 29.8 for $(CD_3)_2CO$]. COSY, HSQC, and HMBC were performed using standard VARIAN pulse sequences. Low-resolution mass spectra were recorded on a Finningan Voyager GC8000^{top} spectrometer. High-resolution mass spectra were recorded on a Autospec-Q spectrometer. Column chromatography was carried out using Merck Silica gel 60 (70–230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrospher Si-60 (Merck) columns in normal phase mode and with LiChrosorb RP-18 (Merck) columns in reversed phase mode using a differential refractometer RI-71. All solvents were spectral grade or distilled prior to use.

3.2. Collection, extraction, and isolation procedure

The sponge *H. intestinalis* was collected by hand using SCUBA at the Gulf of California and liophylized (263.3 g). The material was extracted with acetone/MeOH (1:1, 6.5 L) and the solvent concentrated under reduced pressure to give a residue that was suspended in H_2O and extracted with Et_2O (4×775 mL). The Et_2O extract (5.9 g) was chromatographed on a silica gel column using solvents of increasing polarities from hexane to Et_2O and subsequently $CHCl_3$/MeOH (80:20) and MeOH. The fraction eluted with hexane/Et_2O (60:40) was subjected to normal phase HPLC (hexane/EtOAc, 70:30) to give furoscalarol (**2**, 5 mg, 1.9×10^{-3}%), hyatolide A (**6**, 1.8 mg, 6.8×10^{-4}%), hyatelone A (**3**, 2.0 mg, 7.6×10^{-4}%), and a mixture of hyatelones B and C (**4** and **5**, 4.5 mg, 1.7×10^{-3}%). The fraction of the general chromatography eluted with hexane/Et_2O (50:50) was subjected to HPLC (hexane/EtOAc, 75:25) to give norscalaral A (2 mg, 7.6×10^{-4}%), deoxoscalarin (11.6 mg, 4.4×10^{-3}%), hyatolide B (**7**, 4.5 mg, 1.7×10^{-3}%), 12α-acetoxy-19β-hydroxy-15,17-dien-20,19-olide (10.1 mg, 3.8×10^{-3}%), and 12-*epi*-12-*O*-deacetyl-19-deoxyscalarin (13.5 mg). The fraction of the general chromatography eluted with hexane/Et_2O (30:70) was subjected to repeated HPLC separations (hexane/EtOAc, 70:30; MeOH/H_2O, 85:15 to 95:5) to yield 12-*epi*-12-*O*-acetylscalarolide (8.5 mg, 3.2×10^{-3}%), hyatolide E (**10**, 2.2 mg, 8.4×10^{-4}%), further amounts of 12-*epi*-12-*O*-deacetyl-19-deoxyscalarin (27.6 mg, 0.016% overall), norscalaral B (**14**, 3.1 mg, 1.2×10^{-3}%) and a mixture of hyatolides C and D (**8** and **9**, 3.0 mg, 1.1×10^{-3}%). The fraction of the general chromatography eluted with Et_2O contained scalarin (**1**, 264 mg). The fraction of the general chromatography eluted with $CHCl_3$/MeOH (80:20) was chromatographed on a silica gel column eluted with mixtures of $CHCl_3$/MeOH of increasing polarities from 99.5:0.5 to 92:8. The fraction eluted with $CHCl_3$/MeOH (99.5:0.5) contained further amounts of **1** (311 mg, 0.22% overall). The fraction eluted

with $CHCl_3$/MeOH (99:1) was subjected to repeated HPLC separations (MeOH/H_2O, 90:10; MeCN/H_2O, 80:20) to give 12-*O*-deacetylnorscalaral B (**13**, 1.8 mg, 6.8×10^{-4}%), hyatelactam (**11**, 4.5 mg, 1.7×10^{-3}%) and 12-*O*-deacetyl-19-*epi*-scalarin (**12**, 1.8 mg, 6.8×10^{-4}%).

3.2.1. Hyatelone A (3). Amorphous solid; $[\alpha]_D$ +72.0 (*c* 0.1, $CHCl_3$); IR (film) 3463, 2927, 1784, 1738, 1716, 1249 cm^{-1}; ^1H and ^{13}C NMR (Table 2); EIMS (70 eV) *m*/*z* (rel int.) 461 ([M+H]$^+$, 4), 444 (10), 427 (3), 418 (19), 400 (61), 386 (53), 372 (63), 368 (55), 357 (37), 191 (99), 81 (100); HRCIMS (+) Obsd *m*/*z*=461.2916, (M+H)$^+$, $C_{27}H_{41}O_6$ requires *m*/*z*=461.2903.

3.2.2. Hyatelones B (4) and C (5). ^1H NMR (600 MHz, $CDCl_3$) δ 5.55 (br s, H-19)/5.22* (br s, H-19), 5.28 (br s H-12), 4.35* (dd, *J*=10.5 and 7.3 Hz, H-16)/3.98 (dd, *J*=11.1 and 6.9 Hz, H-16), 4.09* (br s, H-20)/3.84 (br s, H-20), 1.98* (s, C*H*$_3$COO–)/1.97 (s, C*H*$_3$COO–), 1.23 (s, Me-25)/1.19* (s, Me-25), 0.89 (s, Me-24), 0.85 (s, Me-21), 0.80 (s, Me-23 and Me-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 215.3/214.6* (s, C-18), 169.9/169.8* (s, CH_3*C*OO–), 102.6*/96.7 (d, C-19), 84.3*/82.5 (d, C-16), 83.6*/83.4 (s, C-17), 82.6*/78.8 (d, C-20), 74.6 (C-12), 56.7/56.6* (d, C-5), 52.4 (d, C-9), 52.1 (s, C-13), 44.8*/44.7 (d, C-14), 41.9 (t, C-3), 41.0 (t, C-7), 39.6 (t, C-1), 37.9 (s, C-8), 36.9 (s, C-10), 33.3 (s, C-4), 33.2 (q, Me-21), 25.6/25.4* (t, C-15), 21.6 (t, C-11), 21.3 (q, Me-22), 21.2 (q, *C*H$_3$COO–), 19.0/18.8* (q, Me-25), 18.4 (t, C-2), 18.0 (t, C-6), 17.8/17.7* (q, Me-24), 16.0 (q, Me-23). For each duplicated signal, the value with asterisk corresponds to compound **5**. Treatment of the mixture of **4** and **5** with Ac_2O/Py and subsequent HPLC separation (hexane/EtOAc, 65:35) yielded compounds **4a** (1.8 mg) and **5a** (2.8 mg).

3.2.2.1. 19,20-di-*O*-Acetylhyatelone B (4a). Amorphous solid; $[\alpha]_D$ +97.0 (*c* 0.1, $CHCl_3$); IR (film) 2926, 1760, 1743, 1717, 1239 cm^{-1}; ^1H and ^{13}C NMR (Table 2); EIMS (70 eV) *m*/*z* (rel int.) 562 ([M]$^+$, 1), 503 (12), 485 (4), 460 (7), 442 (25), 390 (100), 191 (82); HRCIMS(+) Obsd *m*/*z*=562.3149 (M)$^+$, $C_{31}H_{46}O_9$ requires *m*/*z*=562.3141.

3.2.2.2. 19,20-di-*O*-Acetylhyatelone C (5a). Amorphous solid; $[\alpha]_D$ +122.0 (*c* 0.05, $CHCl_3$); IR (film) 2925, 1760, 1750, 1706, 1232, 1214 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.07 (1H, s, H-19), 5.27 (1H, dd, *J*=3.3 and 2.2 Hz, H-12), 5.12 (1H, s, H-20), 4.40 (1H, dd, *J*=10.9 and 7.6 Hz, H-16), 2.29 (1H, dd, *J*=13.2 and 7.6 Hz, H-15α), 2.11 (3H, s, C*H*$_3$COO-19), 2.02 (3H, s, C*H*$_3$COO-20), 1.97 (3H, s, C*H*$_3$COO-12), 1.89 (1H, ddd, *J*=15.0, 3.3, and 3.0 Hz, H-11α), 1.85 (1H, ddd, *J*=12.8, 3.3, and 3.3 Hz, H-7β), 1.76 (1H, d, *J*=12.5 Hz, H-14), 1.66 (1H, m, H-11β), 1.60 (1H, m, H-6α), 0.86 (3H, s, H-21), 1.56 (3H, m, H-1β, H-2, and H-15β), 1.43 (2H, m, H-2 and H-6β), 1.37 (1H, m, H-3β), 1.22 (1H, m, H-9), 1.21 (3H, s, H-25), 1.12 (1H, ddd, *J*=13.5, 13.5, and 4.1 Hz, H-3α), 1.06 (1H, ddd, *J*=12.8, 12.8, and 3.8 Hz, H-7α), 0.91 (3H, s, H-24), 0.85 (1H, m, H-5), 0.82 (3H, s, H-23), 0.81 (3H, s, H-22), 0.61 (1H, ddd, *J*=13.2, 13.2, and 3.3 Hz, H-1α); ^{13}C NMR (150 MHz, $CDCl_3$) δ 212.9 (s, C-18), 169.7[a] (s, CH_3*C*OO-19), 169.5[a] (s, CH_3*C*OO-12), 168.3 (s, *C*H$_3$COO-20), 98.3 (d, C-19), 85.7 (d, C-16), 82.8 (d, C-20), 82.0 (s, C-17), 74.3 (d, C-12), 56.5 (d, C-5), 52.2 (d, C-9), 52.0 (s, C-13), 44.0 (d, C-14), 41.9 (t, C-3), 40.8 (t, C-7), 39.7 (t, C-1), 37.8 (s, C-8), 36.9 (s, C-10), 33.3 (s, C-4), 33.2 (q, C-21), 24.7 (t, C-15), 21.6 (t, C-11), 21.3 (q, C-22), 21.1[b] (2×q, *C*H$_3$COO-19 and *C*H$_3$COO-12), 20.7[b] (q, *C*H$_3$COO-20), 19.1 (q, C-25), 18.4 (t, C-2), 18.0 (t, C-6), 17.7 (q, C-24), 16.0 (q, C-23), Signals with the same superscript may be interchanged; EIMS (70 eV) *m*/*z* (rel int.) 563 ([M+H]$^+$, 2), 503 (42), 485 (8), 460 (9), 442 (21), 390 (100), 191 (62); HRCIMS (+) Obsd *m*/*z*=562.3129 (M)$^+$, $C_{31}H_{46}O_9$ requires *m*/*z*=562.3141.

3.2.3. Hyatolide A (6). Amorphous solid; $[\alpha]_D$ +27.0 (*c* 0.1, $CHCl_3$); IR (film) 3375, 2926, 1740, 1715, 1260 cm^{-1}; UV (MeOH) 220 (ε 6707) nm; ^1H and ^{13}C NMR (Table 2); EIMS (70 eV) *m*/*z* (rel int.) 400 ([M−AcOH]$^+$, 2), 382 (4), 367 (5), 288 (16), 271 (14), 191 (100); HRCIMS (+) Obsd *m*/*z*=461.2931 (M+H)$^+$, $C_{27}H_{41}O_6$ requires *m*/*z*=461.2903.

3.2.4. Hyatolide B (7). Amorphous solid; $[\alpha]_D$ +14.0 (*c* 0.18, $CHCl_3$); IR (film) 3435, 2928, 1735, 1717 cm^{-1}; UV (MeOH) 220 (ε 6115) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 9.97 (1H, d, *J*=7.7, H-19), 6.58 (1H, dd, *J*=7.7 and 1.5 Hz, H-20), 4.42 (1H, dd, *J*=11.2, 3.5, and 1.5 Hz, H-16), 3.88 (1H, dd, *J*=3.4 and 2.4 Hz, H-12), 2.38 (1H, ddd, *J*=13.4, 3.5, and 3.1 Hz, H-15α), 2.13 (1H, dd, *J*=13.0 and 3.1 Hz, H-14), 1.91 (1H, ddd, *J*=14.3, 3.4, and 2.1 Hz, H-11α), 1.86 (1H, ddd, *J*=12.5, 3.3, and 3.3 Hz, H-7β), 1.80 (1H, ddd, *J*=13.4, 13.0, and 11.2 Hz, H-15β), 1.67 (1H, dd, *J*=13.0 and 2.1 Hz, H-9), 1.63 (1H, m, H-1β), 1.59 (2H, m, H-2 and H-6α), 1.50 (1H, ddd, *J*=14.3, 13.0, and 2.4 Hz, H-11β), 1.44 (1H, m, H-2), 1.41 (3H, s, H-25), 1.38 (2H, m, H-3β and H-6β), 1.16 (2H, m, H-3α and H-7α), 0.92 (1H, m, H-5), 0.88 (1H, m, H-1α), 0.86 (3H, s, H-21), 0.82 (3H, s, H-24), 0.80 (6H, s, H-22 and H-23); ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.9 (d, C-19), 130.5 (d, C-20), 165.7 (s, C-18), 157.8 (s, C-17), 89.3 (s, C-13), 73.9 (d, C-12), 70.0 (d, C-16), 56.0 (d, C-5), 49.7 (d, C-14), 49.5 (d, C-9), 41.8 (t, C-3), 41.3 (t, C-7), 39.6 (t, C-1), 38.8 (s, C-8), 36.8 (s, C-10), 34.2 (t, C-15), 33.2 (q, C-21), 33.2 (s, C-4), 23.9 (t, C-11), 21.6 (q, C-25), 21.2 (q, C-22), 18.5 (2×t, C-2 and C-6), 16.4 (q, C-23), 15.8 (q, C-24); EIMS (70 eV) *m*/*z* (rel int.) 418 ([M]$^+$, 3), 400 (4), 385 (5), 357 (9), 329 (5), 191 (77), 69 (100); HRCIMS (+) Obsd *m*/*z*=418.2734 (M)$^+$, $C_{25}H_{38}O_5$ requires *m*/*z*=418.2719.

3.2.5. Hyatolides C (8) and D (9). ^1H NMR (600 MHz, $CDCl_3$) δ 6.11 (s, H-20)/5.93* (s, H-20), 5.51 (dd, *J*=3.2 and 2.7 Hz, H-12)/5.50* (t, 2.6 Hz, H-12), 4.08* (br d, *J*=4.3 Hz, H-16)/4.05 (dd, *J*=4.5 and 1.0 Hz, H-16), 3.46* (s, –OC*H*$_3$)/3.44 (s, –OC*H*$_3$), 2.13* (m, H-15α)/2.12 (m, H-15α), 1.98* (s, C*H*$_3$COO–)/1.95 (s, C*H*$_3$COO–), 1.85* (dd, *J*=12.8 and 1.3 Hz, H-14)/1.78 (dd, *J*=12.9 and 1.7 Hz, H-14), 1.56 (m, H-15β), 1.15 (s, Me-25), 0.92* (s, Me-24)/0.91 (s, Me-24), 0.85 (s, Me-21), 0.81(s, Me-23 and Me-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9/169.3* (s, C-19), 169.9 (s, CH_3*C*OO–), 155.6/152.5* (s, C-17), 142.0*/139.4 (s, C-18), 97.2*/95.4 (d, C-20) 74.0*/73.6 (d, C-12), 72.0*/69.7 (d, C-16), 56.5 (d, C-5), 52.8*/52.7 (d, C-9), 45.9/45.6* (d, C-14), 42.0 (t, C-3), 41.3 (t, C-7), 39.7 (t, C-1), 39.0 (s, C-13), 37.0 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, Me-21), 21.8 (q, Me-22), 21.3 (t, C-15), 21.2 (q, *C*H$_3$COO–), 20.9 (t, C-11), 19.6/19.3* (q, Me-25), 18.4 (t, C-2), 18.1 (t, C-6), 17.0*/16.9 (q, Me-24), 15.9 (q,

Me-23). For each duplicated signal, the value with asterisk corresponds to compound **9**. Treatment of the mixtures of **8** and **9** with Ac_2O/Py and subsequent HPLC separation (hexane/EtOAc, 80/20) led to recover compound **8a** (1.8 mg).

3.2.5.1. 20-*O*-Acetylhyatolide C (8a). Amorphous solid; $[\alpha]_D$ +13.0 (*c* 0.1, $CHCl_3$); IR (film) 2927, 1780, 1740, 1238 cm^{-1}; UV (MeOH) 210 (ε 7396) nm; ^1H and ^{13}C NMR (Table 3); EIMS (70 eV) *m*/*z* (rel int.) 516 ($[M]^+$, 1), 474 (47), 456 (18), 442 (21), 414 (57), 396 (23), 384 (27), 382 (70), 365 (89), 337 (32), 191 (44), 69 (100); HRCIMS (+) Obsd *m*/*z*=485.2910 $(M+H-CH_3OH)^+$, $C_{29}H_{41}O_6$ requires *m*/*z*=485.2903; Obsd *m*/*z*=457.2952 $(M+H-AcOH)^+$, $C_{28}H_{41}O_5$ requires *m*/*z*=457.2954.

Table 3. Cytotoxicity assay results for sesterterpenes from *H. intestinalis* (GI_{50} values in μg/mL)

	3	4a	6	8a	11	13
MDA-MB-231	5.4	5.3	4.8	4.0	—	4.9
A-549	—	9.3	5.1	7.2	—	4.5
HT-29	9.2	5.1	5.0	6.2	8.1	4.2

3.2.6. Hyatolide E (10). ^1H NMR (600 MHz, $CDCl_3$) δ 6.39 (dd, *J*=9.7 and 2.7 Hz, H-15), 6.31 (dd, *J*=9.7 and 3.1 Hz, H-16), 6.00 (s, H-20), 5.48 (dd, *J*=3.1 and 2.7 Hz, H-12), 2.66 (dd, *J*=3.1 and 2.7 Hz, H-14), 2.00 (s, C*H*$_3$COO–), 1.07 (s, Me-25), 1.03 (s, Me-24), 0.86 (s, Me-21), 0.82 (s, Me-23 and Me-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9 (s, CH_3*C*OO–), 168 (s, C-19), 154 (s, C-17), 138.3 (d, C-15), 133.1 (s, C-18), 118.7 (d, C-16), 95.4 (d, C-20), 72.3 (d, C-12), 56.7 (d, C-5), 53.7 (d, C-14), 52.0 (d, C-9), 41.9 (t, C-3), 40.8 (t, C-7), 39.9 (s, C-13), 39.6 (t, C-1), 37.0 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, Me-21), 21.3 (2×q, Me-22 and *C*H_3COO–), 21.2 (t, C-11), 18.8 (q, Me-24), 18.4 (t, C-2), 17.8 (t, C-6), 17.2 (q, Me-25), 15.8 (q, Me-23). Treatment of **10** with Ac_2O/Py and subsequent HPLC purification (hexane/EtOAc, 75:25) yielded compound **10a** (1.2 mg).

3.2.6.1. 20-*O*-Acetylhyatolide E (10a). Amorphous solid; $[\alpha]_D$ +46.0 (*c* 0.1, $CHCl_3$); IR (film) 2930, 1771, 1740, 1240, 1209 cm^{-1}; UV (MeOH) 294 (ε 8785) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 6.83 (1H, s, H-20), 6.41 (1H, dd, *J*=9.7 and 2.7 Hz, H-15), 6.19 (1H, dd, *J*=9.7 and 3.3 Hz, H-16), 5.47 (1H, dd, *J*=3.0 and 2.4 Hz, H-12), 2.68 (1H, dd, *J*=3.3 and 2.7 Hz, H-14), 2.17 (3H, s, C*H*$_3$COO-20), 2.00 (3H, s, C*H*$_3$COO-12), 2.08 (1H, ddd, *J*=14.9, 3.0, and 2.7 Hz, H-11α),1.94 (1H, dddd, *J*=12.5, 3.3, and 3.3 Hz, H-7β), 1.64 (1H, ddd, *J*=14.9, 13.4, and 2.4 Hz, H-11β), 1.60 (3H, m, H-1β, H-2, and H6α), 1.45 (2H, m, H-2 and H-6β), 1.37 (1H, m, H-3β), 1.24 (1H, dd, *J*=13.4 and 2.7 Hz, H-9), 1.13 (1H, ddd, *J*=13.3, 13.3, and 3.4 Hz, H-3α), 1.09 (3H, s, H-25), 1.04 (3H, s, H-24), 1.03 (1H, m, H-7α), 0.86 (1H, m, H-5), 0.86 (3H, s, H-21), 0.61 (1H, m, H-1α), 0.83 (3H, s, H-23), 0.82 (3H, s, H-22); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.7 (s, CH_3*C*OO-12), 169.4 (s, CH_3*C*OO-20), 167.8 (s, C-19), 152.5 (s, C-17), 138.8 (d, C-15), 133.5 (s, C-18), 118.3 (d, C-16), 91.2 (d, C-20), 72.2 (d, C-12), 56.7 (d, C-5), 53.7 (d, C-14), 52.0 (d, C-9), 41.9 (t, C-3), 40.8 (t, C-7), 40.1 (s, C-13), 39.6 (t, C-1), 37.1 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, C-21), 21.3 (q, C-22), 21.2 (t, C-11), 21.2 (q, *C*H_3COO-12), 20.8 (q, *C*H_3COO-20), 18.8 (q, C-24), 18.4 (t, C-2), 17.7 (t, C-6), 17.1 (q, C-25), 15.8 (q, C-23); EIMS (70 eV) *m*/*z* (rel int.) 485 ($[M+H]^+$, 1), 424 (6), 409 (22), 381 (47), 365 (97), 349 (48), 241 (59), 226 (100); HRCIMS (+) Obsd *m*/*z*=485.2935 $(M+H)^+$, $C_{29}H_{41}O_6$ requires *m*/*z*=418.2903.

3.2.7. Hyatelactam (11). Amorphous solid; $[\alpha]_D$ +56.0 (*c* 0.1, $CHCl_3$); IR (film) 3240, 2926, 1732, 1694, 1242 cm^{-1}; UV (MeOH) 217 (ε 7900), 278 (ε 2500) nm; ^1H and ^{13}C NMR (in $CDCl_3$, Table 3); ^1H NMR (600 MHz, $(CD_3)_2CO$) δ (selected data) 6.93 (1H, br s, NH), 6.21 (1H, dd, *J*=9.6 and 3.1 Hz, H-16), 5.99 (1H, dd, *J*=9.6 and 2.5 Hz, H-15), 5.04 (1H, dd, *J*=3.3 and 2.2 Hz, H-12), 3.96 (1H, d, *J*=20.0 Hz, H-19), 3.84 (1H, d, *J*=20.0 Hz, H-19), 2.69 (1H, br s, H-14), 2.07 (3H, s, C*H*$_3$COO–), 1.91 (1H, ddd, *J*=14.8, 3.3, and 3.0 Hz, H-11α), 1.77 (1H, ddd, *J*=14.8, 13.0, and 2.2 Hz, H-11β), 1.10 (3H, s, H-24), 1.07 (3H, s, H-25), 0.88 (3H, s, H-23), 0.86 (3H, s, H-21), 0.84 (3H, s, H-22); EIMS (70 eV) *m*/*z* (rel int.) 425 ($[M]^+$, 15), 365 (29), 350 (79), 228 (77), 214 (100), 173 (93), 148 (84); HRCIMS (+) Obsd *m*/*z*=426.3013 $(M+H)^+$, $C_{27}H_{40}NO_3$ requires *m*/*z*=426.3008.

3.2.8. 12-*O*-Deacetyl-19-*epi*-scalarin (12). Amorphous solid; $[\alpha]_D$ +129.0 (*c* 0.1, $CHCl_3$); IR (film) 3479, 2931, 1747, 1693 cm^{-1}; UV (MeOH) 227 (ε 8864) nm; ^1H NMR (600 MHz, $CDCl_3$) δ 6.83 (1H, ddd, *J*=3.8, 3.5, and 3.5 Hz, H-16), 5.57 (1H, d, *J*=6.7 Hz, H-19), 4.00 (1H, br s, H-12), 3.38 (1H, dddd, *J*=6.7, 5.0, 3.5, and 3.5 Hz, H-18), 2.30 (1H, dddd, *J*=20.1, 5.6, 3.8, and 3.8 Hz, H-15α), 2.18 (1H, dddd, *J*=20.1, 11.4, 5.0, and 3.2 Hz, H-15β), 1.82 (1H, ddd, *J*=14.6, 13.3, and 2.3 Hz, H-11β), 1.71 (1H, ddd, *J*=12.6, 3.2, and 3.2 Hz, H-7β), 1.66 (1H, m, H-1β), 1.64 (1H, m, H-2), 1.60 (1H, m, H-11α), 1.56 (1H, dd, *J*=11.4 and 5.6 Hz, H-14), 1.54 (1H, m, H-6α), 1.43 (1H, m, H-2), 1.38 (2H, m, H-3β and H-6β), 1.37 (1H, dd, *J*=13.3 and 1.8 Hz, H-9), 1.15 (1H, ddd, *J*=13.6, 13.6, and 4.1 Hz, H-3α), 0.99 (1H, ddd, *J*=12.6, 12.6, and 3.8 Hz, H-7α), 0.99 (3H, s, H-25), 0.94 (3H, s, H-24), 0.88 (1H, dd, *J*=12.6 and 2.3 Hz, H-5), 0.85 (3H, s, H-21), 0.85 (3H, s, H-23), 0.81 (3H, s, H-22), 0.79 (1H, ddd, *J*=13.1, 13.1, and 3.8 Hz, H-1α); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.3 (s, C-20), 135.5 (d, C-16), 127.1 (s, C-17), 106.6 (d, C-19), 72.3 (d, C-12), 56.5 (d, C-5), 51.6 (d, C-9), 50.8 (d, C-14), 46.6 (d, C-18), 42.0 (t, C-3), 41.7 (t, C-7), 40.1 (s, C-13), 39.9 (t, C-1), 37.9 (s, C-8), 36.8 (s, C-10), 33.3 (s, C-4), 33.2 (q, C-21), 25.9 (t, C-11), 24.6 (t, C-15), 21.3 (q, C-22), 18.5 (t, C-2), 18.0 (t, C-6), 16.4 (q, C-23), 16.1 (q, C-24), 15.3 (q, C-25); EIMS (70 eV) *m*/*z* (rel int.) 403 ($[M+H]^+$, 9), 386 (28), 384 (85), 366 (35), 356 (94), 351 (43), 341 (36), 205 (51), 191 (96), 69 (100); HRCIMS (+) Obsd *m*/*z*=384.2673 $(M-H_2O)^+$, $C_{25}H_{36}O_3$ requires *m*/*z*=384.2664; Obsd *m*/*z*=367.2629 $(M+H-2H_2O)^+$, $C_{25}H_{35}O_2$ requires *m*/*z*=367.2637.

3.2.9. 12-*O*-Deacetylnorscalaral B (13). Amorphous solid; $[\alpha]_D$ +30 (*c* 0.1, $CHCl_3$); IR (film) 3703, 3457, 2927, 1668 cm^{-1}; UV (MeOH) 222 (ε 10386) nm; ^1H and ^{13}C NMR (Table 3); EIMS (70 eV) *m*/*z* (rel int.) 358 ($[M]^+$, 8), 356 (29), 346 (44), 342 (22), 338 (16), 324 (11), 235 (46), 217 (47), 206 (23), 192 (93), 136 (100); HRCIMS (+) Obsd *m*/*z*=374.2819 $(M)^+$, $C_{24}H_{38}O_3$ requires *m*/*z*=374.2821.

3.3. Cytotoxicity assays

Compounds **3**, **4a**, **5a**, **6**, **7**, **8a**, and **11–13** were tested against the human tumor cell lines MDA-MB-231 (breast carcinoma), A-549 (lung adenocarcinoma), and HT-29 (colon adenocarcinoma). Cytotoxicity assays were performed by PharmaMar. A colorimetric type of assay using sulforhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability following the method described in the literature.[18]

Acknowledgements

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Influence of intramolecular hydrogen bonds in the enzyme-catalyzed regioselective acylation of quinic and shikimic acid derivatives

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Abstract—Selective mono-functionalization of 3-*epi*, 4-*epi*-, and 5-*epi* quinic and shikimic acid derivatives has been accomplished by enzymatic acylation with *Candida antarctica* lipase A (CAL-A). We propose that the selectivity of this lipase is related to both the inherent receptor selectivity and the degree of intramolecular hydrogen bonding in the ligand. Conformational analysis of the hydroxyl protons has been carried out by ^{1}H NMR spectroscopy. We have shown that exchange of the hydroxyl protons by acid catalysis provides a useful method for the detection of intramolecular hydrogen bonds. The interpretation of exchange rates and coupling constants determines the direction of the H-bonds as conditioned by the relative acceptor and donor properties of the hydroxyl groups. The selectivity of the acylation agrees fully with the effectiveness of H-bonding networks in polyol compounds and with the higher reactivity of the equatorial hydroxyl groups.

1. Introduction

The selective functionalization of one particular hydroxyl group among others of similar reactivity in polyhydroxylated molecules is extremely difficult in organic synthesis. A major problem is the lack of an efficient orthogonal protection–deprotection strategy. In the carbohydrate chemistry, Wong et al.[1] have described the synthesis of a library of oligosaccharides using a designed building-block with four selectively removable protecting groups as acceptors for glycosylation. On the other hand, regioselectivity in sugar chemistry is often solved using an approach based on complexation-induced activation of a particular OH group. For this purpose, tin[2] and boron[3] reagents have been widely used.

In some cases certain hydroxyl groups have shown an anomalous reactivity, with a secondary hydroxyl group being functionalized instead of the usually more reactive primary one.[4] However, steric interactions within themselves are not sufficient to explain this unusual reactivity. The existence of noncovalent intramolecular bonding interactions involving hydroxyls is well recognized in carbohydrate chemistry. The role of intramolecular hydrogen bonding has been used as an explanation for the low reactivity of the primary 5′-OH during the synthesis of 5′-*O*-galactosylated nucleosides. Hydrogens of such primary hydroxyls are shown to be intramolecularly hydrogen-bonded with the heteroaromatic system present in the molecule, and consequently proved to be resistant to hydrogen abstraction.[5] In another example, selective 2-*O*-benzylation of sucrose is due to the persistence of an intramolecular hydrogen bond, which makes the hydroxyl group at the 2-position the most readily deprotonated in aprotic solvents.[6] Hydrogen bonds are also confirmed to play a significant role in the acidic behavior of the various hydroxyl groups of the sucrose molecule.[7] The relative reactivities in the DMAP-catalyzed acetylation were successfully correlated with the calculated proton affinity of each OH group in carbohydrates by Yoshida et al.[8] They found that secondary OH groups were preferentially acetylated in the presence of the primary OH group at position 6. In contrast, the 6-*O*-acetylated product was the major one in the absence of DMAP. In addition, molecular recognition through hydrogen-bonding interactions is one of the challenging goals in supramolecular chemistry.[9]

Enzyme-catalyzed reactions are practical processes for the selective modifications of polyol derivatives. The regioselectivity of enzyme catalysis has been exquisitely exploited in the fields of nucleosides,[10] steroids,[11] and carbohydrates.[12] Enzymes catalyze reactions under mild conditions, diminish undesired side-reactions, and facilitate product recovery. Furthermore, enzymes are proteins, and as such they are completely biodegradable.

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.03.074

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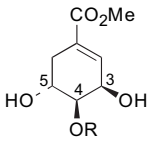

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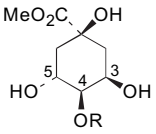
2, R= Ac



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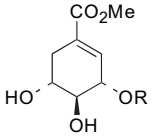

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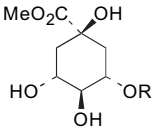

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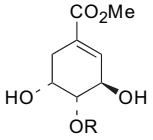
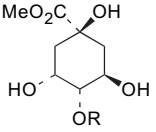
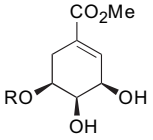
8, R= Ac



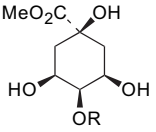
9, R= H, Methyl 4-*epi*-Shikimate
10, R= Ac



11, R= H, Methyl 4-*epi*-Quinate
12, R= Ac



13, R= H, Methyl 5-*epi*-Shikimate
14, R= Ac



15, R= H, Methyl 5-*epi*-Quinate
16, R= Ac

Figure 1. Structures of natural (**1** and **3**), 3-*epi* (**5** and **7**), 4-*epi* (**9** and **11**), and 5-*epi* (**13** and **15**) shikimic and quinic acid derivatives and their regioselective enzymatic acetylated products (**2**, **4**, **6**, **8**, **10**, **12**, **14**, and **16**) with CAL-A using vinyl acetate both as acylating agent and solvent.

In our ongoing research focused on the synthesis of quinic and shikimic acid derivatives, we carried out the regioselective enzymatic acylation of methyl shikimate (**1**, Fig. 1) and methyl quinate (**3**) with *Candida antarctica* lipase A (CAL-A).[13] This lipase allowed the selective acylation of the C-4 hydroxyl group, giving rise to a variety of ester derivatives of both acids, including the cinnamate analogues. Previous studies on the enzymatic acylation of quinic and shikimic acid derivatives have been described by Guyot et al.[14a] and Danieli et al.[14b] Although, the use of enzymes in synthesis is common, the basis of their selectivity is not well understood. The goals of this study are both to regioselectively prepare the valuable monoacyl analogues of the 3-*epi*, 4-*epi*, and 5-*epi* quinic and shikimic acid derivatives, and to identify the molecular basis of the regioselectivity of CAL-A towards these compounds as a function of the degree of intramolecular hydrogen bonding within the ligand.

2. Results and discussion

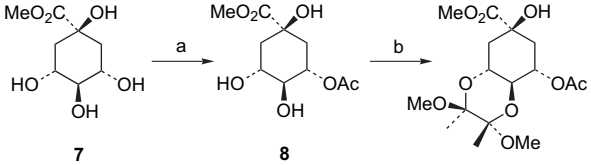
2.1. Regioselective enzymatic acylation of methyl 3-*epi*, 4-*epi*, and 5-*epi* quinic and shikimic acid derivatives

2.1.1. Enzymatic acylation of (−)-methyl 3-*epi*-shikimate (5) and *meso*-methyl 3-*epi*-quinate (7).[15] As previously reported for the natural isomer,[13b] the enzymatic transesterification of methyl 3-*epi*-shikimate (**5**) was carried out at 20 °C with vinyl acetate as acylating agent and solvent, CAL-A, and in the presence of molecular sieves 4 Å. CAL-A exhibited excellent selectivity towards the 3-position, giving rise to methyl 3-*O*-acetyl-3-*epi*-shikimate (**6**) in 88% yield after 0.5 h. In addition, methyl 3,5-di-*O*-acetyl-3-*epi*-shikimate was isolated as a minor compound (92:8 ratio **6**:diacyl derivative calculated by ^{1}H NMR). To avoid the formation of the diacyl derivative other solvents suitable for the enzymatic acylation of methyl shikimate such as *tert*-butylmethyl ether (TBME), toluene, 1,4-dioxane, chloroform or THF were used. In general, longer reaction times were achieved, although CAL-A maintained a similar degree of selectivity.

If as seems, the selectivity of CAL-A is highly dependent on the relative position of the hydroxyl groups, CAL-A would also acylate the 3-position of methyl 3-*epi*-quinate (**7**) as it did with methyl 3-*epi*-shikimate (**5**). This fact was corroborated when the latter was subjected to similar reaction conditions as those described for methyl quinate. That is, with vinyl acetate both as solvent and acylating agent, CAL-A, molecular sieves 4 Å, and at 40 °C. However, since **7** is a *meso* compound, positions 3 and 5 are prochirals. Thus, CAL-A catalyzes the acylation of methyl 3-*epi*-quinate toward the C-3 or C-5 position with 95% regioselectivity (C-3 or C-5 vs C-4) in 4.5 h. Importantly, no traces of 4-*O*-acyl or diacyl derivatives were detected by ^{1}H NMR of the crude reaction mixture.

The enantiopurity of compound **8** was determined by derivatization to the corresponding *trans*-acetal **17** (Scheme 1). In addition, (±)-**17** was synthesized by protection of *trans*-1,2-diol in *meso*-methyl 3-*epi*-quinate and subsequent treatment with acetic anhydride as described in Scheme 2.

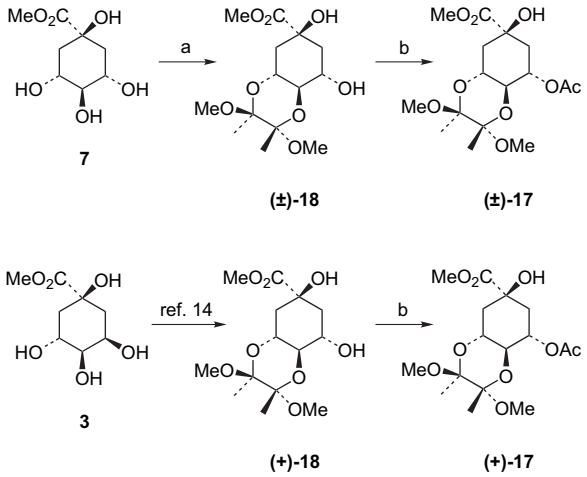
Chiral HPLC analysis of (±)-**17** and derivative of **17** obtained from the enzymatic reaction revealed an enantiomeric excess of 60% (see page S2 in Supplementary data). The assignment of the relative configuration of **17** was determined by comparison of the sign of the optical rotation of our synthesized derivative of **17** and that of the enantiomerically pure derivative prepared according to Scheme 2.[15] This was corroborated by chiral HPLC analysis of both compounds, which clearly establishes the configuration (1*R*,3*S*,4*S*,5*R*) for our compound. This means that acetylation of methyl 3-*epi*-quinate takes place with moderate selectivity towards 3-position. We did not try to find the optimal conditions for asymmetrization of **7** since it was not the aim of this study.



7 **8** **17**

(a) **CAL-A**, CH_2=CHOAc, sieves 4 Å, 40 °C, 4.5 h; (b) butane-2,3-dione, $CH(OMe)_3$, (±)-CSA, MeOH, 65 °C, 2 h

Scheme 1.



(a) butane-2,3-dione, $CH(OMe)_3$, (±)-CSA, MeOH, 65 °C, 2 h;
(b) Ac_2O, DMAP, Py, CH_2Cl_2, 0 °C, 4 h

Scheme 2.

2.1.2. Enzymatic acylation of (−)-methyl 4-*epi*-shikimate (9) and (−)-methyl 4-*epi*-quinate (11).[16] We extended the aforementioned methodology to 4-*epi* derivatives. First, we studied the acylation reaction of methyl 4-*epi*-shikimate (**9**) with vinyl acetate at 20 °C in the presence of molecular sieves, and catalyzed by CAL-A. The transesterification takes place in 1.5 h with total selectivity towards the C-4 hydroxyl group, methyl 4-*O*-acetyl-4-*epi*-shikimate (**10**) being isolated exclusively in 80% yield after flash chromatography.

Similarly, methyl 4-*epi*-quinate (**11**) was subjected to reaction with CAL-A under identical conditions as **7**. However, the presence of corresponding 1,5-lactone **19** (see below, Fig. 8D) was observed and a mixture of acylated derivatives was obtained. Formation of the lactone has been previously observed in the attempted recrystallization of **11**. To avoid this by-product, the reaction was carried out in the absence of molecular sieves since these remove the MeOH and shift the equilibrium towards the lactone. Also, the temperature was decreased from 40 to 20 °C. In these conditions, CAL-A furnished exclusively the 4-*O*-acetyl derivative **12** after 24 h.

2.1.3. Enzymatic acylation of (−)-methyl 5-*epi*-shikimate (13) and *meso*-methyl 5-*epi*-quinate (15).[17] When the reaction was done with methyl 5-*epi*-shikimate (**13**), CAL-A showed total selectivity towards the C-5 hydroxyl group, a 100% conversion being achieved after 0.3 h at 20 °C.

In contrast to other *epi*-isomers, ^{1}H NMR of the 5-*epi*-shikimate derivative revealed different half-chair conformations in MeOH-d_4 and $CDCl_3$. Change from MeOH-d_4 to $CDCl_3$ resulted in an upfield shift of H-4 and a downfield shift of H-5 (see pages S47 and S55 in Supplementary data). This is in line with exchanges in the axial and equatorial positions. Moreover, the coupling constants of H-5 are very significant, indicating an axial position in MeOH-d_4 ($^3J_{HH}$ 9.6 Hz) and an equatorial position in $CDCl_3$ ($^3J_{HH}$ 5.0 Hz). Thus, in MeOH-d_4 solution, triol **13** prefers a conformation with OH-3 and OH-5 in equatorial orientation and OH-4 in axial one (A, Fig. 2). On the other hand, OH-3 and OH-5 are in axial orientation and OH-4 is in equatorial one, which are the proposed conformation in $CDCl_3$ (B, Fig. 2). In that conformation, strong 1,3-diaxial intramolecular hydrogen bonds are present and as a result this should be the favored conformation in nonhydrogen bonding solvents.

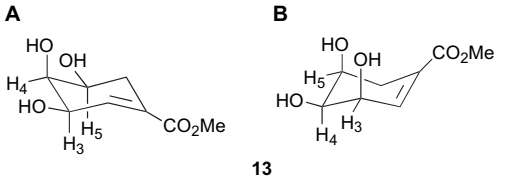


Figure 2. Chair conformations of methyl 5-*epi*-shikimate in MeOH-d_4 (A) and in $CDCl_3$ (B).

A comparison of the acylation of **13** in hydrogen (acetone, vinyl acetate) and nonhydrogen (CH_2Cl_2, $CHCl_3$) bonding solvents has been performed. No changes in the regioselectivity were observed with any of the solvents tested.

Finally, selective acylation at the C-4 position of methyl 5-*epi*-quinate (**15**) was successfully accomplished using CAL-A at 40 °C for 4.5 h. As with the 5-*epi*-shikimate derivative, methyl 5-*epi*-quinate showed different conformation in MeOH-d_4 and $CDCl_3$ solvents. Protons H-6a and H-2a exhibited in MeOH-d_4 a $^3J_{HH} \approx 12.1$ Hz corresponding to an axial–axial vicinal coupling to H-3 and H-5, in addition to a large geminal coupling ($^2J_{HH} \approx 12.1$ Hz). Therefore, OH-3 and OH-5 are in equatorial orientation and OH-4 in axial one (A, Fig. 3). On the other hand, in $CDCl_3$ the value of this vicinal coupling constant is ≈3.8 Hz, which is consistent with an axial position for OH-3 and OH-5, and an equatorial orientation for OH-4 (B, Fig. 3).

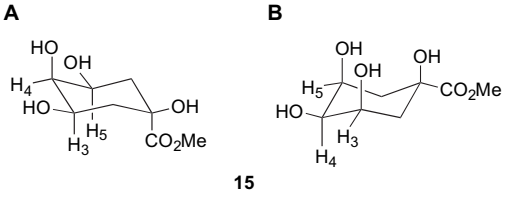


Figure 3. Chair conformations of methyl 5-*epi*-quinate in MeOH-d_4 (A) and in $CDCl_3$ (B).

Although different solvents were tested (acetone, vinyl acetate, CH_2Cl_2, and $CHCl_3$) CAL-A maintains the excellent regioselectivity; methyl 4-*O*-acetyl-5-*epi*-quinate (**16**) in all cases being isolated.

2.2. Conformational analysis of intramolecular hydrogen bonds and their influence in the enzymatic catalysis

In order to explain the regioselectivity exhibited by CAL-A in quinic and shikimic acid derivatives, we next study the role of intramolecular hydrogen bonds between vicinal alcohols within each ligand.

IR and ^{1}H NMR spectroscopy are common methods to determine intra- and intermolecular H-bonding. IR allows direct

observation of the free and hydrogen-bonded hydroxyl stretch resonances (ν_{OH}), which are subsequently used to determine the extent of intramolecular hydrogen bonding.[18] Since our molecules possess several OH groups this technique is not adequate due to the superposition of the OH bands. NMR spectra potentially provide more useful information in the form of coupling constants, chemical shifts, temperature coefficients, and NOEs, although in order to obtain this data the suppression of intermolecular exchange of hydroxyl groups is necessary. In these conditions, vicinal coupling constants (^3J) for hydroxyl protons calculated by ^{1}H NMR can provide important structural information in terms of hydrogen-bonding. Since the magnitude of ^3J depends on the dihedral angle, according to the Karplus equation[19] derived for hydroxyl protons,[20] the values of $^3J_{CH,OH}$ can predict the orientation of the hydroxyl groups.

For the detection of intramolecular hydrogen bonds sample dilution is a powerful method.[21] The limiting chemical shifts and the concentrations required for fast exchange are characteristically different for protons that are intramolecularly hydrogen-bonded. Intramolecular H-bonds can also be detected by a weak dependence of the chemical shift of OH groups upon the temperature.[22] Due to the low solubility of our substrates in chloroform or methylene chloride (commonly used deuterated solvents), which are nonhydrogen-bonding, a concentration dependent hydroxyl proton exchange is not adequate. Similarly, since a conformational equilibrium is present in the target molecules, a dependence upon the temperature of the OH signals in ^{1}H NMR is not suitable. These facts oblige us to study the exchange process by acid catalysis. As a consequence, rigorous removal of acid and water from sample, solvent, and NMR tube should be controlled. To the best of our knowledge, it is the first time that hydrogen bond interactions are determined by exchange of the hydroxyl protons with acid in an organic solvent solution.

2.2.1. Study of hydrogen bonds network in methyl shikimate (1). After careful preparation of the NMR samples (see Section 4) the rate of exchange of the hydroxyl protons with the solvent is slow enough, because of which the coupling pattern is detectable. Figure 4 shows the ^{1}H NMR spectra of methyl shikimate (**1**) in $CDCl_3$ solution before (spectrum A) and after addition of subsequent portions of 0.05 mM trifluoroacetic acid in $CDCl_3$ solution (spectra B and C). Ester

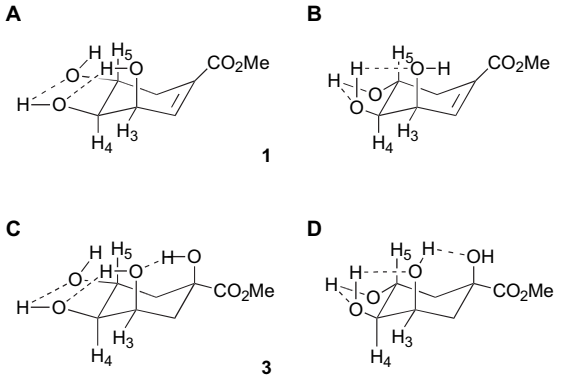


Figure 5. Clockwise and reverse clockwise orientations of methyl shikimate (A and B) and methyl quinate (C and D) hydrogen-bonding networks.

1 exists preferentially in the chair conformation indicated in Figure 5 (A and B). To assign the spectra and determine the structures, ^{1}H–^{1}H homonuclear correlation experiments have been performed. The analysis of the spectrum of **1** provides the coupling constants of the hydroxyl groups: $^3J_{H\text{-}3,OH\text{-}3}$=4.7 Hz, $^3J_{H\text{-}4,OH\text{-}4}$=5.5 Hz, and $^3J_{H\text{-}5,OH\text{-}5}$= 3.5 Hz. Comparison of spectra A–C in Figure 4 suggests that the signal corresponding to OH-5 is altered first, since it appears as a singlet instead of a doublet. Spectrum C shows that the next hydroxyl to loose its coupling is OH-3, whereas OH-4 still maintains certain residual coupling. These results of hydroxyl exchange indicated an acidity order of OH-5>OH-3>OH-4.

There are two possible orientations for the hydrogen bonds in the cyclohexene ring of **1** (A and B, Fig. 5). The hydroxyl OH-5 forms a weak H-bond with the adjacent hydroxyl OH-4 both are in equatorial position. Diequatorial *trans*-1,2-diols are expected to form weaker intramolecular bonds. The strong hydrogen bond is formed by OH-3 and OH-4, which are in cis orientation. Equatorial–axial vicinal diols in six-membered rings have stronger intramolecular hydrogen bonds than equatorial–equatorial diols. On the other hand, OH-3 is more acidic than OH-4 and this suggests that the axial OH-3 and not the equatorial OH-4 acts preferentially as H-acceptor.[22b] Thus, the directional hydrogen-bonding network is that indicated in Figure 5B. In addition, an equatorial OH group in a six-membered ring system can be

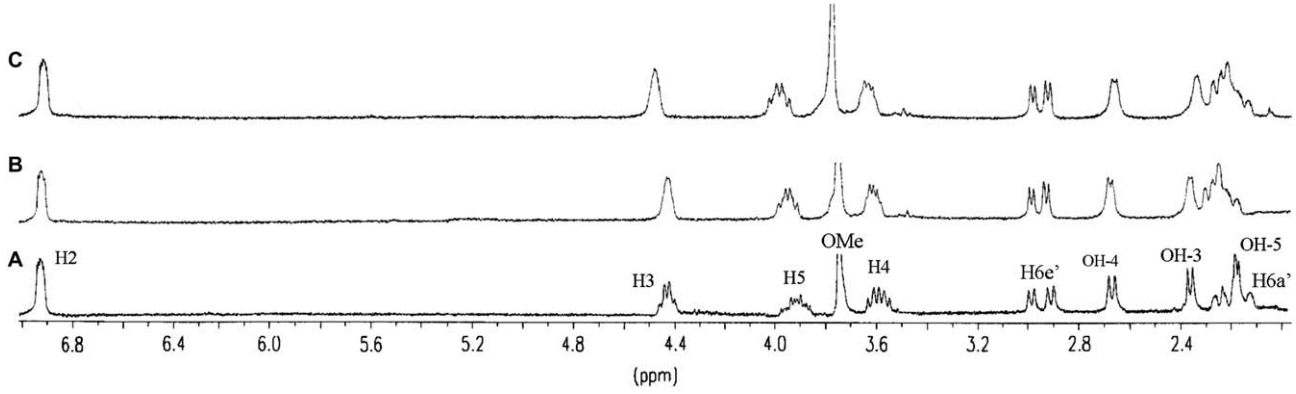


Figure 4. ^{1}H NMR spectra (300 MHz) of **1** in $CDCl_3$ solution (2 mM): (A) in absence of acid; (B) first change observed after acid addition; and (C) second change observed after acid addition.

functionalized preferentially in the presence of secondary axial partners.

According to the points mentioned above, when OH-4 results acylated the positive charge in the transition state is delocalized through a strong intramolecular hydrogen bond. In contrast, OH-5 forms a less-efficient hydrogen bond to delocalize the charge. If these parameters have an influence in the transition state of the enzymatic acylation, the major regioisomer formed should be the 4-*O*-acetylated derivative. In fact, acylation of **1** catalyzed by CAL-A takes place with excellent selectivity at OH-4.[13b]

2.2.2. Study of hydrogen bonds network in methyl quinate (3). The ^{1}H NMR spectra of methyl quinate (**3**) in $CDCl_3$ or MeOH-d_4 solution shows that the preferred chair conformation is close to the one observed for methyl shikimate (C and D, Fig. 5). Compound **3** is characterized by two large $^3J_{H\text{-}3,OH\text{-}3}$=8.6 Hz and $^3J_{H\text{-}4,OH\text{-}4}$=9.1 Hz, and a smaller $^3J_{H\text{-}5,OH\text{-}5}$=2.5 Hz (A, Fig. 6). Comparison of the ^{1}H NMR spectra A–C (Fig. 6) reveal that the order of acidity of the hydroxyl groups is OH-5>OH-4>OH-3.

Since we have an additional hydroxyl proton in the molecule (OH-1) for which ^{1}H NMR spectrum does not provide coupling information (it is a tertiary alcohol), acidity does not indicate the direction of the hydrogen bonds. For that, vicinal coupling constants were employed. As previously mentioned, the value of $^3J_{CH,OH}$ depends on the dihedral angle between the hydroxyl proton and the hydrogen of the vicinal carbon. $^3J_{CH,CH}$ values of 11–15 Hz are characteristic of axial–axial vicinal couplings, while the presence of electronegative atoms give rise to ^3J values of 8–10 Hz. According to the Karplus equation, the large $^3J_{H\text{-}4,OH\text{-}4}$=9.1 Hz evidences a predominant conformation in which the O–H bond is almost *anti* to the C–H bond (dihedral angle close to 150–180°).[23] The same interpretation can be applied to OH-3, suggesting that the direction of the hydrogen bonds is that which is indicated in Figure 5D. OH-3 and OH-1 form a strong 1,3-diaxial hydrogen bond. Also, the hydrogen-bonding between OH-4 and OH-3 is effective. Thus, the OH-4 should exhibit much higher reactivity than the other hydroxyl groups in the enzyme-catalyzed acetylation reaction. The positive charge in the transition state is stabilized through two consecutive efficient hydrogen bonds. The OH-5 group does not effectively participate in the hydrogen-bonding network (trans-diequatorial hydrogen bond between OH-5 and OH-4), and acetylation at the OH-3 reduces the extent of charge delocalization (through only one effective hydrogen bond). Interestingly, in the presence of CAL-A, the regioselectivity of the acetylation of **3** agrees fully with cooperative H-bond effects and by a higher reactivity of the equatorial groups, methyl 4-*O*-acetylquinate (**4**) being obtained exclusively.[13a]

2.2.3. Study of hydrogen bonds network in methyl 3-*epi*-shikimate (5). The ^{1}H NMR spectra of methyl 3-*epi*-shikimate (**5**) in MeOH-d_4, acetone-d_6, or $CDCl_3$ solutions show the chair conformation indicated in Figure 7A, in which the large coupling constants of H-4 (dd, $^3J_{HH}$=9.8, 7.8 Hz) and H-5 (ddd, $^3J_{HH}$=9.7, 9.7, 6.2 Hz) suggest an axial disposition (see pages S44 and S51 in Supplementary data).

The exchange of hydroxyl protons of alcohols after addition of acid to NMR sample implies an acidity order of OH-4≈OH-5>OH-3 (see pages S62 and S63 in Supplementary data). The two hydrogen bonds are between trans-diequatorial OH groups, and, evidently, none of them are involved in a strong intramolecular H-bond. The fact that OH-3 is the lesser acidic hydroxyl, and thus a H-bond donor, indicates the direction of the H-bonds (Fig. 7A). The enzymatic acylation of **5** led almost exclusively to 3-acetyl derivative **6**. This is in keeping with the more efficient hydrogen bond

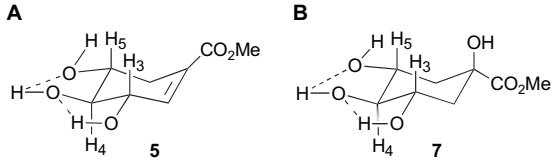


Figure 7. Hydrogen-bonding orientations of methyl 3-*epi*-shikimate (A) and methyl 3-*epi*-quinate (B).

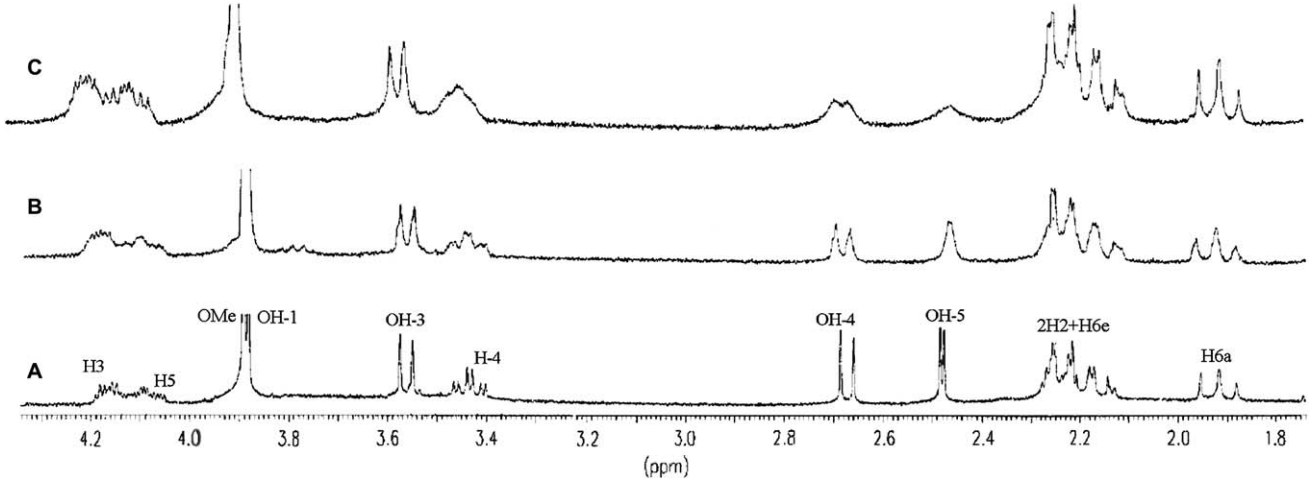


Figure 6. ^{1}H NMR spectra (300 MHz) of **3** in $CDCl_3$ solution (2 mM): (A) in absence of acid; (B) first change observed after acid addition; (C) second change observed after acid addition.

between OH-3 and OH-4 due to the *pseudo*equatorial position of OH-3.

2.2.4. Study of hydrogen bonds network in methyl 3-*epi*-quinate (7). As with the shikimate derivative, analysis of the coupling constants of *meso*-methyl 3-*epi*-quinate (**7**) evidences the preferred chair conformation, shown in Figure 7B. Large coupling constant values are observed for axial H-2 and H-6 (dd, $^{2}J_{HH}$=12.5 Hz, $^{3}J_{HH}$=12.5 Hz), and thus H-3 and H-5 are in axial orientation.

The three secondary OH groups of **7** are involved in weak intramolecular H-bonds. Hydroxyl exchange is similar, although the H-bond formed by OH-3 (or OH-5) seems to be more stable (see pages S64 and S65 in Supplementary data). In addition, the two possible orientations of the hydroxyl network are equivalent (Fig. 7B), since the molecule possesses a plane of symmetry.

Acetylation of **7** catalyzed by CAL-A led to exclusive acylation at OH-3 or OH-5. The larger value of the coupling constant for OH-3 (or OH-5) ($^{3}J_{H\text{-}3,OH\text{-}3}$=3.7 Hz and $^{3}J_{H\text{-}4,OH\text{-}4}$=2.9 Hz) indicates a more adequate dihedral angle for effective hydrogen-bonding.

2.2.5. Study of hydrogen bonds network in methyl 4-*epi*-shikimate (9). The analysis of (−)-methyl 4-*epi*-shikimate (**9**) by ^{1}H NMR in several deuterated solvents ($CDCl_3$, MeOH-d_4, and acetone-d_6) determines the chair conformation indicated in Figure 8A. The coupling constants of H-5 with the two H-6 protons show values ≤5 Hz, which indicate an equatorial position for H-5. This was corroborated by $^{3}J_{HH}$=∼7.8 Hz between H-3 and H-4, which is in agreement with an axial–*pseudo*axial disposition for both protons.

The study of hydroxyl exchange indicates an acidity order of OH-5>OH-3≈OH-4 (see pages S66 and S67 in Supplementary data). The analysis of the acidity order determines the direction of the hydrogen bonds. Since OH-5 is the most acidic group, it is an acceptor, and as a consequence the direction of the hydrogen bonds network is as shown in Figure 8A. Thus, the enzymatic acylation of **9** should proceed regioselectively towards the 4-position: OH-4 is equatorial, and the positive charge in the transition state is stabilized through the most efficient hydrogen bond. In practice, CAL-A catalyzes the acylation at OH-4 with total selectivity, the 4-*O*-acetyl derivative **10** being isolated with excellent yield.

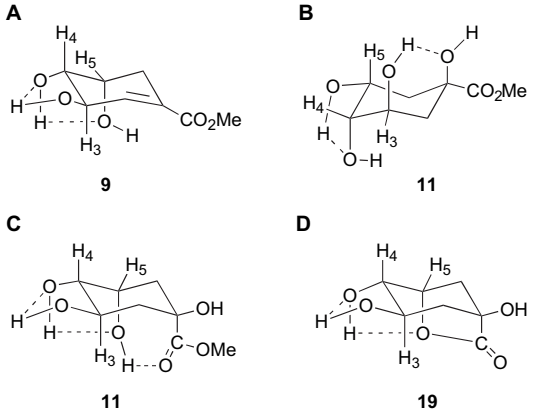


Figure 8. Hydrogen bond networks of methyl 4-*epi*-shikimate (A), methyl 4-*epi*-quinate (B and C), and methyl 4-*epi*-quinate lactone derivative **19** (D).

2.2.6. Study of hydrogen bonds network in methyl 4-*epi*-quinate (11). The hydroxyl exchange study of (−)-methyl 4-*epi*-quinate (**11**) was more difficult than in previous cases because of the low solubility of this derivative in $CDCl_3$, and even in CD_2Cl_2. The ^{1}H NMR of **11** in $CDCl_3$ shows the coupling constants of two hydroxyls ($^{3}J_{CH,OH}$=8.7 Hz and $^{3}J_{CH,OH}$=3.1 Hz), which were assigned by COSY spectrum as OH-3 and OH-4, respectively, (page S69 in Supplementary data). Taking into account that proton spectra in $CDCl_3$, MeOH-d_4, and acetone-d_6 evidence the chair conformation indicated in Figure 8B, the small value of the coupling constant for OH-4 indicates that the axial OH-4, and not the equatorial OH-5, acts preferentially as H-acceptor. In addition, the large value of $^{3}J_{H\text{-}3,OH\text{-}3}$ suggests a dihedral angle of 180°, showing the donor character of OH-3, and compatible with a strong 1,3-diaxial H-bond (see Fig. 8B). In accordance with this, the acylation should take place at OH-3, although this hydroxyl is in axial configuration. However, CAL-A exhibited total selectivity towards OH-4. This is in keeping with the chair conformation similar to that previously shown for methyl 4-*epi*-shikimate (Fig. 8C). In this case, an efficient hydrogen bond between OH-5 and the oxygen of the ester group increases the hydrogen-bonding network. In addition, OH-4 is in equatorial orientation. Similarly, enzymatic acetylation of the corresponding lactone **19** (Fig. 8D), which is restricted in this chair conformation, also afforded exclusively the 4-*O*-acetyl derivative **20** (Fig. 9).

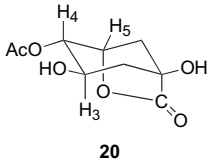


Figure 9.

Furthermore, we previously observed that the selectivity of CAL-A was independent of the solvent employed in the acylation of 5-*epi*-derivatives, despite both compounds having different conformations in different solvents. These facts suggest that the enzyme active site fits the most effective hydrogen-bonding network conformation, since the later better stabilizes the transition state.

2.2.7. Study of hydrogen bonds network in methyl 5-*epi*-shikimate (13). The ^{1}H NMR spectrum of **13** in $CDCl_3$ exhibits well-resolved OH signals ($^{3}J_{H\text{-}3,OH\text{-}3}$=9.9 Hz, $^{3}J_{H\text{-}4,OH\text{-}4}$=6.3 Hz and $^{3}J_{H\text{-}5,OH\text{-}5}$=5.5 Hz). Hydroxyl exchange after acid addition provided an acidity order of OH-5>OH-4>OH-3 (see pages S70 and S71 in Supplementary data). Thus, OH-5 should be the acceptor of OH-3, the two hydroxyl groups being involved in the molecule's strongest hydrogen bond, which is almost 1,3-diaxial. This observation is consistent with the large coupling constant value of OH-3, suggesting a dihedral H–C–O–H angle close to

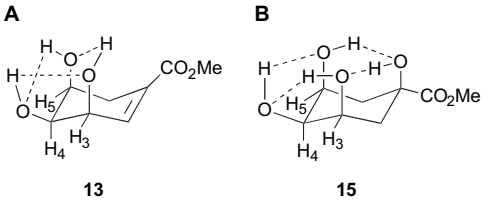


Figure 10. Hydrogen bonds network of methyl 5-*epi*-shikimate (A) and methyl 5-*epi*-quinate (B).

180°. The coupling constants of OH-4 and OH-5 also corroborated the direction of the hydrogen bonds indicated in Figure 10A.

Thus, if acylation takes place at the equatorial OH-4 group, the transition state is stabilized through an effective hydrogen bond network. However, we isolated exclusively the 5-*O*-acyl derivative. This result would be explained due to acyl migration from position 4 to 5 (acyl migrations were previously observed for methyl *O*-acylshikimate derivatives in certain conditions[13]). To confirm this fact, we tried to prepare the corresponding 4-*O*-acyl analogue by independent synthesis. On doing so, 3,5-di-*O*-TBDMS protection of methyl 5-*epi*-shikimate and subsequent acetylation of the OH-4 group afforded the 4-acetyl-3,5-di-*O*-TBDMS derivative. All attempts to obtain methyl 4-*O*-acetyl-5-*epi*-shikimate, after silyl deprotection, gave rise to a mixture of 3-, 4-, and 5-*O*-acetyl derivatives, in which the 5-*O*-acyl derivative was the major compound.

2.2.8. Study of hydrogen bonds network in methyl 5-*epi*-quinate (15). Finally, we also studied the hydrogen bonds in methyl 5-*epi*-quinate (**15**). The exchange of the hydroxyl protons of alcohols by acid shows the hydrogen bonds to be of similar strength. As previously mentioned, in $CDCl_3$ the chair conformation is indicated in Figure 10B. The $J_{CH,OH}$ values for hydroxyl groups ($^3J_{H\text{-}3,H\text{-}5,OH\text{-}3,OH\text{-}5}$= 7.6 Hz and $^3J_{H\text{-}4,OH\text{-}4}$=8.2 Hz) imply that the O–H bond is almost *anti* to the C–H bond and confirm that OH-1, OH-3, and OH-5 are involved in 1,3-diaxial hydrogen bonding. As methyl 3-*epi*-quinate, this is a *meso* form and the two possible orientations of the hydroxyl network are equivalent. Since equatorial hydroxyls are more reactive than axials, the acylation reaction should take place towards that position. In fact, the enzymatic process proceeds selectively at the OH-4 group.

In view of these results, we propose that the cooperative effect of hydrogen bonds is responsible for the enzyme–substrate interactions, and leads to the selectivity detected. Unusually strong hydrogen bonds, known as low barrier hydrogen bonds (LBHB), have emerged as a new rationale for the exceptional catalytic abilities of some enzymes.[24] It has been suggested that these LBHB are significant contributors to the intermediate stabilization and catalytic power of the enzyme.

The catalytic mechanism of lipases is based on a 'catalytic triad' composed of a nucleophilic serine residue activated by a hydrogen bond in relay with histidine and aspartate or glutamate. The interaction between the enzyme and the acylating agent yields the acyl–enzyme intermediate. A nucleophile attacks the latter intermediate, assisted by the catalytic histidine, yielding a second tetrahedral intermediate (TI-2). This intermediate collapses to expel the acylated product and gives the free enzyme.

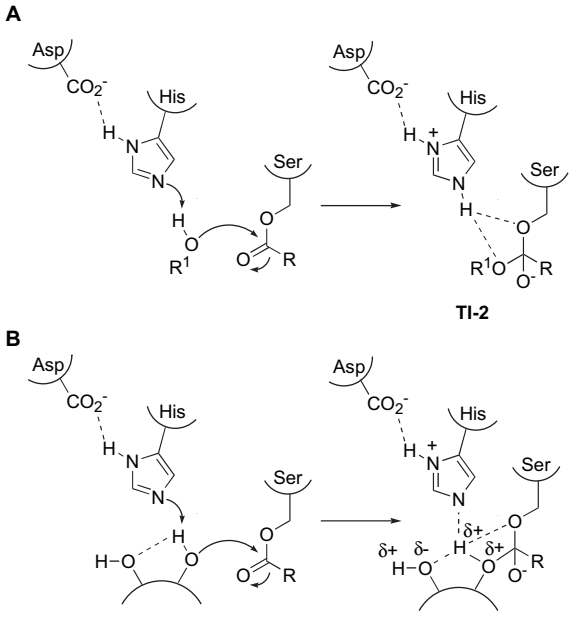


Figure 11. (A) Tetrahedral intermediate (TI-2) in the serine mechanism catalysis. (B) General representation of TI-2 in which positive charge is delocalized through hydrogen-bonding networks in quinic and shikimic acid derivatives.

We propose that transition state hydrogen-bonding plays an important role in enzyme selectivity, and can be understood as one result of the effectiveness of hydrogen-bonding networks (the original hydrogen bond network from the enzyme conjugates with intramolecular hydrogen bonding in the ligand). It is worth noting that a web of hydrogen-bonding interactions links the substrate binding sites to the catalytic triad. Two key hydrogen bonds in the TI-2 are that from His to the oxygen of Ser, and that with the OR^1 group (A, Fig. 11). In the case of quinic and shikimic acid derivatives, the positive charge in the transition state can also be delocalized through a hydrogen-bonding network present in the polyhydroxylated molecule (B, Fig. 11). The acylation reaction takes place preferentially at the hydroxyl group that participates in the more effective hydrogen-bonding network.

Acylation of quinic and shikimic acid derivatives was also carried out in the presence of DMAP as catalyst and in 1 mM concentration in $CHCl_3$, suitable conditions for efficient intramolecular hydrogen bonding.[8] In some cases, the regioselectivity of the acetylation agrees with the H-bonding scheme shown and a rise in the reactivity of certain OHs was observed. However, the reaction is not as selective as the process catalyzed by CAL-A and a mixture of acylated products was obtained.

3. Summary

Selective acylated derivatives of 3-*epi*, 4-*epi*, and 5-*epi*-isomers of quinic and shikimic acids have been efficiently synthesized in a one-step enzymatic transesterification reaction

with vinyl acetate as acyl donor and *C. antarctica* lipase A as catalyst. These analogues are useful as chiral building blocks and as optically active synthetic precursors for natural products. A study of the exchange rate of hydroxyl protons by ^{1}H NMR spectroscopy has been performed to determine the strength of the intramolecular H-bonds in these substrates. In addition, vicinal coupling constants $J_{CH,OH}$ provide structural information in terms of dihedral H–O–C–H angles and evidence the directional hydrogen-bonding network. We have established that selectivity of *C. antarctica* lipase A is related to both the inherent receptor selectivity and the degree of intramolecular hydrogen bonding in the ligand. We found a satisfactory correlation between reactivity of hydroxyl groups and effectiveness of the corresponding hydrogen-bonding network.

4. Experimental

4.1. General spectroscopy and experimental data

C. antarctica lipase A (CAL-A, chirazyme L-5, c-f, lyophilized, 25 U/g using 1-phenylacetate) was obtained from Roche. TLC chromatograms were visualized by heating after spraying with a 5% aqueous sulfuric acid solution containing cerium sulfate (1%) and molybdophosphoric acid (2.5%). Column chromatography was performed over silica 60 Å (230–400 mesh) except for **10**, **12**, **14**, and **20** in which silica 60 Å (32–63 μm) pH 7 was used. Molecular sieves 4 Å were dried at 180 °C in a vacuum over 2 h. Methyl 3-*epi*-, 4-*epi*-, and 5-*epi*-shikimate and quinate derivatives were dried in a high vacuum (10^{-5} mbar) before use. ^{1}H and ^{13}C NMR signals assignment is based on selective homonuclear and heteronuclear decoupling experiments.

Chiral HPLC analysis were performed using a chiralcel OD-H column, a flow of 0.5 mL/min, 5% EtOH/hexane as eluent, and at 35 °C. Sample concentration was 0.5 mg/mL. For (±)-**17**, t_R 12.7 and 13.9 min.

4.1.1. Sample preparation for ^{1}H NMR hydroxyl exchange studies. $CDCl_3$ was filtered over anhydrous K_2CO_3, which serves to deacidify and partially dry the solvent. $CDCl_3$ was stored under N_2 over K_2CO_3 and powdered 4 Å molecular sieves (predried under vacuum at 180 °C during 2 h) to completely remove the water. Syringes and NMR tubes were dried on a vacuum line at room temperature. The samples were prepared by dissolving a portion of the quinic and shikimic acid derivative in $CDCl_3$ to make a 2 mM solution. For the hydroxyl exchange study successive portions of 0.05 mM trifluoroacetic acid solution in $CDCl_3$ were added to the NMR tube and corresponding ^{1}H NMR spectra (300.13 MHz) were recorded.

4.2. Enzymatic acylation of methyl 3-*epi*-, 4-*epi*-, and 5-*epi*-shikimate and quinate derivatives. General procedure for the synthesis of 6, 8, 10, 12, 14, and 16

In a standard procedure, vinyl acetate (0.75 mL) was added to an Erlenmeyer flask that contained **5**, **7**, **9**, **11**, **13**, or **15** (0.069 mmol), CAL-A (13 mg), and 4 Å molecular sieves (13 mg) under nitrogen. The suspension was shaken at 250 rpm at 20 °C (except for **7** and **15** at 40 °C) for 0.5 h for **5**, 4.5 h for **7**, 1.5 h for **9**, 24 h for **11**, 0.3 h for **13**, and 4.5 h for **15**. The mixture was filtered, and the organic solvent was evaporated. Then, the reaction crude was subjected to flash chromatography (gradient eluent 50–80% Et_2O/CH_2Cl_2 for **6**; gradient eluent EtOAc–3% MeOH/EtOAc for **8**; 40% acetone/CH_2Cl_2 for **10**; 45% acetone/CH_2Cl_2 for **12**; 20% acetone/CH_2Cl_2 for **14**; and gradient eluent EtOAc–5% MeOH/EtOAc for **16**) to give **6** (white solid, 88% yield), **8** (colorless oil, 84% yield), **10** (colorless oil, 80% yield), **12** (colorless oil, 82% yield), **14** (colorless oil, 88% yield), and **16** (colorless oil, 85% yield).

4.2.1. Methyl 3-*O*-acetyl-3-*epi*-shikimate (6). R_f (Et_2O): 0.13; mp: 106–107 °C; IR (NaCl): υ 3406, 2952, 2921, 1719, 1654, 1438, 1374, 1240, and 1072 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 2.17 (s, 3H, OAc), 2.30 (dddd, 1H, $H_{6a'}$, $|^2J_{HH}|$ 17.8, $^3J_{HH}$ 9.8, $|^4J_{HH}|$ 3.7, $^5J_{HH}$ 3.1 Hz), 2.95 (ddd, 1H, $H_{6e'}$, $|^2J_{HH}|$ 17.7, $^3J_{HH}$ 5.7, $|^4J_{HH}|$ 1.1 Hz), 3.71 (dd, 1H, H_4, $^3J_{HH}$ 10.0, 8.0 Hz), 3.78 (s, 3H, OMe), 3.87 (ddd, 1H, H_5, $^3J_{HH}$ ~10.0, ~10.0, 5.9 Hz), 5.43 (m, 1H, H_3), and 6.62 (m, 1H, H_2) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.0 (C_{10}), 31.7 (C_6), 52.2 (OMe), 69.5 (C_5), 74.7, 74.9 (C_3+C_4), 130.4 (C_1), 134.4 (C_2), 166.0 (C_7), and 171.5 (C_9) ppm; $[\alpha]_D^{20}$ +38 (*c* 1.07, $CHCl_3$); MS (ESI$^+$, *m/z*): 253 [(M+Na$^+$), 100%] and 269 [(M+K$^+$), 6%]; Anal. Calcd (%) for $C_{10}H_{14}O_6$: C, 52.16; H, 6.13. Found: C, 52.2; H, 6.1.

4.2.2. Methyl 3-*O*-acetyl-3-*epi*-quinate (8). R_f (EtOAc): 0.18; IR (KBr): υ 3396, 2956, 2927, 1732, 1435, 1371, 1259, 1130, 1067, and 1036 cm^{-1}; ^{1}H RMN (300 MHz, acetone-d_6): δ 1.76 (ddd, 2H, H_{6a}+H_{2a}, $|^2J_{HH}|$ 12.9, $^3J_{HH}$ 11.7, $|^2J_{HH}|$ 5.7 Hz), 1.98 (s, 3H, OAc), 2.01–2.1 (m, 2H, H_{6e}+H_{2e}, under solvent), 2.9 (br s, 1H, OH), 3.40 (dd, 1H, H_4, $^3J_{HH}$ ~9.2, ~9.2 Hz), 3.71 (s, 3H, OMe), 3.83 (ddd, 1H, H_5, $^3J_{HH}$ 11.6, 9.0, 4.6 Hz), and 5.02 (ddd, 1H, H_3, $^3J_{HH}$ 11.6, 9.6, 4.8 Hz) ppm; ^{13}C RMN (75.5 MHz, acetone-d_6): δ 21.7 (C_{10}), 39.4, 42.0 (C_2+C_6), 53.3 (OMe), 70.7 (C_5), 73.7 (C_3), 74.8 (C_1), 78.8 (C_4), 171.5 (C_9), and 176.2 (C_7) ppm; $[\alpha]_D^{20}$ (compound from the enzymatic reaction) +19 (*c* 1.37, MeOH); $[\alpha]_D^{20}$ (compound enantiomerically pure) +31 (*c* 1.31, MeOH); MS (ESI$^+$, *m/z*): 271 [(M+Na$^+$), 100%], 287 [(M+K$^+$), 7%], and 287 [(M+H$^+$), 1%]; Anal. Calcd (%) for $C_{10}H_{16}O_7$: C, 48.39; H, 6.5. Found: C, 48.4; H, 6.6.

4.2.3. Methyl 4-*O*-acetyl-4-*epi*-shikimate (10). R_f (CH_2Cl_2): 0.13; IR (NaCl): υ 3420, 2950, 1710, 1655, 1430, 1358, 1250, and 1182 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 2.16 (s, 3H, OAc), 2.58 (dd, 1H, $H_{6a'}$, $|^2J_{HH}|$ 18.5, $^3J_{HH}$ 4.5 Hz), 2.68 (dddd, 1H, $H_{6e'}$, $|^2J_{HH}|$ 18.6, $^3J_{HH}$ 4.4, $|^4J_{HH}|$ ~2.2, $^5J_{HH}$ ~2.2 Hz), 3.78 (s, 3H, OMe), 4.28 (ddd, 1H, H_5, $^3J_{HH}$ 4.5, 4.3, 2.4 Hz), 4.59 (m, 1H, H_3), 4.91 (dd, 1H, H_4, $^3J_{HH}$ 6.9, 2.1 Hz), and 6.84 (m, 1H, H_2) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.1 (C_{10}), 31.1 (C_6), 52.1 (OMe), 66.4 (C_5), 67.3 (C_3), 76.8 (C_4), 128.6 (C_1), 136.9 (C_2), 166.7 (C_9), and 171.4 (C_7) ppm; $[\alpha]_D^{20}$ −56 (*c* 0.86, $CHCl_3$); MS (ESI$^+$, *m/z*): 253 [(M+Na$^+$), 100%] and 231 [(M+H$^+$), 3%]; Anal. Calcd (%) for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13. Found: C, 52.2; H, 6.1.

4.2.4. Methyl 4-*O*-acetyl-4-*epi*-quinate (12). R_f (EtOAc): 0.2; IR (KBr): υ 3402, 2946, 1733, 1447, 1434, 1237, 1128, and 1091 cm^{-1}; ^{1}H RMN (300 MHz, MeOH-d_4): δ 1.70 (dd, 1H, H_{2e}, $|^2J_{HH}|$ 13.8, $^3J_{HH}$ 6.6 Hz), 1.87 (dd, 1H, H_{6e}, $|^2J_{HH}|$ 13.3, $^3J_{HH}$ 3.4 Hz), 2.11 (s, 3H, H_{10}), 2.28–2.36

(m, 2H, H_{2a}+H_{6a}), 3.75 (s, 3H, OMe), 4.11 (1H, m, H_3), 4.23 (ddd, 1H, H_5, $^3J_{HH}$ ~6.4, ~6.4, 4.1 Hz), and 4.89 (dd, 1H, H_4, $^3J_{HH}$ 6.4, 2.9 Hz) ppm; ^{13}C RMN (75.5 MHz, MeOH-d_4): δ 19.6 (C_{10}), 36.7 (C_2), 38.6 (C_6), 51.38 (OMe), 63.8 (C_5), 66.7 (C_3), 74.1 (C_1), 75.7 (C_4), 171.1 (C_9), and 174.0 (C_7) ppm; $[\alpha]_D^{20}$ −19 (*c* 0.48, H_2O); MS (ESI$^+$, *m/z*): 271 [(M+Na)$^+$, 100%] and 249 [(M+H)$^+$, 10%]; Anal. Calcd (%) for $C_{10}H_{16}O_7$: C, 48.39; H, 6.5. Found: C, 48.2; H, 6.5.

4.2.5. Methyl 5-*O*-acetyl-5-*epi*-shikimate (14). R_f (Et_2O): 0.18; IR (NaCl): υ 3422, 2955 1718, 1654, 1438, 1372, 1259, and 1036 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 2.13 (s, 3H, OAc), 2.52–2.78 (m, 2H, $H_{6a'}$+$H_{6e'}$), 3.78 (s, 3H, OMe), 4.12 (m, 1H, H_4), 4.42 (m, 1H, H_3), 5.07 (ddd, 1H, H_5, $^3J_{HH}$ 8.2, 6.0, 2.0 Hz), and 6.80 (m, 1H, H_2) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.1 (C_{10}), 26.0 (C_6), 52.1 (OMe), 67.5 (C_3), 68.5 (C_4), 70.9 (C_5), 128.6 (C_1), 137.5 (C_2), 166.3 (C_7), and 170.3 (C_9) ppm; $[\alpha]_D^{20}$ −60 (*c* 0.5, MeOH); MS (ESI$^+$, *m/z*): 253 [(M+Na)$^+$, 100%] and 231 [(M+H)$^+$, 5%]; Anal. Calcd (%) for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13. Found: C, 52.2; H, 6.1.

4.2.6. Methyl 4-*O*-acetyl-5-*epi*-quinate (16). R_f (10% MeOH/EtOAc): 0.30; IR (KBr): υ 3387, 2957, 1727, 1651, 1455, 1435, 1258, 1128, and 1045 cm^{-1}; ^{1}H RMN (300 MHz, acetone-d_6): δ 1.81 (dd, 2H, H_{6a}+H_{2a}, $|^2J_{HH}|$ ~12.0, $^3J_{HH}$ ~12.0 Hz), 2.09 (s, 3H, OAc), 2.18 (dd, 2H, H_{6e}+H_{2e}, $|^2J_{HH}|$ 11.9, $^3J_{HH}$ 4.6 Hz), 3.71 (s, 3H, OMe), 3.80 (m, 2H, H_3+H_5), 3.99 (d, 2H, OH_3+OH_5, $^3J_{HOH}$ 6.6 Hz), 4.82 (s, 1H, OH_1), and 5.27 (dd, 1H, H_4, $^3J_{HH}$ ~2.4, ~2.4 Hz) ppm; ^{13}C RMN (75.5 MHz, acetone-d_6): δ 21.2 (C_{10}), 39.4 (C_2+C_6), 52.5 (OMe), 66.91 (C_3+C_5), 73.58 (C_1), 75.0 (C_4), 171.0 (C_9), and 175.3 (C_7) ppm; MS (ESI$^+$, *m/z*): 271 [(M+Na)$^+$, 100%]; Anal. Calcd (%) for $C_{10}H_{16}O_7$: C, 48.39; H, 6.5. Found: C, 48.4; H, 6.4.

4.3. Methyl (1*S*,3*R*,4*R*,5*S*,2′*S*,3′*S*)-3-acetoxy-4,5-[2,3-dimethoxybutan-2,3-di(yloxy)]-1-hydroxycyclohexan-1-carboxylate (17)

A solution of **8** (20 mg, 0.081 mmol) in MeOH (1 mL) was treated with trimethyl orthoformate (43 μL, 0.39 mmol), 2,3-butanedione (14 μL, 0.165 mmol), and camphorsulfonic acid (0.93 mg, 0.004 mmol). The mixture was refluxed under nitrogen for 2 h. The resulting solution was treated with powdered $NaHCO_3$ (spatula tip) and filtered. The filtrate was concentrated and subjected to flash chromatography (gradient eluent 50–80% Et_2O/CH_2Cl_2) to afford **17** as a colorless oil (89% yield).

4.3.1. Synthesis of (±)-17. Acetic anhydride (0.3 mL, 0.312 mmol) was added to a mixture of (±)-**18** (50 mg, 0.156 mmol), pyridine (63 μL, 0.78 mmol), and DMAP (1 mg, 1.56 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 4 h at 0 °C, the mixture was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was reextracted with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. Filtration and concentration afforded a crude, which was purified by flash chromatography (gradient eluent 50–80% Et_2O/CH_2Cl_2) to give (±)-**17** (80% yield).

R_f (20% Et_2O/CH_2Cl_2): 0.29; IR (NaCl): υ 3448, 2954, 1739, 1438, 1370, 1240, and 1132 cm^{-1}; ^{1}H RMN (300 MHz, $CDCl_3$): δ 1.30, 1.31 (2s, 6H, 2Me), 1.83 (dd, 1H, H_{2a}, $|^2J_{HH}|$ 12.9, $^3J_{HH}$ 11.2 Hz), 1.88–1.99 (m, 2H, H_{6a}+H_{6e}), 2.08 (s, 3H, OAc), 2.18 (m, 1H, H_{2e}), 3.26, 3.29 (2s, 6H, OMe), 3.70 (dd, 1H, H_4, $^3J_{HH}$ ~9.9, ~9.9 Hz), 3.80 (s, 3H, OMe), 4.08 (ddd, 1H, H_5, $^3J_{HH}$ 11.7, 9.9, 5.1 Hz), and 5.17 (ddd, 1H, H_3, $^3J_{HH}$ 11.2, 10.1, 5.1 Hz) ppm; ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 17.6, 17.63 (C_{13}+C_{14}), 21.0 (C_{18}), 37.3, 38.5 (C_2+C_6), 53.1 (OMe), 47.5, 47.8 (C_{15}+C_{16}), 65.3 (CH), 69.2 (CH), 72.9 (CH), 73.2 (C_1), 99.5, 99.54 (C_9+C_{10}), 170.1 (C_{17}), and 175.1 (C_7) ppm; MS (ESI$^+$, *m/z*): 385.1 [(M+Na$^+$), 100%] and 401.1 [(M+K$^+$), 33%]; Anal. Calcd (%) for $C_{16}H_{26}O_9$: C, 53.03; H, 7.23. Found: C, 52.9; H, 7.2.

4.4. Methyl (1*S*,3*R*,4*R*,5*S*,2′*S*,3′*S*)-1,3-dihydroxy-4,5-[2,3-dimethoxybutan-2,3-di(yloxy)]cyclohexan-1-carboxylate [(±)-18]

The same procedure as previously described for **17** (from **8**) afford (±)-**18** starting from **7**. The crude was purified by flash chromatography (70% EtOAc/hexane). White solid. Yield: 91%. Spectroscopical data in agreement with the enantiomerically pure compound previously described.[15,25] R_f (20% MeOH/EtOAc): 0.6; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.3, 1.34 (2s, 6H, Me), 1.92 (dd, 1H, H_{6a}, $|^2J_{HH}|$ 13.4, $^3J_{HH}$ 10.1 Hz), 2.08 (m, 3H, H_{6e}, H_{2a}, H_{2e}), 3.26 (2s, 6H, OMe), 3.59 (dd, 1H, H_4, $^3J_{HH}$ 8.6, 3.2 Hz), 3.77 (s, 3H, OMe), 4.18 (m, 1H, H_3), and 4.28 (ddd, 1H, H_5, $^3J_{HH}$ 10, 8.6, 4.5 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.5, 17.7 (C_{11}+C_{12}), 37.3 (CH_2), 38.5 (CH_2), 47.8, 52.8 (C_{13}+C_{14}), 62.3 (CH), 69.0 (CH), 72.6 (CH), 75.7 (C_1), 99.6, 100.2 (C_9+C_{10}), and 174.1 (C═O) ppm.

4.5. (1*S*,3*R*,4*S*,5*R*)-1,3,4-Trihydroxycyclohexan-1,5-carbolactone (19)

This compound was obtained in the attempted recrystallization of **11**. R_f (EtOAc): 0.35; mp: 148–150 °C; IR (KBr): υ 3396, 3311, 2997, 2963, 1765, 1445, 1362, 1256, 1122, and 1062 cm^{-1}; ^{1}H NMR (300 MHz, MeOH-d_4): δ 1.96 (dd, 1H, H_{2a}, $|^2J_{HH}|$ 11.9, $^3J_{HH}$ 10.6 Hz), 2.27 (d, 1H, H_{6a}, $^3J_{HH}$ 11.5 Hz), 2.42–2.29 (m, 1H, H_{2e}), 2.63 (ddd, 1H, H_{6e}, $|^2J_{HH}|$ 11.7, $^3J_{HH}$ 6.7, $|^4J_{HH}|$ 3.5 Hz), 3.68–3.86 (m, 2H, H_3+H_4), and 4.79 (d, 1H, H_5, $^3J_{HH}$ 6.6 Hz) ppm; ^{13}C NMR (75.5 MHz, MeOD-d_4): δ 41.9 (C_2), 42.3 (C_6), 71.0 (C_3), 74.1 (C_1), 76.1 (C_4), 80.5 (C_5), and 179.5 (C_7) ppm; MS (ESI$^+$, *m/z*): 371 [(2M+Na)$^+$, 100%] and 197 [(M+Na)$^+$, 70%]; $[\alpha]_D^{20}$ −94 (*c* 0.51, MeOH); Anal. Calcd (%) for $C_7H_{10}O_5$: C, 48.28; H, 5.79. Found: C, 48.2; H, 5.8.

4.6. (1*S*,3*R*,4*S*,5*R*)-4-Acetoxy-1,3-dihydroxycyclohexane-1,5-carbolactone (20)

Using the general procedure for enzymatic acylation previously described yield **20** (colorless oil, 70% yield) from **19**. The reaction mixture was stirred at 40 °C for 4.5 h. The crude material was purified by flash chromatography (40% acetone/CH_2Cl_2). R_f (EtOAc): 0.31; IR (NaCl): υ 3358, 2995, 2971, 1771, 1440, 1453, 1360, 1258, 1122, and 1067 cm^{-1}; ^{1}H NMR (300 MHz, MeOH-d_4): δ 2.03 (dd, 1H, H_{2a}, $|^2J_{HH}|$ 12.3, $^3J_{HH}$ 11.2 Hz), 2.31 (s, 3H, OAc), 2.38 (d, 1H, H_{6a}, $|^2J_{HH}|$ 11.7 Hz), 2.39 (m, 1H, H_{2e}), 2.67 (ddd, H_{6e}, $|^2J_{HH}|$ 11.7, $^3J_{HH}$ 6.7, $|^4J_{HH}|$ 3.5 Hz), 3.98

(ddd, 1H, H_3, $^3J_{HH}$ 11.0, 8.3, 7.3 Hz), 4.89 (dd, 1H, H_5, $^3J_{HH}$ 6.7, 1.1 Hz), and 4.96 (dd, 1H, H_4, $^3J_{HH}$ 8.3, 1.1 Hz) ppm; ^{13}C NMR (75.5 MHz, MeOH-d_4): δ 20.9 (C_9), 41.6 (C_2), 42.0 (C_6), 68.2 (C_3), 73.8 (C_1), 77.3 (C_5), 78.1 (C_4), 172.3 (C_8), and 178.9 (C_7) ppm; $[\alpha]_D^{20}$ −116 (*c* 0.48, MeOH); MS (ESI$^+$, *m*/*z*): 239 [(M+Na)$^+$, 100%]; Anal. Calcd (%) for $C_9H_{12}O_6$: C, 50.0; H, 5.59. Found: C, 50.1; H, 6.0.

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Tandem aza-Michael additions under high pressure: a shortcut to the azanorbornyl skeleton

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Abstract—The hyperbaric aza-Michael addition of mono- and diamines on α,β-unsaturated β,β-disubstituted mono- and diesters has been studied. While in the case of monoester, this reaction provides a β-aminoester presenting a quaternary center, a direct and efficient access to diester or lactams featuring an azanorbornyl skeleton was obtained when starting from a diester, following an unprecedented double aza-Michael addition.

1. Introduction

The β-lactam, which was derived directly from β-amino-acids, and the azanorbornane skeletons are nitrogen heterocycles sharing two characteristics. First, their biological activity and their extensive use as fundamental building blocks in the synthesis of pharmaceuticals and analogues of natural products such as alkaloids and antibiotics fuels up a permanent attention from the organic chemistry community.[1] For instance, the β-lactam nuclei featuring new characteristics such as quaternary centers in α of the nitrogen position[2] or spirocyclic arrangements[3] are regarded as valuable assets in antibiotherapy. Spiro-β-lactams exhibit other attractive biological activities illustrated by the cholesterol absorption inhibitor Sch 58053 (Fig. 1, left).[4] It is also now well established that the replacement of α-aminoacid residues with β-amino or α,β-diaminoacid residues at specific positions in some peptides leads to improved biological activity due to increased metabolic stability against peptidases.[5]

Meanwhile, the discovery of the alkaloid, epibatidine, found in trace amounts from skin extracts of an Ecuadoran poison frog, *Epipedobates tricolor*, has generated considerable interest in the 7-azanorbornane nucleus.[6] Indeed, epibatidine (Fig. 1, right) was first reported to be a highly potent, nonopioid analgesic, and nicotinic acetylcholine receptor agonist, but its high toxicity soon prompted the development and screening of new analogues.[7]

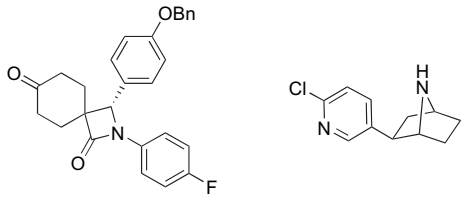

Figure 1. Cholesterol absorption inhibitor Sch 58053 (left) and analgesic epibatidine (right).

On a synthetic point of view, and this constitutes the second common point between β-aminoacids and azanorbornanes, the access to analogues of above structures can be envisaged through an aza-Michael addition on simple or double acetylidenic cyclohexanes (Fig. 2). The combination of this reaction with a substitution–condensation sequence (tandem aza-Michael reactions) can thus provide a short and atom-economical route to cyclic and polycyclic complex molecules including these heterocyclic moieties.

The aza-Michael addition on unsaturated esters is probably the most direct method to prepare β-aminoesters.[8] However, and despite its atom-economical character, this reaction is severely limited by steric factors such as those imposed by the presence of α and/or β-substituent(s), as evidenced by Pfau's pioneering results.[9] An elegant way to circumvent this problem is to resort to the addition of enantiomerically pure chiral lithium amides as homochiral ammonia equivalents. This strategy has proved to be remarkably general and is probably regarded as the most efficient route to these

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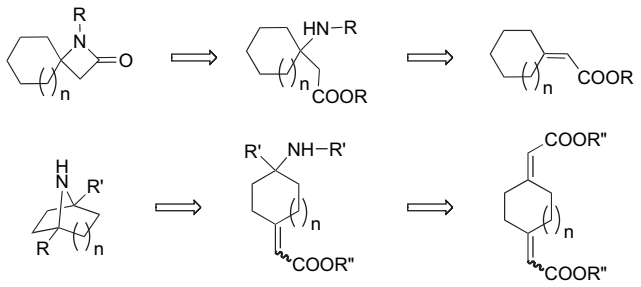


Figure 2. Access to spiro-β-lactams and to aza-bridged carbocyclic structures by aza-Michael additions.

compounds to date.[10] Another alternative consists in resorting to high pressure, which is known to help overcoming steric barriers. It has been previously shown that this technique indeed opens direct routes toward tertiary β-aminoesters,[11] and Jenner has measured a highly negative (≈ -50 mL/mol)[12] activation volume associated to this reaction, explaining its efficiency under hyperbaric conditions.

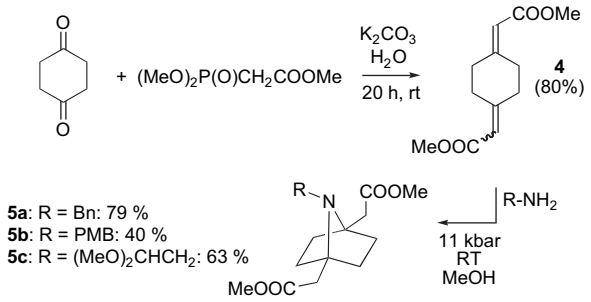
Our first results in the field led us to suggest that *exo*-cyclic olefins bearing an electron-withdrawing group are substrates for the hyperbaric (11 kbar) conjugate addition of primary amines, provided the reaction is run in alcohols.[13] Actually, α-brominated α,β-unsaturated esters give access, in these same conditions, to spiroaziridines in high yields and stereoselectivities.[14] In this paper, we report developments dealing with polyfunctional nucleophiles allowing an aza-Michael–condensation reaction sequence.

2. Results and discussion

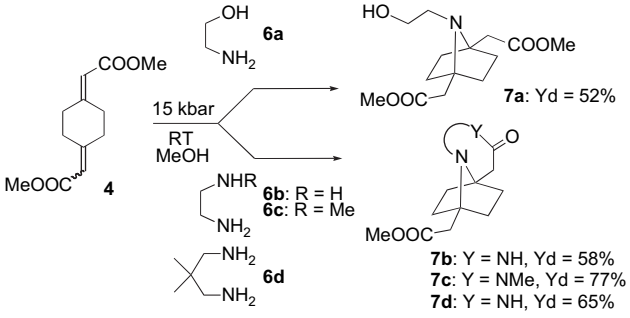
In a preliminary step, we checked that simple *exo*-cyclic olefins such as **1** react with nucleophilic primary amines such as benzylamine in comparable conditions. The expected β-aminoester **2** (featuring a quaternary center) was indeed obtained in 46% yield under 11 kbar (Scheme 1). Actually, the nucleophilic attack of the amine on the ester group (providing the corresponding unsaturated benzylamide) is the main competing reaction. No double 1,2- and 1,4-additions were noted. An efficient conjugate addition was also observed with a secondary amine such as morpholine.[15]

We next moved to binucleophiles and compressed *N*-methylethylenediamine with **1** in identical conditions. The spirolactam **3** was directly obtained in 57% yield (Scheme 1). The two diastereomers of the resulting lactams, present in a 5:1 ratio, could be separated by flash chromatography. Their NMR analysis showed that the major isomer **3a** resulted from an axial attack of the amine, in contrast with our own previous observations.[14a] About 15% of β-methoxyester **2c**, resulting from the conjugate addition of methanol on **1**, was also obtained in this case.

Bielectrophiles were also expected to trigger cascade reactions, of potential interest in the perspective of the synthesis of polyheterocyclic cation-chelating molecules. We thus turned our hands to the known[16] diester **4**, which was prepared and reacted with primary amines (Scheme 2) as well as with commercially available *N*,*N*- and *N*,*O*-binucleophiles (Scheme 3).

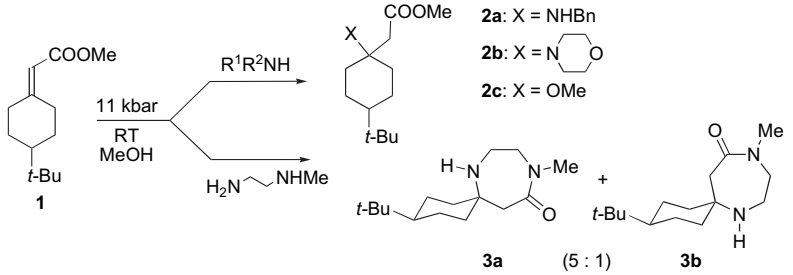


Scheme 2. Synthesis of diester **4** and their reactions with primary amines.

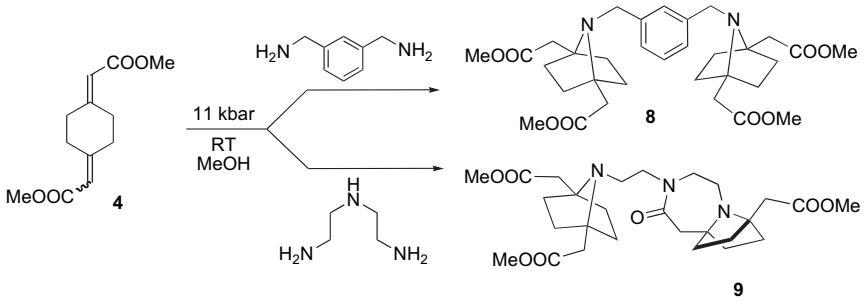


Scheme 3. Reactions of diester **4** with *N*,*N*- and *N*,*O*-binucleophiles.

The diester **4** was obtained efficiently as a 1:1 mixture of (*Z*)- and (*E*)-isomers from 1,4-cyclohexanedione resorting to the aqueous Horner–Emons conditions described by Villieras and Rambaud (Scheme 2).[17] The room temperature hyperbaric addition of a series of primary amines led to the bridged structure **5** in relatively good yields. This direct access to bridged β-aminodiesters, never described before to our knowledge, was relatively surprising considering the low



Scheme 1. Reactions of ester **1** with *N*-nucleophiles.



Scheme 4. Reactions of diester **4** with *N*,*N*-binucleophiles.

reactivity of secondary amines in conjugate additions, even under high pressure.[11a] Note that the reaction requires a protic solvent such as methanol and that only small amounts of the corresponding amides are formed, except in the case of *p*-methoxybenzylamine. Compound **5b** was indeed recovered together with 28% of its monoamide derivative.

We next wondered whether the combination of binucleophiles and bielectrophiles could afford polycyclic molecules in a single hyperbaric step. Thus, diamines **6** and diester **4** in methanol were put under 15 kbar at room temperature. The tricyclic derivatives **7b–d** were isolated in reasonable yields (Scheme 3). It is worth underlining that: (i) the same reaction can be performed under atmospheric pressure at refluxing methanol. However, the conversion rate is only 50% after one week; (ii) neither in the case of **6b** nor in that of **6d**, any product resulting from the double aza-Michael addition of the two primary amines on the two double bonds was obtained; (iii) in all cases, the intramolecular conjugate addition of the intermediate secondary amine on the remaining double bond takes place efficiently while no intermolecular addition of a secondary amine could be observed in comparable conditions. Note also that resorting to 2-aminoethanol **6a** led to the double addition of the amine while no lactonization was observed. The corresponding aminoalcohol **7a** was recovered in 52% yield after 24 h at 15 kbar and room temperature.

Extending this reaction to binucleophiles in which longer tethers separate the two primary amines revealed impossible. Actually, two separate additions take place leading to dimeric bridged structures, a supplementary lactam ring closure occurring with diethylenetriamine (Scheme 4). However, these reactions lead to large amounts of polymeric

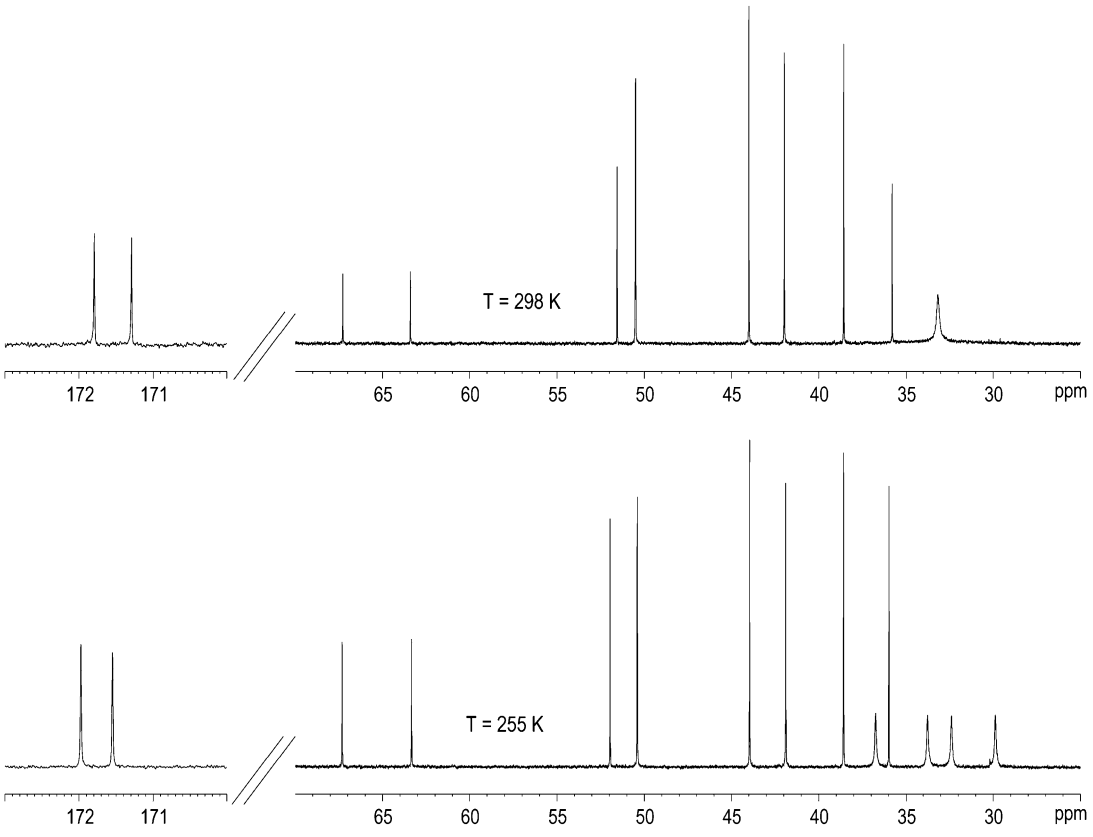


Figure 3. ^{13}C (150 MHz) spectrum of **7c** at variable temperature.

materials, adducts **8** and **9** being recovered in 10 and 16%, respectively.

Some physicochemical properties of tricyclic derivatives **7b–d** deserve comments. First, the floppiness of their seven-membered ring lactams is associated to poorly resolved ^{1}H and ^{13}C NMR spectra at room temperature. At 255 K, a dramatic improvement of the resolution was observed (see for instance the 20–40 ppm region of the ^{13}C NMR spectrum of **7c** in Fig. 3).

Interestingly, an elemental analysis revealed that the folded lactamic ring traps one molecule of water. This stable cluster can be dehydrated in refluxing benzene using a Dean–Stark apparatus. The water removal could be confirmed by a quick IR spectrum recording as well as by elemental analysis. The ability of this highly chelating ring to trap polar molecules (and ions?) could be of interest in the perspective of supramolecular applications.

3. Conclusion

In conclusion, the conjugate addition of primary amines on α,β-unsaturated β,β-disubstituted esters can be efficient under hyperbaric conditions, even in the case of β,β-disubstituted esters reacting with secondary amines. Resorting to *N*,*N*-binucleophiles and bielectrophiles such as diester **4** leads to polycyclic lactams in reasonable yields through a yet unknown double aza-Michael step. Extensions of these results to other substrates, their application to the synthesis of skeletons of biological relevance, and efforts toward an asymmetric version[18] of this tandem reaction are in progress.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded at 300 (default) or 600 (specified) MHz and ^{13}C NMR spectra at 75 (default) or 150 (specified) MHz; chemical shifts (δ) are given in parts per million (ppm) and the coupling constants (J) in hertz. The solvent was deuterochloroform. IR spectra were recorded by transmission. The mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing potential; ammonia was used for chemical ionization (CI). The silica gel used for flash chromatography was 230–400 mesh. High pressure reactions were performed in a piston–cylinder type apparatus, designed for pressures up to 20 kbar. All reagents were of reagent grade and were used as such or distilled prior to use. Methyl 4-*tert*-butylcyclohexylidene acetate **1** and diester **4** were prepared as reported previously.[13,14a]

4.2. Typical procedure for the treatment of esters 1 and 4 with amines and binucleophiles

A solution of the ester (1 mmol) and amine or binucleophile (1 mmol) in methanol (1–1.5 mL) was allowed to stand under 11–15 kbar at room temperature. After reversion to atmospheric pressure, the solvent was evaporated. The residue was chromatographed (CH_2Cl_2/MeOH 9:1) to yield the corresponding products. The following compounds were all prepared according to this procedure.

4.2.1. Methyl 2-[1-(benzylamino)-4-(*tert*-butyl)cyclohexyl]acetate (2a). Oil. IR (KBr, ν, cm^{-1}): 1731 (C=O). ^{1}H NMR ($CDCl_3$): δ 0.91 (s, 9H), 1.25–1.07 (m, 3H), 1.47 (br t, 2H), 1.77 (br d, 2H), 1.89 (br d, 2H), 2.67 (s, 2H), 3.73 (s, 3H), 3.79 (s, 2H), 7.82–7.24 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 23.8 ($C(CH_3)_3$), 27.9 ($C(CH_3)_3$), 32.7, 37.1 (CH_2 cycl.), 44.1 (C_q), 46.2 (CH_2CO), 48.3 (CH), 51.7 (CH_3O), 55.0 (NCH_2), 127.9, 128.8, 129.1, 141.6 (C_6H_5), 172.9 (C=0). MS (CI) *m*/*z* (relative intensity): 318 (M^++1), 244 (7), 106 (20), 91 (60), 57 (100). Calcd for $C_{20}H_{32}NO_2$: C, 75.67; H, 9.84; N, 4.41; found C, 75.65; H, 9.89; N, 4.49.

4.2.2. Methyl 2-[4-(*tert*-butyl)-1-morpholinocyclohexyl] acetate (2b). Oil. ^{1}H NMR ($CDCl_3$): δ 0.77 (s, 9H), 0.90–1.15 (m, 3H), 1.30 (br t, 2H), 1.63 (br d, 2H), 1.85 (br d, 2H), 2.43 (s, 2H), 2.50–2.60 (m, 4H), 3.50–3.60 (m, 4H), 3.57 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 23.62 ($C(CH_3)_3$), 27.58 ($C(CH_3)_3$), 32.32, 37.42 (CH_2 cycl.), 36.82 (C_q), 45.84 (CH_2CO), 47.67 (CH), 51.24 (CH_3O), 58.70 (NCH_2), 68.10 (OCH_2), 172.70 (C=O).

4.2.3. Methyl 2-[4-(*tert*-butyl)-1-methoxycyclohexyl] acetate (2c). Oil. IR (KBr, ν, cm^{-1}): 1741 (C=O). ^{1}H NMR ($CDCl_3$): (major diastereomer): δ 0.83 (s, 9H), 1.00–1.10 (m, 2H), 1.30–1.50 (m, 2H), 1.70–1.80 (m, 2H), 1.90–2.00 (m, 2H), 2.58 (s, 2H), 3.26 (s, 3H), 3.66 (s, 3H); (minor diastereomer): δ 0.82 (s, 9H), 1.20–1.35 (m, 7H), 1.45–1.55 (m, 2H), 1.90–2.00 (m, 2H), 2.42 (s, 2H), 3.19 (s, 3H), 3.65 (s, 3H). ^{13}C NMR ($CDCl_3$): (major diastereomer): δ 24.31 (CH_2 cycl.), 27.77 (CH_3, Bu^t), 35.20 (CH_2 cycl.), 37.56 (CH_2CO), 47.69 (CH), 49.09 (OCH_3), 51.70 (OCH_3), 76.20 (C–O cycl.), 171.76 (C=O); (minor diastereomer): δ 22.26 (CH_2 cycl.), 27.72 (CH_3, Bu^t), 34.60 (CH_2 cycl.), 43.04 (CH_2CO), 47.50 (CH), 48.93 (OCH_3), 51.70 (OCH_3), 74.14 (C–O cycl.), 171.51 (C=O). MS (EI) *m*/*z* (relative intensity): 210 (16, M^+-CH_3OH), 169 (49), 143 (98), 57 (100). Calcd for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81; found C, 69.19; H, 10.72.

4.2.4. 4-(*tert*-Butyl)-10-methyl-7,10-diazaspiro[5,6]dodecan-11-one (3a). Solid. Mp 148 °C. IR (KBr, ν, cm^{-1}): 1624 (C=O), 3309 (NH). ^{1}H NMR ($CDCl_3$): δ 0.81 (s, 9H), 0.90–1.15 (m, 2H), 1.04 (dt, J=3.0, 13.5 Hz, 2H), 1.45–1.85 (m, 5H), 2.54 (s, 2H), 2.85–2.90 (m, 2H), 2.95 (s, 3H), 3.35–3.40 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 22.02 (CH_2 cycl.), 27.69 ($C(CH_3)_3$), 32.50 ($C(CH_3)_3$), 36.10 (NCH_3), 42.30 (CH_2 cycl.), 47.83 (C^4), 50.20 (C^3), 52.98 (CH_2N), 53.79 (CH_2N), 173.33 (C=O). MS (EI) *m*/*z* (relative intensity): 252 (38, M^+), 237 (18), 153 (100). Calcd for $C_{15}H_{28}N_2O$: C, 71.38; H, 11.18; N, 11.10; found C, 71.24; H, 11.26; N, 11.08.

4.2.5. 3-(*tert*-Butyl)-10-methyl-7,10-diazaspiro[5,6]dodecan-11-one (3b). Solid. Mp 110 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.79 (s, 9H), 1.01 (m, 1H), 1.32 (m, 4H), 1.74 (br s, 2H), 1.97 (br d, J=7.3 Hz, 2H), 2.80 (s, 2H), 3.06 (s, 3H), 3.13 (t, J=4.6 Hz, 2H), 3.55 (br s, 2H), 5.10 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 23.28 (CH_2 cycl.), 27.67 ($C(CH_3)_3$), 32.44 ($C(CH_3)_3$), 35.94 (NCH_3), 42.19 (CH_2 cycl.), 43.67 (C^4), 47.86 (C^3), 51.76 (CH_2N), 53.00

(CH_2N), 172.14 (C=O). MS (EI) *m/z* (relative intensity): 252 (35, M^+), 237 (18), 153 (100).

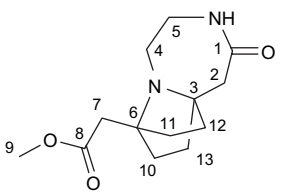
4.2.6. Methyl 2-[7-benzyl-4-(2-methoxy-2-oxoethyl)-7-azabicyclo[2.2.1]hept-1-yl]acetate (5a). Solid. Mp 65–66 °C. IR (KBr, ν, cm^{-1}): 1751 (C=O). ^1H NMR ($CDCl_3$): δ 1.64–1.78 (m, 8H), 2.44 (s, 2H), 3.52 (s, 2H), 3.56 (s, 6H), 7.10–7.40 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 33.90 (CH_2 cycl.), 40.50 (CH_2), 46.80 (NCH_2), 51.80 (OCH_3), 67.40 (C cycl.), 126.70, 128.10, 128.50, 142.10 (C_6H_5), 172.10 (C=O). MS (EI) *m/z* (relative intensity): 331 (1, M^+), 272 (2), 258 (12). Calcd for $C_{19}H_{25}NO_4$: C, 68.86; H, 7.60; N, 4.23; found C, 68.94; H, 7.59; N, 4.28.

4.2.7. Methyl 2-[7-(4-methoxybenzyl)-4-(2-methoxy-2-oxoethyl)-7-azabicyclo[2.2.1]hept-1-yl]acetate (5b). Solid. Mp 62–64 °C. IR (KBr, ν, cm^{-1}): 1742 (C=O). ^1H NMR ($CDCl_3$): δ 1.63–1.78 (m, 8H), 2.46 (s, 2H), 3.47 (s, 2H), 3.59 (s, 6H), 3.77 (s, 3H), 6.82–7.27 (dd, J=8.7 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ 33.90 (CH_2 cycl.), 40.50 (CH_2), 46.20 (NCH_2), 51.80 (OCH_3), 55.60 (OCH_3 phenyl), 67.30 (C cycl.), 113.90, 129.10 (C_6H_5), 172.20 (C=O). MS (EI) *m/z* (relative intensity): 361 (34, M^+), 330 (2), 232 (31), 121 (100). Calcd for $C_{20}H_{27}NO_5$: C, 66.46; H, 7.53; N, 3.88; found: C, 66.65; H, 7.66; N, 4.02.

4.2.8. Methyl 2-[7-(2,2-dimethoxyethyl)-4-(2-methoxy-2-oxoethyl)-7-azabicyclo[2.2.1]hept-1-yl]acetate (5c). Oil. IR (KBr, ν, cm^{-1}): 1747 (C=O). ^1H NMR ($CDCl_3$): δ 1.64–1.78 (m, 8H), 2.38 (d, J=4.9 Hz, 1H), 2.64 (s, 2H), 3.37 (s, 6H), 3.65 (s, 6H), 4.22 (t, J=4.9 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 33.70 (CH_2 cycl.), 40.40 (CH_2), 46.10 (NCH_2), 51.90 (OCH_3), 55.20 (OMe), 67.50 (C cycl.), 106.40 (CHO), 172.30 (C=O). MS (EI) *m/z* (relative intensity): 298 (4, $[M-MeO]^+$), 254 (100), 224 (6), 193 (15), 151 (17). Calcd for $C_{16}H_{27}NO_6$: C, 58.34; H, 8.26; N, 4.25; found C, 58.07; H, 8.31; N, 4.32.

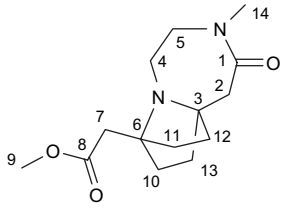
4.2.9. Methyl 2-[7-(2-hydroxyethyl)-4-(2-methoxy-2-oxoethyl)-7-azabicyclo[2.2.1]hept-1-yl]acetate (7a). Oil. IR (KBr, ν, cm^{-1}): 1737 (C=O), 3449 (OH). ^1H NMR ($CDCl_3$): δ 1.63 (br s, 8H), 2.45 (t, J=5.3 Hz, 2H), 2.61 (s, 4H), 3.46 (t, J=5.3 Hz, 2H), 3.60 (br s, 1H), 3.64 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 33.40 (CH_2 cycl.), 39.48 (CH_2), 43.69 (NCH_2), 51.80 (OCH_3), 61.36 (C cycl.), 67.29 (OCH_2), 171.73 (C=O). MS (EI) *m/z* (relative intensity): 254 (100, M^+-CH_3OH), 198 (25); (CI) *m/z* (relative intensity): 286 (100, M^+), 254 (17, M^+-CH_3OH). Calcd for $C_{14}H_{23}NO_5$: C, 58.93; H, 8.12; N, 4.91; found C, 58.89; H, 8.16; N, 4.92.

4.2.10. Methyl 2-(6-oxo-2,5-diazatricyclo[6.2.2.0^{2,8}]-dodec-1-yl)acetate (7b).



Solid. Mp 151 °C. IR (KBr, ν, cm^{-1}): 1660, 1732 (C=O). ^1H NMR (600 MHz, $CDCl_3$, T=298 K): δ 1.50–1.80 (m, 8H), 2.56 (s, 2H), 2.60 (br s, 2H), 2.77 (br s, 2H), 3.35 (br s, 2H), 3.63 (s, 3H), 6.88 (br s, 1H); (T=255 K): δ 1.50–1.90 (m, 8H, CH_2-10–CH_2-13), 2.37, 2.89 (2m, 2H, CH_2-4), 2.54, 3.13 (2m, CH_2-2), 2.64 (s, 2H, CH_2-7), 3.32, 3.53 (2m, 2H, CH_2-5), 3.70 (s, 3H, OCH_3), 7.58 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$, T=298 K): δ 33.35 (br s, C-10–C-13), 38.84 (C-7), 41.69 (C-5), 42.18 (C-2), 46.03 (C-4), 51.82 (OCH_3), 63.25 (C-3), 67.68 (C-6), 171.56 (C-8), 175.05 (C-1); (T=255 K): δ 29.96, 32.40, 33.77, 36.77 (C-10–C-13), 38.68 (C-7), 41.53 (C-2), 45.69 (C-4), 41.85 (C-5), 52.00 (OCH_3), 63.01 (C-3), 67.54 (C-6), 171.62 (C-8), 175.50 (C-1). MS (EI) *m/z* (relative intensity): 252 (35, M^+), 224 (30), 179 (37), 165 (100). Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.10; found C, 61.93; H, 8.03; N, 10.89.

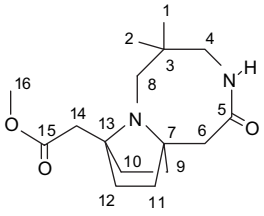
4.2.11. Methyl 2-(5-methyl-6-oxo-2,5-diazatricyclo[6.2.2.0^{2,8}]dodec-1-yl)acetate (7c).



Solid. IR (KBr, ν, cm^{-1}): 1650, 1736 (C=O). ^1H NMR (600 MHz, $CDCl_3$, T=298 K): δ 1.45–1.75 (m, 8H), 2.56 (s, 2H), 2.60 (br s, 2H), 2.82 (br s, 2H), 2.96 (s, 3H), 3.50 (br s, 2H), 3.64 (s, 3H); (T=255 K): δ 1.50–1.80 (m, 8H, CH_2-10,11,12,13), 2.40, 2.90 (2m, 2H, CH_2-4), 2.65, 3.12 (2m, 2H, CH_2-2), 2.67 (s, 2H, CH_2-7), 2.96 (s, 3H), 3.30, 3.85 (2m, 2H, CH_2-5), 3.64 (s, 3H, OCH_3). ^{13}C NMR (150 MHz, $CDCl_3$, T=298 K): δ 33.37 (br s, C-10,11,12,13), 36.04 (NCH_3), 38.81 (C-7), 42.21 (C-2), 44.23 (C-4), 50.74 (C-5), 51.81 (OCH_3), 63.58 (C-3), 67.46 (C-6), 171.56 (C-8), 172.03 (C-1); (T=255 K): δ 29.89, 32.40, 33.77, 36.77 (C-10,11,12,13), 36.01 (NCH_3), 38.61 (C-7), 41.92 (C-2), 43.98 (C-4), 50.44 (C-5), 51.97 (OCH_3), 63.34 (C-3), 67.31 (C-6), 171.59 (C-8), 172.01 (C-1). MS (EI) *m/z* (relative intensity): 266 (59, M^+), 238 (42), 193 (63), 179 (100); (CI) *m/z* (relative intensity): 267 (100, MH^+). Calcd for $C_{14}H_{22}N_2O_3$: C, 63.13; H, 8.33; N, 10.52; found C, 63.03; H, 8.39; N, 10.27.

7c·1H$_2$O: Solid. IR (KBr, ν, cm^{-1}): 1650, 1736 (C=O), 3467 (OH). ^1H NMR (CD_3CN, T=298 K): δ 1.45–1.75 (m, 8H), 2.20 (br s, 2H), 2.56 (s, 2H), 2.60 (br s, 2H), 2.82 (br s, 2H), 2.96 (s, 3H), 3.50 (br s, 2H), 3.64 (s, 3H); (T=323 K): δ 1.50–1.80 (m, 8H, CH_2-10–CH_2-13), 2.10 (br s, 2H), 2.40, 2.90 (2m, CH_2-4), 2.62 (s, 2H, CH_2-7), 2.65, 3.12 (2m, 2H, CH_2-2), 2.96 (s, 3H, NCH_3), 3.30, 3.85 (2m, 2H, CH_2-5), 3.64 (s, 3H, OCH_3); ($(CD_3)_2CO$, T=298 K): δ 1.45–1.75 (m, 8H), 2.20 (br s, 2H), 2.56 (s, 2H), 2.60 (br s, 2H), 2.77 (br s, 2H), 2.82 (br s, 2H), 2.96 (s, 3H), 3.50 (br s, 2H), 3.64 (s, 3H); (T=323 K): δ 1.50–1.80 (m, 8H, CH_2-10–CH_2-13), 2.10 (br s, 2H), 2.40, 2.90 (2m, CH_2-4), 2.47 (br s, 2H), 2.62 (s, 2H, CH_2-7), 2.65, 3.12 (2m, 2H, CH_2-2), 2.96 (s, 3H, NCH_3), 3.30, 3.85 (2m, 2H, CH_2-5), 3.64 (s, 3H, OCH_3). Calcd for $C_{14}H_{24}N_2O_4$: C, 59.13; H, 8.51; N, 9.85; found C, 58.91; H, 8.43; N, 9.75.

4.2.12. Methyl 2-(4,4-dimethyl-7-oxo-2,6-diazatricyclo-[7.2.2.0^{2,9}]tridec-1-yl)acetate (7d).



Solid. Mp 179 °C. IR (KBr, ν, cm^{-1}): 1661, 1736 (C=O), 31.98, 32.91 (NH). ^{1}H NMR (600 MHz, $CDCl_3$, T=260 K) major isomer: δ 0.80 (s, 3H), 0.90 (s, 3H), 1.45–1.75 (m, 8H, CH_2-9–CH_2-12), 1.83 (A-part of AB system, J=14.8 Hz, 1H), 2.04 (B-part of AB system, J=14.8 Hz, 1H), 2.40 (A-part of AB system, J=13.2 Hz, 1H), 2.56 (m, 2H), 2.60 (d, J=4.4 Hz, 1H), 2.88 (B-part of AB system, J=13.2 Hz, 1H), 3.38 (dd, J=14.8, 11.1 Hz, 1H), 3.67 (s, 3H), 6.62 (br s, 1H); minor isomer: δ 0.78 (s, 3H), 0.98 (s, 3H), 1.45–1.75 (m, 8H, CH_2-9–CH_2-12), 2.06 (A-part of AB system, J=14.8 Hz, 1H), 2.24 (B-part of AB system, J=14.8 Hz, 1H), 2.29 (A-part of AB system, J=13.0 Hz, 1H), 2.54 (m, 2H), 2.72 (d, J=7.2 Hz, 1H), 2.75 (B-part of AB system, J=13.0 Hz, 1H), 3.20 (dd, J=13.5, 7.2 Hz, 1H), 3.67 (s, 3H), 6.70 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$, T=298 K) major isomer: δ 23.16, 24.01 (C-1, C-2), 30.54 (C-9 or C-11), 32.73 (C-10 or C-12), 33.70 (C-10 or C-12), 36.57 (C-3), 38.08 (C-9 or C-11), 39.99 (C-14), 40.15 (C-6), 48.79 (C-8), 50.51 (C-4), 51.40 (C-16), 67.46 (C-7), 68.11 (C-13), 171.75 (C-15), 174.93 (C-5); minor isomer: δ 22.05, 27.03 (C-1, C-2), 32.18, 33.31, 33.87, 34.58 (C-9–C-11), 35.33 (C-3), 36.57 (C-6), 39.99 (C-14), 51.40 (C-16), 53.53 (C-8), 54.76 (C-4), 67.16 (C-13), 67.86 (C-7), 171.82 (C-15), 174.33 (C-5). MS (EI) *m/z* (relative intensity): 294 (11, M$^+$), 265 (42), 221 (100). Calcd for $C_{16}H_{26}N_2O_3$: C, 65.28; H, 8.90; N, 9.52; found C, 65.02; H, 8.97; N, 9.48.

Compound ***8***: Solid. IR (KBr, ν, cm^{-1}): 1738 (C=O). ^{1}H NMR ($CDCl_3$): δ 1.55–1.80 (m, 16H), 2.39 (s, 8H), 3.47 (AB system, 4H), 3.52 (s, 12H), 7.10–7.25 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 33.70 (CH_2 cycl.), 40.34 (CH_2), 46.70 (NCH_2), 51.60 (OCH_3), 67.12 (C cycl.), 126.19, 127.11, 128.12, 141.64 (C_6H_4), 171.98 (C=O). MS (EI) *m/z* (relative intensity): 584 (100, M$^+$), 553 (43), 511 (32).

Compound ***9***: Solid. IR (KBr, ν, cm^{-1}): 1650, 1736 (C=O). ^{1}H NMR ($CDCl_3$): δ 1.51–1.70 (m, 16H), 2.36 (t, J=2.6 Hz, 2H), 2.59 (br s, 2H), 2.68 (br s, 6H), 2.75–2.85 (m, 2H), 3.25–3.35 (m, 2H), 3.55–3.65 (m, 2H), 3.65 (s, 6H), 3.66 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 33.51 (br s, C-10–C-13), 38.75, 39.70 ($CH_2C(O)O$), 40.23 (CH_2CON), 42.31, 45.12, 50.23, 51.00 (CH_2N), 51.71, 51.83 (OCH_3), 63.61, 67.27, 67.56 (C_q), 171.53, 171.83, 171.88 (C=O). MS (EI) *m/z* (relative intensity): 519 (5, M$^+$), 488 (10), 254 (100).

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Synthesis and crystal structures of extremely crowded oligophenylenes as model precursors to 'cubic graphite'

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Abstract—By using drastic conditions for a Diels–Alder cycloaddition reaction, it was possible to synthesize an oligophenylene with an extremely dense packing of the benzene rings. Crystallographic data could be obtained and a projection of the structure on the plane of the central phenyl ring reveals that the molecule retained its theoretical threefold symmetry with only minor deviations. Due to its dense packing of interlocked benzene rings, this oligophenylene could be furthermore used as a suitable precursor for constructing a subunit of 'cubic graphite'.

1. Introduction

The search for novel three-dimensional polyphenylene structures also includes a carbon phase so-called 'cubic graphite', proposed and attempted to synthesize by Gibson et al.[1] The name cubic graphite implies both, the cubic symmetry of such a phase and the close relationship between its solid-state structure and that of conventional graphite. In such a phase each benzene ring is connected to six other different rings and each benzene ring is part of three equivalent polyparaphenylene chains.

Four neighboring benzene rings are arranged to a tetraphenylene ring with a strong mutual twist between them (Fig. 1). Thus, within such a novel phase, all carbon atoms become equivalent. This material would present the perfect three-dimensional polyphenylene structure[2,3] and should possess high thermal and mechanical stability. These features and the presence of large diffusion channels for lithium cations and its high predicted maximum charge storage capacity make 'cubic graphite' a candidate for rechargeable batteries, battery-like supercapacitors, and electrochromic displays.[2,3]

Although this particular carbon phase might never be accessible due to the extremely crowded three-dimensional arrangement of hexaphenylbenzene (HPB) units, highly dense polycyclic aromatic hydrocarbons, and nanosized dendritic or hyperbranched polyphenylenes have gained even more attention as fascinating synthetic targets.[3–11] The biggest oligophenylene nanostructures from which crystallographic data could be obtained are based on the Diels–Alder cycloaddition between tetraphenylcyclopentadienones and arylacetylenes.[5,6] This approach in combination with palladium-catalyzed Hagihara–Sonogashira coupling reactions[12] leads to a great diversity of higher generation polyphenylene dendrimers, and when applied to a functionalizable core to a versatile synthesis and self-assembly of star-type hexabenzocoronenes.[13] A different strategy to novel three-dimensional polyphenylene structures utilizes the Suzuki coupling reaction of dibromo-hexaphenylbenzene units with complementary bis(boronic acid) derivatives. This route affords a novel oligophenylene

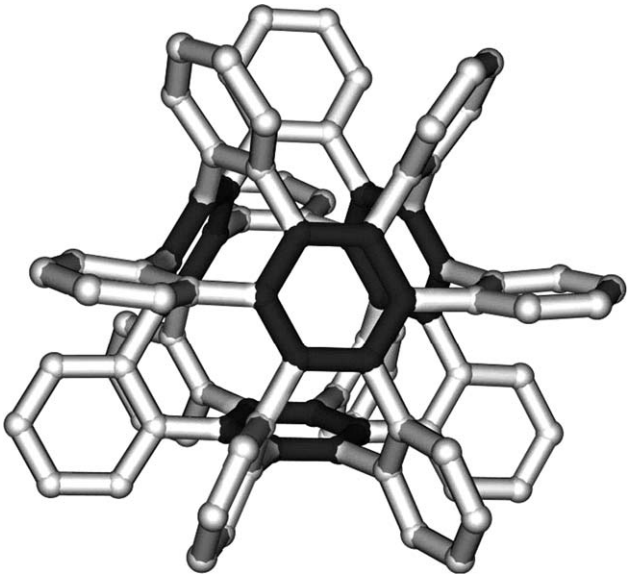

Figure 1. Three-dimensional representation of a subunit of cubic graphite.

Keywords: Cubic graphite; Oligophenylene; Dendrimer; Dense packing; Diels–Alder; Cycloaddition.
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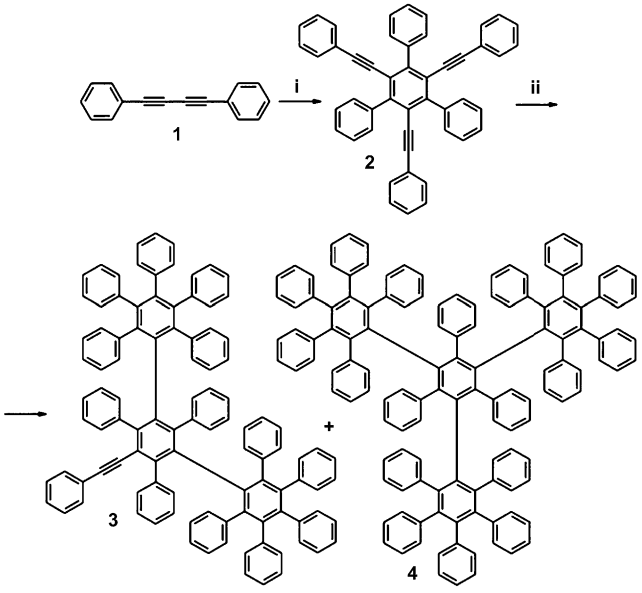
macrocycle related to 'phenylogous cubic graphite' with a large central cavity and the formation of channels within the crystal.[14] A further approach to crowded polyphenylenes is based on the thermal ring opening of biphenylene units with subsequent dimerization to tetraphenylene and leads to 'cubic graphite' related structures.[15]

Herein, we present the straightforward synthesis and crystal structures[16] of the densely packed oligophenylenes **3** and **4**. The 1,3,5-tris-(phenylethynyl)-2,4,6-triphenylbenzene (**2**) was synthesized via a cobaltoctacarbonyl mediated cyclotrimerization of diphenylbutadiyne **1** following a literature procedure[17] (Scheme 1). Looking at the sterical demands of **4**, a threefold Diels–Alder reaction with **2** seemed to be impossible. Indeed, reaction of both components in refluxing diphenyl ether under argon atmosphere proceeded very slowly. The generation of the monoadduct was detected by thin layer chromatography (TLC) and field-desorption mass spectrometry (FDMS) soon after the reaction started. However, the formation of the bisadduct **3** required four days. Under these reaction conditions, target compound **4** was not formed even after an extremely prolonged reaction time of one month. Instead, decomposition became rampant and therefore a rather uncommon preparative method was chosen. A mixture of the acetylene derivative **2** and excess tetraphenylcyclopentadienone was placed in a glass ampoule. After evacuation (10^{-5} mbar), the ampoule was sealed and heated for 72 h at 265 °C. A complex reaction mixture was obtained, which made a full separation and characterization of all components impossible. After column chromatography and washing, only minor amounts of **4** could be isolated, and the amount of double Diels–Alder product **3** was also very modest. Although the yield of **4** was rather low, these conditions seemed to provide an optimum for the formation of **4**. While a decrease in temperature prevented the formation of a melt, and therefore, good mixing, an increase resulted in more drastic conditions, favoring undesired side reactions. These harsh conditions and the resulting low yields stand in contrast to other ethynyl precursor molecules, where the products also show a dense packing of the benzene rings.[6] However, one major difference is that the ethynyl functionalities in these examples were situated spatially less close than for the precursor **2**, which indicates the strong influence of the steric situation upon the Diels–Alder cycloaddition.



Scheme 1. Reaction conditions: (i) $Co_2(CO)_8$, dioxane, 8 h, reflux; (ii) tetraphenylcyclopentadienone, 265 °C, 72 h, **3** (34%), **4** (2%).

The molecular structure of both compounds could be assigned by MS, ^{1}H NMR, and ^{13}C NMR spectroscopy; however, absolute certainty provided their crystal structures. Whereas isolation of **3** via column chromatography did not provide any difficulties, the fraction containing **4** consisted of several components. Field-desorption and MALDI-TOF MS analysis showed the presence of a peak at *m*/*z* 1706 Da, most likely corresponding to the norbornadienone, which is subsequently transferred to the final product **4** under CO extrusion. The presence of such a norbornadienone is substantiated by a recent publication[10] demonstrating that significant steric crowding supports the stability of that particular class of compounds. Additionally, one has to consider that applying the above described reaction conditions, it is not possible to estimate to what extent partial cyclodehydrogenation will also come into play, making an unambiguous structural assignment even more difficult.

The ^{1}H NMR spectra of both oligophenylenes, **3** and **4**, show resonances at unusually high-field positions for aromatic ring protons implying that they are placed above the centers of neighboring aromatic rings. While the aromatic proton resonances assigned to the 'core' of **3** resonate at δ=5.74, those attributable to core **4** (Fig. 3 b, d, f) appear at δ=4.83. These strong high-field shifts are caused by the magnetic anisotropy as a result of the ring currents of the surrounding *ortho*-phenyl rings of the pentaphenyl units. Similar effects have been reported in the literature.[5,7] The ^{13}C NMR spectrum of **3** indicated a C_{2v} symmetry on the timescale of the solution-based NMR experiment, as it displays 34 resonances. This is consistent with a fast, unrestricted rotation of all phenyl groups including the ones directly connected to the central phenyl ring. Due to the very poor solubility of **4** in all organic solvents, the acquisition of ^{13}C NMR spectral data had to be performed at 373 K using $C_2D_2Cl_4$ as solvent. The spectrum shows 22 signals corresponding to a D_{3h} symmetry of the molecule, which would be expected within the fast exchange limit for all single bond rotations.

Crystals of **3** and **4** were grown from tetrachloroethane and methylenechloride, respectively, by slow evaporation at room temperature (Fig. 2). They crystallize as solvates and especially **3**, which contains five solvent molecules per asymmetric unit, decomposes quickly under ambient conditions.[18] A projection of the structure of **4** on the plane of the central phenyl ring reveals that here the molecule retains its theoretical threefold symmetry with only minor deviations. This can be explained by the rigidity of the intramolecular packing in this overcrowded molecule, which reduces the distortions by outer forces, e.g., packing effects. Thus the molecular shape is almost cylindrical. As a consequence **3** crystallizes in a pseudohexagonal packing ($a \approx c$, $\beta \approx 120°$), which is expected for an array of disks. Compound **3** appears to be much less rigid owing to its reduced inner

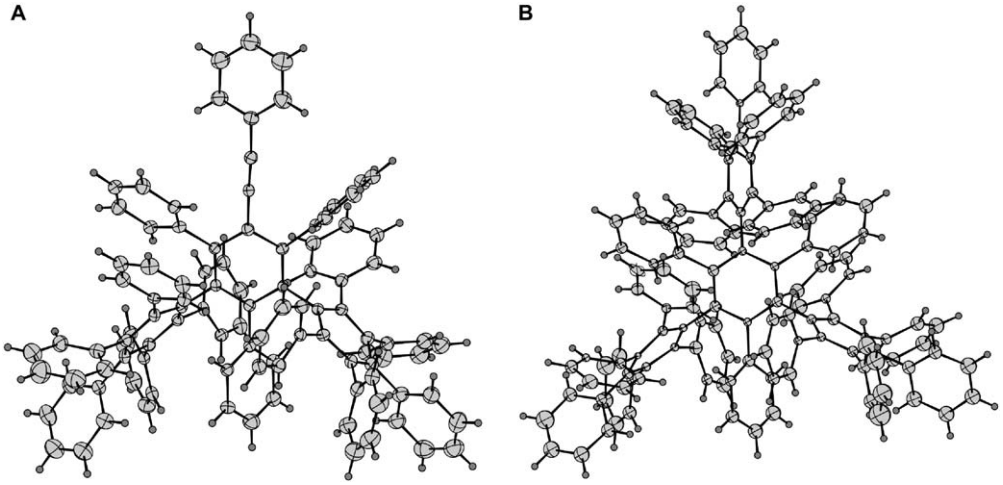


Figure 2. Crystal structures of (A) compound **3** and (B) compound **4** (projection along the central phenyl ring).

packing density and especially the angles, which are determined by the three phenyl substituents (Fig. 3 b, d, f) with the central phenyl ring, seem to be affected considerably by intermolecular contacts.

In summary, the results presented herein show that, by using rather drastic synthetic conditions, highly dense oligophenylenes are accessible. Compound **4**, due to its dense packing of benzene rings, is furthermore a suitable precursor for constructing a subunit of 'cubic graphite'. The key structural motif comprises of a HPB subunit having in ortho- and ortho′-positions each of the three alternating (Fig. 3 a, c, e) benzene rings connected with further two benzene rings. The latter ones would allow for the formation of six alternating tetraphenylene rings in a final cyclodehydrogenation step involving the other three alternating (Fig. 3 b, d, f) benzene rings of the central HPB unit. After the formation of the red dotted bonds in Figure 3A, one would obtain a subunit of 'cubic graphite', represented schematically in Figure 3B.

However, the extremely low yield towards **4** forces us to seek for more efficient synthetic approaches to create suitable precursors of this extremely challenging carbon framework. This will also promote efforts into the creation of related[14,15] and new, intriguing graphite models. A promising approach to achieve the latter goal includes spatial restricted cyclodehydrogenations applied to polyphenylene dendrimers. Thus, a suitable arrangement of the dendrons, predetermined by the core, would just allow for oxidative cyclodehydrogenation within the wings and not between them. This concept might create not only giant propeller-shaped molecules with blades comprised of PAHs, but also large channels and cages for storage and transport are feasible.[19]

2. Experimental

2.1. General

Freshly powdered 1,3,5-tris(ethynylphenyl)-2,4,6-trisphenylbenzene (**2**) (75 mg, 0.124 mmol) and tetraphenylcyclopentadienone (245 mg, 0.637 mmol) were placed in a glass ampoule. The mixture was thoroughly mixed, and the ampoule afterward evacuated to 10^{-5} mbar. After sealing,

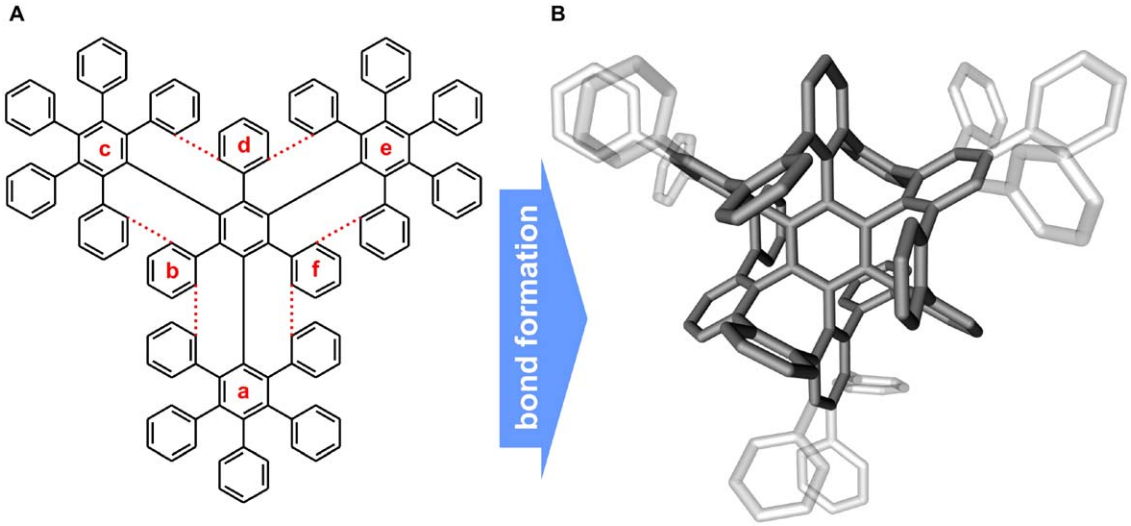


Figure 3. (A) Necessary bond formations from **4** (red dotted lines) towards a subunit of 'cubic graphite'; (B) schematic representation of the theoretically formed product (semi-empirical AM1 calculation).

it was heated in an electric oven at 2 °C/min to 265 °C. This temperature was maintained for 72 h. The ampoule was cooled to room temperature and carefully opened. The solid red-brownish residue was removed from the ampoule and subjected to column chromatography (silica gel, *n*-hexane/dichloromethane 2:1) to yield, after removal of the solvent by rotary evaporation and careful washings with *n*-pentane, the double- and triple-Diels–Alder product **3** (55 mg, 0.042 mmol, 34%) and **4** (5 mg, 0.003 mmol, 2%), respectively.

2.1.1. Compound 3. Colorless solid; mp>300 °C; ^{1}H NMR (700 MHz, $C_2D_2Cl_4$, 306 K): δ=7.16–6.93 (m, 16H), 6.88–6.73 (m, 12H), 6.72–6.55 (m, 27H), 6.54–6.41 (m, 8H), 6.28 (s, 3H), 5.74(w, 4H); ^{13}C NMR (176 MHz, $C_2D_2Cl_4$, 318 K): δ=144.32, 142.59, 141.97, 141.52, 141.26, 141.16, 141.03, 139.98, 139.69, 138.35, 137.64, 136.54, 133.89, 133.37, 132.45, 131.92, 131.73, 131.57, 131.02, 128.23, 127.88, 127.02, 126.62, 126.49, 126.37, 126.01, 125.83, 125.41, 124.96, 124.74, 123.86, 123.74, 96.10, 92.05; FDMS (8 kV): *m*/*z* (%): 1320 (100%) [M$^+$].

2.1.2. Compound 4. Colorless solid; mp>300 °C; ^{1}H NMR (700 MHz, $C_2D_2Cl_4$, 306 K): δ=6.95 (t, ^{3}J=7.3 Hz, 6H), 6.88 (d, ^{3}J=7.7 Hz, 7H), 6.85 (t, ^{3}J=7.3 Hz, 7H), 6.78 (t, ^{3}J=7.7 Hz, 9H), 6.58 (d, ^{3}J=6.0 Hz, 12H), 6.52 (w, 20H), 6.38 (d, ^{3}J=5.1 Hz, 9H), 6.16 (d, ^{3}J=8.5 Hz, 14H), 4.83 (w, 6H); ^{13}C NMR (176 MHz, $C_2D_2Cl_4$, 373 K): δ=142.15, 141.89, 141.72, 141.66, 141.54, 139.75, 139.59, 138.69, 137.90, 135.89, 135.79, 132.29, 131.62, 131.52, 126.49, 126.24, 126.01, 125.55, 125.15, 124.97, 124.71, 124.22; FDMS (8 kV): *m*/*z* (%): 1676 (100%) [M$^+$].

Acknowledgements

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16. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CSD 285923 and CSD 285924 and can be obtained free of charge, on application to: CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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18. Crystals were mounted in closed capillaries and the data collections were carried out at 120 K on a Nonius KappaCCD diffractometer with graphite monochromated Mo Kα radiation. 19,149 and 19,089 unique reflections were collected for **4** and **3**, respectively, of which 4121 and 14,881 reflections were considered observed (I>22(I)). The structures were solved by direct methods and refined by full-matrix least squares analyses on F^2 with anisotropic temperature factors for all non-hydrogen atoms. The H atoms were refined with fixed isotropic temperature factors in the riding mode. Final R values for all reflections were 0.115 (R_w 0.194) (**4**) and 0.068 (R_w 0.117) (**3**). The R values for the observed reflections were 0.069 (R_w 0.107) (**4**) and 0.055 (R_w 0.099) (**3**).
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Stereoselective synthesis of pachastrissamine (jaspine B)

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Dedicated to Professor J. Barluenga, from the University of Oviedo, on the occasion of his 65th birthday

Abstract—A stereoselective synthesis in enantiopure form of the natural anhydrophytosphingosine pachastrissamine (jaspine B), a metabolite isolated from sponges, is described. The chiral epoxide (*R*)-glycidol was the starting material. Key steps of this synthesis are a Sharpless asymmetric epoxidation, an intramolecular stereospecific epoxide opening mediated by a trichloroacetimidate group, and the formation of a tetrahydrofuran ring via intramolecular nucleophilic displacement.

1. Introduction

Sponges of the genus *Jaspis* (family Coppatiidae) have received in recent times a considerable attention from scientists because of the interesting pharmacological properties of its chemical components. Among these, modified peptides[1] and nucleosides,[2] sterols and other triterpene derivatives,[3] unusual bisoxazoles,[4] cytotoxic macrolides,[5] styryl sulfates,[6] unusual terpenes of mixed biogenetic origin,[7] α-amino-ε-lactam derivatives (bengamides),[8] and brominated tyrosine derivatives[9] are particularly worth mentioning. Very recently, two cytotoxic anhydrophytosphingosine derivatives, named jaspine A **1** and B **2**, have been isolated from a new species of the aforementioned genus.[10] Compound **2** was found highly active against the A5409 human lung carcinoma cell line (IC_{50}=0.34 μM). Compound **2** had also been isolated shortly before from another sponge species of the *Pachastrissa* genus (family Calthropellidae) and received the name pachastrissamine.[11,12] It was assayed against the P388, HT29, MEL28 tumoral cell lines and found cytotoxic at the submicromolar level. The valuable pharmacological properties and novel structural features of **2**[13] have prompted us to undertake its total synthesis in enantiopure form (Fig. 1).

Very recently, total syntheses of **2** starting from the chiral pool have been reported.[14] As a part of our research program aimed at developing stereocontrolled syntheses of naturally occurring bioactive compounds in enantiopure form,[15] we here report our own synthesis of pachastrissamine (jaspine B) **2** from (*R*)-glycidol as the chiral starting material.[16]

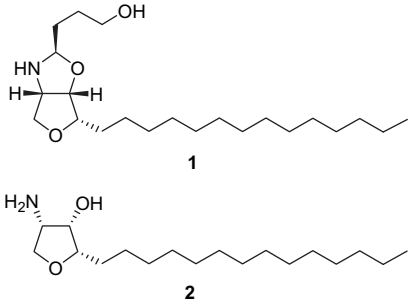
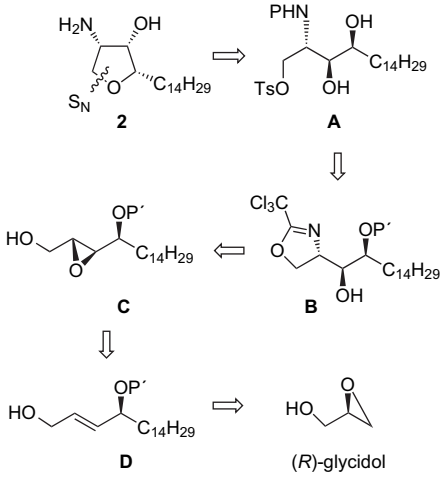


Figure 1. Structures of jaspines A and B.

The retrosynthetic strategy for the synthesis of **2** is outlined in Scheme 1 (P, P′=protecting groups). Retrocleavage of one of the C–O bonds in the tetrahydrofuran ring via



Scheme 1. Retrosynthetic plan for the synthesis of pachastrissamine (jaspine B) **2**.

Keywords: Pachastrissamine (jaspine B); (*R*)-Glycidol; Sharpless asymmetric epoxidation; Imidate epoxide opening.
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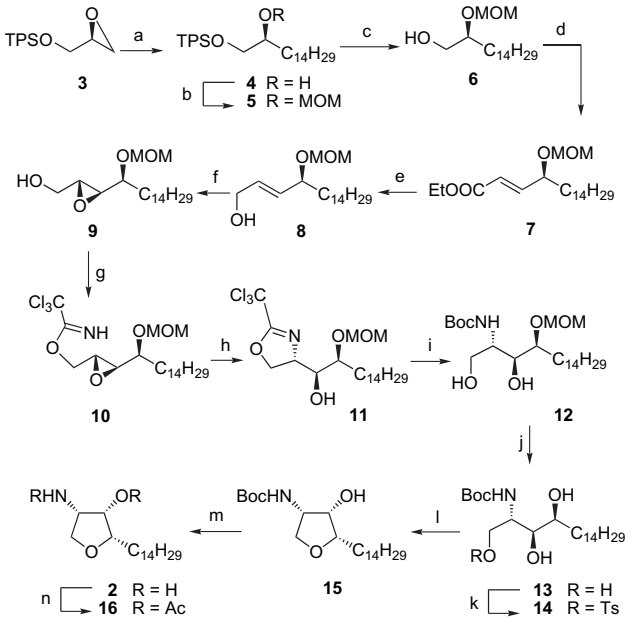
intramolecular nucleophilic displacement gives the open chain intermediate **A**. The nitrogen atom is then introduced (in the retrosynthetic sense) by means of another intramolecular nucleophilic displacement in epoxy alcohol **C** via oxazoline **B**. Compound **C** can be obtained through Sharpless epoxidation of (*E*)-allylic alcohol **D**, to be prepared in turn from (*R*)-glycidol.

2. Results and discussion

The synthesis of **2** is illustrated in Scheme 2. Commercial (*R*)-glycidol was transformed into its known[17] TPS ether **3** (for nonstandard acronyms, see Scheme 2). Epoxide opening in **3** with a *n*-tridecyl cuprate reagent[18] afforded alcohol **4**, which was then protected as its MOM derivative **5**.[19] Desilylation of the latter with TBAF furnished alcohol **6**, which was transformed into the (*E*)-unsaturated ester **7** as reported by sequential Swern oxidation and olefination.[20] DIBAL reduction of **7** to (*E*)-allylic alcohol **8** followed by Sharpless asymmetric epoxidation with *t*-BuO_2H in the presence of Ti(*i*PrO)$_4$ and (−)-diethyl tartrate[21] provided epoxy alcohol **9**. Upon reaction with trichloroacetonitrile in the presence of DBU, **9** furnished the corresponding imino ester derivative **10**.[22] The nucleophilic intramolecular epoxide opening in **10** was first attempted in the presence of catalytic amounts of $BF_3\cdot Et_2O$ but only decomposition was observed. Success was finally achieved through reaction of imidate **10** in the presence of Et_2AlCl to yield oxazoline **11**.[23] Acidic hydrolysis of **11** followed by *N*-Boc protection furnished diol **12**. Removal of the MOM group could be performed with TMSBr[24,25] to provide triol **13**, which was then selectively tosylated at the primary alcohol group. Basic treatment of monotosylate **14** with K_2CO_3 in MeOH caused intramolecular tosylate displacement and gave rise to tetrahydrofuran **15**. Cleavage of the *N*-Boc group in **15** with TFA yielded compound **2**, which had spectral properties identical to those reported in the bibliography for either pachastrissamine[11] or jaspine B.[10] Furthermore, acetylation of **2** provided the *O*,*N*-diacetyl derivative **16**, with spectral properties also identical to those reported in the bibliography.[10,11]



Scheme 2. Synthesis of pachastrissamine (jaspine B) **2**. Reaction conditions: (a) $CH_3(CH_2)_{12}MgBr$, CuI, THF, −10→0 °C, 82%; (b) MOMCl, EtN*i*Pr$_2$, CH_2Cl_2, 24 h, rt, 94%; (c) TBAF, THF, 3 h, rt, 94%; (d) Ref. 20; (e) DIBAL, hexane, 0 °C, 2.5 h, 95%; (f) *t*-BuO_2H, (−)-diethyl tartrate, Ti(*i*PrO)$_4$, CH_2Cl_2, −20 °C, 24 h, 89%; (g) Cl_3CCN, DBU, CH_2Cl_2, 30 min, 0 °C; (h) Et_2AlCl, CH_2Cl_2, 0 °C→rt, 5 h, 72% overall yield from **9**; (i) aq 1 M HCl, THF, 5 h, rt, then $NaHCO_3$, Boc_2O, 16 h, rt, 96% overall yield from **11**; (j) TMSBr, CH_2Cl_2, −78 °C, 30 min, 75%; (k) TsCl, Et_3N, DMAP, CH_2Cl_2, 20 min, rt; (l) K_2CO_3, MeOH, 16 h, rt, 70% overall from **13**; (m) TFA, CH_2Cl_2, 0 °C→rt, 45 min, 75%; (n) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 16 h, rt (Acronyms: CSA=camphorsulfonic acid; TBAF=tetra-*n*-butyl ammonium fluoride hydrate; TPS=*tert*-butyldiphenylsilyl).

3. Conclusions

We have performed a stereoselective synthesis of the natural anhydrophytosphingosine pachastrissamine (jaspine B) with (*R*)-glycidol as the chiral starting material. Since both antipodes of glycidol as well of diethyl tartrate (for the Sharpless asymmetric epoxidation step) are available, our synthetic concept is flexible enough as to be adaptable to the preparation of the non-natural antipode of **2** or else diastereomers thereof. Furthermore, ring-opening of the protected glycidol with organocopper reagents of various structures would provide a library of pachastrissamine analogues for structure–activity studies.

4. Experimental

4.1. General

$^1H/^{13}C$ NMR spectra were measured at 500/125 MHz in $CDCl_3$ solution at 25 °C. The signals of the deuterated solvent ($CDCl_3$) were taken as the reference (the singlet at δ 7.25 for ^1H NMR and the triplet centered at 77.00 ppm for ^{13}C NMR data). Carbon atom types (C, CH, CH_2, CH_3) were determined with the DEPT pulse sequence. Mass spectra were run by the electron impact (EIMS, 70 eV) or the fast atom bombardment mode (FABMS, *m*-nitrobenzyl alcohol matrix) on a VG AutoSpec mass spectrometer. IR data are given only for compounds with relevant functions (OH, C=O) and were recorded as oily films on NaCl plates (oils) or as KBr pellets (solids). Optical rotations were measured at 25 °C. Reactions which required an inert atmosphere were carried out under N_2 with flame-dried glassware. Et_2O and THF were freshly distilled from sodium–benzophenone ketyl and transferred via syringe. Dichloromethane was freshly distilled from CaH_2. Tertiary amines were freshly distilled from KOH. Toluene was freshly distilled from sodium wire. Commercially available reagents were used as received. Unless detailed otherwise, 'work-up' means pouring the reaction mixture into brine, followed by extraction with the solvent indicated in parenthesis. If the reaction medium was acidic (basic), an additional washing with 5% aq $NaHCO_3$ (aq NH_4Cl) was performed. Drying over anhydrous Na_2SO_4 and elimination of the solvent under reduced pressure were followed by chromatography of the residue on a silica gel column (60–200 μm) with the indicated eluent. Where solutions were filtered through a Celite pad, the pad was additionally washed with the same solvent used, and the washings incorporated to

the main organic layer. Nonstandard acronyms are explained in the caption of Scheme 2.

4.1.1. (2*S*)-1-(*tert*-Butyldiphenylsilyloxy)hexadecan-2-ol (4). Magnesium turnings (365 mg, ca. 15 mmol) were suspended under N_2 in dry THF (10 mL) at room temperature. A few drops of 1,2-dibromoethane were added to the suspension via syringe until an effervescence started. A solution of *n*-tridecyl bromide (3.6 mL, 14 mmol) in THF (10 mL) was then added dropwise via syringe. The resulting mixture was stirred at 50 °C for 30 min.

Powdered CuI (1.33 g, 7 mmol) was gently heated in vacuo until the solid turned light yellow. The flask was then filled with Ar and cooled to −30 °C, followed by addition of dry THF (30 mL). The previously prepared solution of *n*-tridecylmagnesium bromide was then added dropwise via syringe. The mixture was then stirred for 15 min at −30 °C. The TPS derivative **3** of (*R*)-glycidol (1.1 g, ca. 3.5 mmol) was dissolved in dry THF (10 mL) and added dropwise to the solution of organocopper reagent. The reaction mixture was then stirred for 15 min at −10 °C and then for further 4 h at 0 °C. Work-up (Et_2O) and column chromatography on silica gel (hexane–EtOAc, 9:1) afforded pure **4** (1.42 g, 82%): oil; $[\alpha]_D$ +1.5 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70–7.60 (4H, m), 7.45–7.35 (6H, m), 3.72 (1H, m), 3.68 (1H, dd, *J*=10, 3.3 Hz), 3.52 (1H, dd, *J*=10, 7.5 Hz), 2.50 (1H, br s, OH), 1.50–1.20 (26H, br m), 1.08 (9H, s), 0.89 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.3, 133.2, 19.3 (C), 135.6, 135.5, 129.8, 127.8, 71.9 (CH), 68.0, 32.8, 32.0, 29.7 (several overlapped peaks), 25.5, 22.7 (CH_2), 26.9 (×3), 14.1 (CH_3); IR ν_{max} 3460 (br, OH) cm^{-1}; HREIMS *m/z* (rel int.) 439.3036 (M^+-tBu, 9), 199 (100), 139 (36). Calcd for $C_{32}H_{52}O_2Si-t$Bu, 439.3032. Anal. Calcd for $C_{32}H_{52}O_2Si$: C, 77.36; H, 10.55. Found: C, 77.37; H, 10.66.

4.1.2. (2*S*)-1-(*tert*-Butyldiphenylsilyloxy)-2-(methoxymethoxy)hexadecane (5). Alcohol **4** (1.24 g, 2.5 mmol) was dissolved in dry CH_2Cl_2 (25 mL) and treated with MOM chloride (570 μL, ca. 7.5 mmol), DMAP (12 mg, 0.1 mmol), and triethyl amine (1.26 mL, ca. 9 mmol). The resulting solution was stirred at room temperature for 24 h. Work-up (CH_2Cl_2) and column chromatography on silica gel (hexane–EtOAc, 19:1) provided compound **5** (1.27 g, 94%): oil; $[\alpha]_D$ −25.4 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz) δ 7.70–7.60 (4H, m), 7.45–7.35 (6H, m), 4.78 (1H, d, *J*=6.8 Hz), 4.65 (1H, d, *J*=6.8 Hz), 3.70–3.60 (3H, m), 3.36 (3H, s), 1.650–1.20 (26H, br m), 1.07 (9H, s), 0.89 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 133.6 (×2), 19.2 (C), 135.7, 135.6, 129.7, 127.7, 78.0 (CH), 96.2, 66.5, 32.0, 31.8, 29.7 (several overlapped peaks), 25.4, 22.7 (CH_2), 55.5, 26.9 (×3), 14.1 (CH_3); HREIMS *m/z* (rel int.) 483.3266 (M^+-tBu, 1), 213 (96), 153 (32), 91(50), 71 (100). Calcd for $C_{34}H_{56}O_3Si-t$Bu, 483.3294. Anal. Calcd for $C_{34}H_{56}O_3Si$: C, 75.50; H, 10.44. Found: C, 75.27; H, 10.32.

4.1.3. (2*S*)-2-(Methoxymethoxy)hexadecan-1-ol (6). Silyl ether **5** (1.19 g, 2.2 mmol) was dissolved in dry THF (20 mL) and treated with a solution of TBAF (680 mg, 2.6 mmol) in dry THF (5 mL). The reaction mixture was stirred at room temperature until consumption of the starting material (about 3 h, TLC monitoring!). After removal of all volatiles in vacuo, the residue was chromatographed on silica gel (hexane–EtOAc, 4:1) to yield **6** (625 mg, 94%): solid; mp 39–40 °C, lit.[20] mp 36.9–38.1 °C; $[\alpha]_D$ +28.7 (*c* 1.6, $CHCl_3$), lit.[20] $[\alpha]_D$ +33.3 (*c* 2.3, $CHCl_3$); spectral data identical to those reported.[20]

4.1.4. Ethyl (2*E*,4*S*)-4-(methoxymethoxy)octadec-2-enoate (7). Prepared from **6** as reported.[20]

4.1.5. (2*E*,4*S*)-4-(Methoxymethoxy)octadec-2-en-1-ol (8). Ester **7** (650 mg, 1.75 mmol) was dissolved in dry hexane (5 mL) and cooled to 0 °C. Then DIBAL (4 mL, 1 M in hexane) was added and the reaction mixture was stirred at 0 °C for 2.5 h. Work-up (CH_2Cl_2) and column chromatography on silica gel (hexane–EtOAc, 19:1, then 4:1) furnished alcohol **8** (546 mg, 95%); solid; mp 44–45 °C; $[\alpha]_D$ −54.8 (*c* 2, $CHCl_3$); ^{1}H NMR (500 MHz) δ 5.80 (1H, dt, *J*=15.5, 5.3 Hz), 5.56 (1H, ddt, *J*=15.5, 8, 1.3 Hz), 4.69 (1H, d, *J*=6.8 Hz), 4.52 (1H, d, *J*=6.8 Hz), 4.15 (2H, d, *J*=4.5 Hz), 4.00 (1H, dt, *J*=8, 6.5 Hz), 3.36 (3H, s), 1.70–1.35 (6H, m), 1.35–1.20 (21H, s), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 131.9, 131.7, 76.4 (CH), 93.8, 62.9, 35.6, 31.9, 29.6 (several overlapped peaks), 25.5, 22.7 (CH_2), 55.4, 14.1 (CH_3); IR ν_{max} 3400 (br, OH) cm^{-1}; HREIMS *m/z* (rel int.) 297.2784 (M^+-CH_2OH, 1), 267 (4), 225 (7), 178 (25), 131 (100), 83 (20). Calcd for $C_{20}H_{40}O_3-CH_2OH$, 297.2793. Anal. Calcd for $C_{20}H_{40}O_3$: C, 73.12; H, 12.27. Found: C, 73.02; H, 12.24.

4.1.6. (2*R*,3*R*,4*S*)-2,3-Epoxy-4-(methoxymethoxy) octadecan-1-ol (9). Titanium tetraisopropoxide (0.6 mL, ca. 2 mmol) and D-(−)-diethyl tartrate (0.38 mL, ca. 2.2 mmol) were sequentially added at −20 °C to a stirred suspension of powdered 4 Å molecular sieves (250 mg) in dry CH_2Cl_2 (5 mL). After stirring for 5 min, a solution of allylic alcohol **8** (526 mg, 1.6 mmol) in dry CH_2Cl_2 (10 mL) was added, and the mixture was stirred for 30 min. After this, *tert*-butyl hydroperoxide (2.5 mL of a freshly prepared ≈4 M solution in toluene, 10 mmol) was added dropwise and the reaction mixture was further stirred at −20 °C for 24 h. Work-up (CH_2Cl_2) and column chromatography on silica gel (hexane–EtOAc, 7:3) afforded epoxy alcohol **9** (491 mg, 89%): solid; mp 40–41 °C; $[\alpha]_D$ −13.4 (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz) δ 4.83 (1H, d, *J*=6.8 Hz), 4.63 (1H, d, *J*=6.8 Hz), 3.92 (1H, dq, *J*=12.8, 2.2 Hz), 3.63 (1H, dq, *J*=12.8, 4.4 Hz), 3.38 (3H, s), 3.36 (1H, m), 3.02 (1H, dd, *J*=7, 2.2 Hz), 2.99 (1H, dt, *J*=4.4, 2.2 Hz), 2.10 (1H, br s, OH), 1.65–1.50 (2H, m), 1.40–1.20 (24H, br m), 0.87 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 77.1, 58.0, 55.6 (CH), 95.5, 61.2, 32.3, 31.9, 29.6 (several overlapped peaks), 25.4, 22.7 (CH_2), 55.5, 14.1 (CH_3); IR ν_{max} 3450 (br, OH) cm^{-1}; HRFABMS *m/z* 345.3009 (M+H)$^+$. Calcd for $C_{20}H_{41}O_4$, 345.2999. Anal. Calcd for $C_{20}H_{40}O_4$: C, 69.72; H, 11.70. Found: C, 69.92; H, 11.88.

4.1.7. (1*S*,2*S*)-2-(Methoxymethoxy)-1-[(4*S*)-(2-trichloromethyl-4,5-dihydrooxazol-4-yl)]hexadecan-1-ol (11). A solution of epoxy alcohol **9** (482 mg, 1.4 mmol) in dry CH_2Cl_2 (5 mL) was cooled at 0 °C and treated dropwise with trichloroacetonitrile (200 μL, 2 mmol) and DBU (45 μL, 0.3 mmol). The mixture was then stirred for 30 min at 0 °C. Work-up (CH_2Cl_2) and rapid column chromatography on silica gel (hexane–EtOAc, 4:1) gave

trichloroacetimidate **10**, which was directly used in the following reaction.

A solution of the trichloroacetimidate from above in dry CH_2Cl_2 (10 ml) was cooled at 0 °C and treated with diethylaluminum chloride (1 mL of a 1 M solution in hexane, 1 mmol). The reaction mixture was stirred for 5 h at room temperature and then filtered through a pad of Celite. Solvent removal in vacuo and column chromatography on silica gel (hexane–EtOAc, 9:1) yielded **11** (493 mg, 72% overall yield from **9**): oil; $[\alpha]_D$ +32.7 (*c* 1.8, $CHCl_3$); ^1H NMR (500 MHz) δ 4.75–4.70 (3H, m), 4.64 (1H, t, *J*=9 Hz), 4.42 (1H, dt, *J*=9, 5.5 Hz), 3.75–3.65 (2H, m), 3.41 (s, 3H), 2.65 (1H, d, *J*=7 Hz, OH), 1.60 (2H, m), 1.35–1.20 (24H, br m), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 163.6 (C), 79.2, 73.6, 69.1 (CH), 96.7, 73.0, 32.0, 31.1, 29.7 (several overlapped peaks), 25.2, 22.7 (CH_2), 56.0, 14.1 (CH_3) (the quaternary carbon signal from the CCl_3 group was not detected); IR ν_{max} 3440 (br, OH), 1662 (C=N) cm^{-1}; HRFABMS *m/z* 488.2111 $(M+H)^+$. Calcd for $C_{22}H_{41}^{35}Cl_3NO_4$, 488.2101. Anal. Calcd for $C_{22}H_{40}Cl_3NO_4$: C, 54.05; H, 8.25. Found: C, 54.02; H, 8.11.

4.1.8. (2*S*,3*S*,4*S*)-4-(Methoxymethoxy)-2-(*tert*-butyloxycarbonylamino)octadecan-1,3-diol (12). Oxazoline **11** (489 mg, 1 mmol) was dissolved in THF (6 ml) and treated with 1 M aq HCl (1.2 mL). The reaction mixture was stirred at room temperature for 5 h. Solid $NaHCO_3$ (1 g) was then added, followed by addition of di-*tert*-butyl dicarbonate (3 mL of a 1 M solution in THF). The reaction mixture was then stirred for 16 h at room temperature. Work-up (EtOAc) and column chromatography on silica gel (hexanes–EtOAc, 1:1) furnished **12** (443 mg, 96%): oil; $[\alpha]_D$ +14.4 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz) δ 5.40 (1H, br d, *J*=9 Hz), 4.76 (1H, d, *J*=6.7 Hz), 4.70 (1H, d, *J*=6.7 Hz), 3.90 (1H, m), 3.75–3.65 (3H, m), 3.55 (1H, m), 3.41 (s, 3H), 1.65–1.55 (2H, m), 1.44 (9H, s), 1.45–1.20 (26H, br m), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 156.0, 79.7 (C), 81.4, 74.6, 52.5 (CH), 97.7, 62.8, 32.0, 31.4, 29.7 (several overlapped peaks), 25.3, 22.7 (CH_2), 56.0, 28.4, 14.1 (CH_3); IR ν_{max} 3440 (br, OH), 1714 (C=O) cm^{-1}; HREIMS *m/z* (rel int.) 462.3781 ($M+H^+$, 1), 430 (2), 269 (34), 160 (37), 264 (100), 134 (52), 104 (44), 60 (76), 57 (100). Calcd for $C_{25}H_{52}NO_6$, 462.3794. Anal. Calcd for $C_{25}H_{51}NO_6$: C, 65.04; H, 11.13. Found: C, 65.02; H, 11.11.

4.1.9. (2*S*,3*S*,4*S*)-2-(*tert*-Butyloxycarbonylamino) octadecan-1,3,4-triol (13). A solution of diol **12** (438 mg, 0.95 mmol) in dry CH_2Cl_2 (10 mL) was cooled at −78 °C. Trimethylsilyl bromide (185 μL, 1.4 mmol) was then added dropwise. The reaction mixture was stirred for 30 min at −78 °C. Work-up (EtOAc) and column chromatography on silica gel (hexane–EtOAc, 1:1) afforded the protected aminotriol **13** (298 mg, 75%): oil; $[\alpha]_D$ −8.3 (*c* 1, $CHCl_3$); ^1H NMR (500 MHz) δ 5.25 (1H, br d, *J*=9 Hz), 4.05 (1H, br d, *J*=10.5 Hz), 3.76 (1H, dd, *J*=10.5, 4 Hz), 3.63 (1H, m), 3.53 (1H, m), 3.40 (1H, m), 2.50 (1H, br s, OH), 1.70–1.60 (2H, m), 1.45 (9H, s), 1.40–1.20 (26H, br m), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 157.2, 80.5 (C), 69.7, 62.1, 53.5 (CH), 73.0, 32.9, 31.9, 29.7 (several overlapped peaks), 26.1, 22.7 (CH_2), 28.4, 14.1 (CH_3); IR ν_{max} 3400 (br, OH), 3250 (br, NH), 1671 (C=O) cm^{-1}; HRFABMS *m/z* 418.3547 $(M+H)^+$. Calcd for $C_{23}H_{48}NO_5$, 418.3532. Anal. Calcd for $C_{23}H_{47}NO_5$: C, 66.15; H, 11.34. Found: C, 66.06; H, 11.40.

4.1.10. (2*S*,3*S*,4*S*)-4-(*tert*-Butyloxycarbonylamino)-2-(tetradecyl)tetrahydrofuran-3-ol (15). Triol **13** (250 mg, 0.6 mmol) was diluted in dry CH_2Cl_2 (20 mL), cooled to 0 °C and treated with Et_3N (420 μL, 3 mmol), DMAP (6 mg, 0.05 mmol), and tosyl chloride (343 mg, 1.8 mmol). The reaction mixture was then stirred at room temperature until consumption of the starting material (about 20–30 min). Work-up (CH_2Cl_2) provided the crude monotosylate **14**, which was used directly in the next step.

A solution of crude **14** in MeOH (8 mL) was cooled at 0 °C and treated with K_2CO_3 (415 mg, 3 mmol). The reaction mixture was stirred for 16 h at room temperature and then filtered through a pad of Celite. Solvent removal in vacuo and column chromatography on silica gel (hexane–EtOAc, 4:1) afforded **15** (168 mg, 70% overall yield from **13**): solid, mp 110–111 °C; $[\alpha]_D$ +6.8 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz) δ 5.10 (1H, br d, *J*=8 Hz), 4.30 (1H, br s), 4.07 (1H, m), 4.04 (1H, t, *J*=8.5 Hz), 3.79 (1H, td, *J*=7, 2.8 Hz), 3.58 (1H, t, *J*=8.5 Hz), 2.00 (1H, br s, OH), 1.65–1.55 (2H, m), 1.45 (9H, s), 1.40–1.20 (24H, br m), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 155.7 (C), 82.2 ($+C_q$), 71.9, 54.3 (CH), 70.3, 31.9, 29.7 (several overlapped peaks), 26.1, 22.7 (CH_2), 28.4, 14.1 (CH_3); IR ν_{max} 3365 (br, OH, NH), 1688 (C=O) cm^{-1}; HRFABMS *m/z* 400.3463 $(M+H)^+$. Calcd for $C_{23}H_{46}NO_4$, 400.3426. Anal. Calcd for $C_{23}H_{45}NO_4$: C, 69.13; H, 11.35. Found: C, 69.20; H, 11.42.

4.1.11. Pachastrissamine (jaspine B) (2). A solution of **15** (120 mg, 0.3 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C and treated with TFA (220 μL, ca. 3 mmol). The reaction mixture was then stirred for 45 min at room temperature. After this time, 2.5 M NaOH in MeOH (2 mL) was added and the stirring was continued for 5 min. Work-up (CH_2Cl_2) and column chromatography on silica gel ($CHCl_3$–MeOH–aq NH_4OH, 95:4:1) furnished **2** (67 mg, 75%): amorphous solid; $[\alpha]_D$ +9 (*c* 1.5, $CHCl_3$), lit.[10] $[\alpha]_D$ +7 (*c* 0.1, $CHCl_3$), lit.[11] $[\alpha]_D$ +18 (*c* 0.1, EtOH); ^1H NMR (500 MHz) δ 3.92 (1H, dd, *J*=8.5, 7.5 Hz), 3.86 (1H, dd, *J*=5, 3.5 Hz), 3.73 (1H, ddd, *J*=7.5, 7.5, 3.5 Hz), 3.65 (1H, m), 3.51 (1H, dd, *J*=8.5, 7 Hz), 2.00 (1H, br s, OH), 1.70–1.60 (2H, m), 1.45–1.20 (26H, br m), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 83.3, 71.8, 54.4 (CH), 72.4, 31.9, 29.7 (several overlapped peaks), 26.4, 22.7 (CH_2), 14.1 (CH_3); IR ν_{max} 3340 (br, OH, NH) cm^{-1}; HREIMS *m/z* (rel int.) 299.2774 (M^+, 9), 282 (26), 265 (21), 226 (17), 60 (100). Calcd for $C_{18}H_{37}NO_2$, 299.2824.

4.1.12. *N*,*O*-diacetylpachastrissamine (*N*,*O*-diacetyl-jaspine B) (16). Compound **2** was acetylated under the standard conditions (Ac_2O, Et_3N, DMAP, CH_2Cl_2, room temperature). Work-up (CH_2Cl_2) and column chromatography on silica gel (hexane–EtOAc, 1:1) yielded **16**[10,11]: amorphous solid; $[\alpha]_D$ −28.4 (*c* 1, $CHCl_3$); ^1H NMR (500 MHz) δ 5.60 (1H, br d, *J*=8.2 Hz), 5.40 (1H, dd, *J*=5.3, 3.5 Hz), 4.83 (1H, qd, *J*=8.2, 5.3 Hz), 4.09 (1H, t, *J*=8.2 Hz), 3.90 (1H, ddd, *J*=8.2, 5.3, 3.5 Hz), 3.60 (1H, t, *J*=8.2 Hz), 2.17 (3H, s), 2.00 (3H, s), 1.55–1.40 (2H, m), 1.35–1.20 (24H, br m), 0.88 (3H, t, *J*=7 Hz); ^{13}C NMR (125 MHz) δ 169.9,

169.8 (C), 81.2, 73.6, 51.4 (CH), 70.0, 31.9, 29.6 (several overlapped peaks), 26.0, 22.7 (CH_2), 23.2, 20.7, 14.1 (CH_3); IR ν_{max} 3215 (NH), 1741, 1642 (C=O) cm^{-1}; HREIMS *m/z* (rel int.) 384 ($M+H^+$, 14), 383.3034 (M^+, 4), 340 (14), 323 (27), 264 (100), 157 (30), 114 (44). Calcd for $C_{22}H_{41}NO_4$, 383.3035.

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Synthesis of 4-(3-hydroxyalkyl)pyrimidines by ring transformation reactions of 2-alkylidenetetrahydrofurans with amidines

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Abstract—Domino reactions of amidines with 2-alkylidenetetrahydrofurans, prepared by cyclization of 1,3-dicarbonyl dianions or 1,3-bis-silyl enol ethers with various dielectrophiles, provided an efficient access to 4-(3-hydroxyalkyl)pyrimidines.

1. Introduction

Functionalized pyrimidines[1] play an important role as analgesic,[1a] antihypertensive,[1b] antipyretic,[1c] and anti-inflammatory drugs,[1d] as pesticides,[1e] herbicides,[1f] and plant growth regulators.[1g] For example, the naturally occurring L-lathyrine shows a wide range of biological activity, such as pollen growth inhibition, antitumor, and hypoglycaemic activity (Fig. 1).[2] Although pyrimidine syntheses are known for a long time,[3] the development of alternative and more efficient strategies is of considerable relevance.

2-Alkylidenetetrahydrofurans represent useful synthetic building blocks.[4–6] They are, for example, available by one-pot cyclizations of free and masked 1,3-dicarbonyl dianions with 1,2-dielectrophiles.[7] 2-Alkylidenetetrahydrofurans have been functionalized by lithiation and subsequent alkylation;[8] in addition, palladium(0) catalyzed cross-coupling reactions of 2′-bromo-2-alkylidenetetrahydrofurans have been reported.[9] Recently, we have reported the synthesis of 6-bromo-3-oxoalkanoates and functionalized benzofurans by reaction of 2-alkylidenetetrahydrofurans with boron tribromide (BBr_3).[10] Furans and benzofurans have been prepared based on elimination[11] or oxidation[12] reactions of 2-alkylideneterahydrofurans. Some years ago, Detty reported the synthesis of functionalized pyrazoles by reaction of 2-alkylidenetetrahydrofurans with hydrazine.[13] Herein, we report an efficient synthesis of functionalized 6-phenyl-4-(3-hydroxypropyl)pyrimidines by transformation reactions of 2-alkylidenetetrahydrofurans with amidines. The starting materials are readily available by one-pot cyclizations developed in our laboratory.

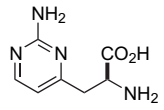
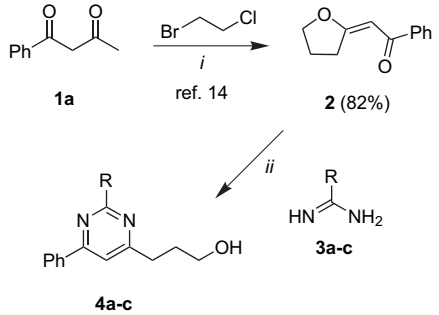


Figure 1. L-Lathyrine.

2. Results and discussion

2-(Benzoylmethylidene)tetrahydrofuran (**2**) was prepared, following our recently reported procedure,[14] by cyclization of the dianion of benzoylacetone (**1a**) with 1-bromo-2-chloroethane. The reaction of **2** with amidines **3a–c** (NEt_3, EtOH, reflux) afforded the 2-phenyl-, 2-methyl-, and 2-dimethylamino-4-(3-hydroxypropyl)pyrimidines **4a–c** (Scheme 1, Table 1).



Scheme 1. Synthesis of **4a–c**; i: (1) 2.3 equiv LDA, THF, 0 °C, 1 h, (2) $Br(CH_2)_2Cl$, −78 → 20 °C, 14 h, then reflux, 12 h; ii: NEt_3, EtOH, reflux, 12 h.

Keywords: Amidines; Heterocycles; Pyrimidines; Ring transformation; Tetrahydrofurans.
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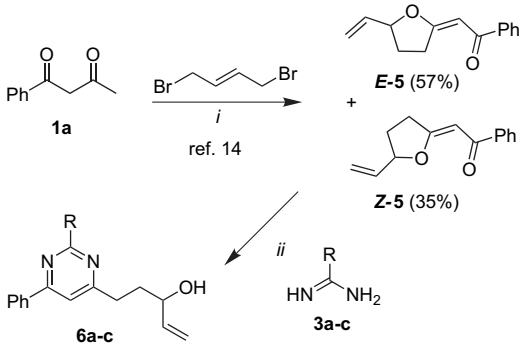
Table 1. Products and yields

4	R	% (4)[a]
a	Ph	60
b	Me	41
c	NMe_2	56

[a] Yields of isolated products.

The cyclization of dilithiated **1a** with 1,4-dibromo-2-butene gave, again following a known protocol,[14] the novel 5-vinyl-2-alkylidenetetrahydrofuran **5** as a separable mixture of *E*/*Z* diastereomers *E*-**5** and *Z*-**5**. The reaction of amidines **3a–c** with *E*-**5** afforded the functionalized pyrimidines **6a–c** (Scheme 2, Table 2).



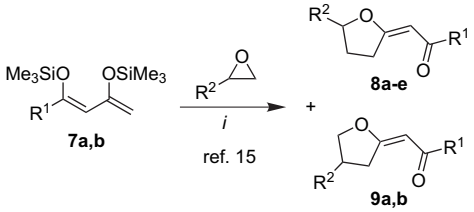
Scheme 2. Synthesis of **6a–c**; i: (1) 2.3 equiv LDA, THF, 0 °C, 1 h, (2) $BrCH_2CH{=}CHCH_2Br$, −78→20 °C, 14 h, then at 20 °C, 24 h; ii: NEt_3, EtOH, reflux, 12 h.

Table 2. Products and yields

6	R	% (6)[a]
a	Ph	76
b	Me	51
c	NMe_2	51

[a] Yields of isolated products.

The 5-alkyl- and 4-alkyl-2-alkylidenetetrahydrofurans **8a–d** and **9a**,**b** were prepared, following a recently reported procedure,[15] by $TiCl_4$ mediated cyclization of 1,3-bis-silyl enol ether **7a** (available from benzoylacetone) with various epoxides (Scheme 3, Table 3). The cyclization of **7** with 1,2-epoxypropane and 1,2-epoxybutane afforded the 4-methyl- and 4-ethyl-2-alkylidenetetrahydrofurans **9a** and **9b**, respectively; besides, a small amount of regioisomers **8a** and **8b** was isolated. The 5-chloromethyl- and 5-bromomethyl-2-alkylidenetetrahydrofurans **8c**,**d** were prepared from epichloro- and epibromohydrin, respectively. The $TiCl_4$ mediated cyclization of 1,3-bis-silyl enol ether **7b**, prepared from acetylacetone, with epibromohydrin afforded **8e**.



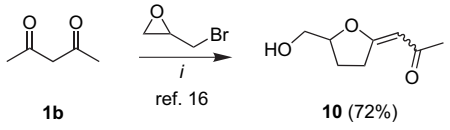
Scheme 3. Synthesis of **8a–e** and **9a**,**b**, i: $TiCl_4$ (2 equiv), CH_2Cl_2, −78→20 °C, 14 h, 20 °C, 3 h.

Table 3. Products and yields

8	9	R^1	R^2	% (8)[a]	% (9)[a]
a	**a**	Ph	Me	6	62[b]
b	**b**	Ph	Et	9[b]	65
c	**c**	Ph	CH_2Cl	54[b]	0
d	**d**	Ph	CH_2Br	56[b]	0
e	**e**	Me	CH_2Br	45	0

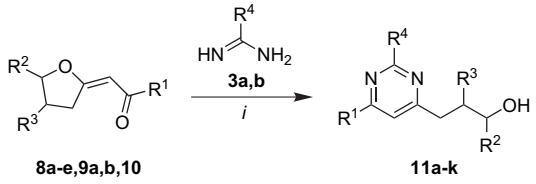
[a] Yields of isolated products.
[b] Known compound (Ref. 15)

The cyclization of dilithiated acetylacetone (**1b**) with epibromohydrin gave, following a known protocol,[16] the 5-hydroxymethyl-2-alkylidenetetrahydrofuran **10** as an inseparable mixture of *E*/*Z* diastereomers (Scheme 4).



Scheme 4. Synthesis of **10**, i: (1) NaH, *n*BuLi, THF, 0 °C, 1 h, (2) epibromohydrin, $LiClO_4$, −78→−40 °C, (3) −40 °C, 8 h, (4) −40→20 °C, (5) 20 °C, 10 h.

The reaction of 4- and 5-alkyl substituted 2-alkylidenetetrahydrofurans with amidines was studied next (Scheme 5, Table 4). The reaction of **8a** with benzamidine (**3a**) and acetamidine (**3b**) afforded the 2-phenyl- and 2-methyl-4-(3-hydroxyalkyl)pyrimidines **11a** and **11b**, respectively. Pyrimidine **11c** was prepared from **8b** and **3a**. The reaction of **3a** with **8c**,**d** gave the (4-halo-3-hydroxybutyl)pyrimidines **11d**,**e**. (3-Hydroxyalkyl)pyrimidines **11f–i** were prepared from **9a**,**b** and **3a**,**b**. The reaction of **8e** with **3a** afforded the (4-bromo-3-hydroxybutyl)pyrimidine **11j**. Likewise, pyrimidine **11k** was prepared by reaction of 5-hydroxymethyl-2-alkylidenetetrahydrofuran **10** with benzamidine (**3a**).



Scheme 5. Synthesis of **11a–k**, i: NEt_3, EtOH, reflux, 12 h.

Table 4. Products and yields

Substrate	11	R^1	R^2	R^3	R^4	% (11)[a]
8a	**a**	Ph	Me	H	Ph	45
8a	**b**	Ph	Me	H	Me	41
8b	**c**	Ph	Et	H	Ph	55
8c	**d**	Ph	CH_2Cl	H	Ph	85
8d	**e**	Ph	CH_2Br	H	Ph	79
9a	**f**	Ph	H	Me	Ph	50
9a	**g**	Ph	H	Me	Me	47
9b	**h**	Ph	H	Et	Ph	57
9b	**i**	Ph	H	Et	Me	42
8e	**j**	Me	CH_2Br	H	Ph	91
10	**k**	Me	CH_2OH	H	Ph	88

[a] Yields of isolated products.

In conclusion, we have reported an efficient synthesis of 4-(3-hydroxyalkyl)pyrimidines based on ring transformation reactions of amidines with 2-alkylidenetetrahydrofurans which are readily available by one-pot cyclization reactions of free and masked 1,3-dicarbonyl dianions.

3. Experimental

3.1. General

All solvents were dried by standard methods and all reactions were carried out under an inert atmosphere. For the ^{1}H and ^{13}C NMR spectra the deuterated solvents indicated were used. Chemical shifts δ are reported in parts per million relative to C*H*Cl$_3$ (^{1}H, 7.26 ppm) and CDCl$_3$ (^{13}C, 77.0 ppm) as internal standards. ^{13}C NMR spectral assignments are supported by DEPT analysis. Mass spectral data (MS) were obtained by electron ionization (EI, 70 eV), chemical ionization (CI, H_2O) or electrospray ionization (ESI). For preparative scale chromatography silica gel (60–200 mesh) was used. Melting points are uncorrected.

3.1.1. General procedure for the cyclization of 1,3-dicarbonyl dianions with 1-bromo-2-chloroethane and *trans*-1,4-dibromo-2-butene. A THF solution of LDA was prepared by addition of *n*BuLi (2.5 equiv) to a THF solution (10 mL/mmol) of diisopropylamine (2.5 equiv) at 0 °C. To the LDA solution was added the 1,3-dicarbonyl compound (1.0 equiv) at 0 °C and the solution was stirred at 0 °C for 1 h. To the solution was added 1-bromo-2-chloroethane (or *trans*-1,4-dibromo-2-butene) (1.2 equiv) at −78 °C. Subsequently, the temperature was allowed to rise to 20 °C during 14 h and the solution was stirred at 20 °C for 24 h. To the reaction mixture was added an aqueous solution of HCl (10%, 10 mL/mmol) and the mixture was extracted with diethylether (2×10 mL/mmol) and then dichloromethane (3×10 mL/mmol). The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/EtOAc) to give 2-alkylidenetetrahydrofuran **2** (or **5**).

3.1.1.1. (Tetrahydrofuran-2(3*H*)-ylidene)acetophenone (2).[7a] Starting with benzoylacetone (2.50 g, 15.4 mmol), diisopropylamine (7.03 mL, 50 mmol), *n*BuLi (31.4 mL, 50 mmol, 15% in *n*-hexane), and 1-bromo-2-chloroethane (2.40 mL, 17 mmol) in THF (150 mL), **2a** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=75:1→1:1) as a yellow oil (2.367 g, 82%). ^{1}H NMR (CDCl$_3$, 300 MHz): δ=2.16 (quint, J=7.2 Hz, 2H, CH_2 at C-4), 3.29 (t, J=7.8 Hz, 2H, CH_2 at C-3), 4.30 (t, J=7.2 Hz, 2H, OCH_2 at C-5), 6.55 (s, 1H, HC=C), 7.40–7.52 (m, 3H, 3×CH of Ph), 7.86–7.92 (m, 2H, 2×CH of Ph). ^{13}C NMR (CDCl$_3$, 75 MHz): δ_C=23.5 (C-3), 31.2 (C-4, CH_2), 71.7 (C-5, OCH_2), 94.9 (H*C*=C–O), 127.4 (2C), 128.1 (2C), 131.5 (CH of Ph), 139.6 (C of Ph), 179.0 (O–*C*=CH), 189.9 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=2985 (w), 2900 (w), 1656 (s), 1599 (s), 1588 (s), 1570 (s), 1447 (w), 1388 (m), 1363 (w), 1269 (w), 1283 (w), 1166 (s), 1054 (w), 1016 (m), 967 (s), 929 (m), 885 (w), 786 (w), 704 (s), 655 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=204 (4.21), 250 (3.93), 284 (4.26). MS (EI, 70 eV): *m*/*z* (%)=188 (M$^+$, 23), 77 (75), 70 (100). HRMS (ESI): calcd for $C_{12}H_{12}O_2$ [M$^+$]: 188.0832; found: 188.0822. Anal. Calcd for $C_{12}H_{12}O_2$ (188.226): C 76.57, H 6.43. Found: C 76.31, H 5.95.

3.1.1.2. (5-Vinyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (5). Starting with benzoylacetone (1.622 g, 10 mmol), diisopropylamine (3.51 mL, 25 mmol), *n*BuLi (10 mL, 25 mmol, 2.5 M in *n*-hexane), and *trans*-1,4-dibromo-2-butene (2.781 g, 13 mmol) in THF (100 mL), *E*-**5** (1.221 g, 57%) and *Z*-**5** (0.747 g, 35%) were isolated after chromatography (silica gel, *n*-hexane/EtOAc=75:1→1:1) as a yellow solid and dark yellow oil, respectively (combined yield: 92%). *E*-**5**: mp=60.5 °C. ^{1}H NMR (CDCl$_3$, 300 MHz): δ=1.86–1.98 (m, 1H, CH_2 at C-4), 2.28–2.40 (m, 1H, CH_2 at C-4), 3.14–3.26 (m, 1H, CH_2 at C-3), 3.40–3.51 (m, 1H, CH_2 at C-3), 4.87–4.94 (m, 1H, OCH at C-5), 5.25–5.41 (m, 2H, CH_2=CH), 5.85–5.96 (m, 1H, C*H*=CH_2), 6.57 (m, 1H, C*H*=C), 7.40–7.52 (m, 3H, 3×CH of Ph), 7.86–7.92 (m, 2H, 2×CH of Ph). ^{13}C NMR (CDCl$_3$, 50 MHz): δ_C=29.7 (C-3), 31.4 (C-4, CH_2), 84.1 (C-5, OCH), 95.1 (H*C*=C–O), 117.6 (*C*H_2=CH), 127.6 (2C), 128.3 (2C, CH of Ph), 131.7 (*C*H=CH_2), 135.9 (CH of Ph), 139.8 (C of Ph), 178.5 (O–*C*=CH), 190.1 (C=O). IR (KBr, cm^{-1}): $\tilde{\nu}$=3059 (w), 2941 (w), 1640 (s), 1591 (s), 1566 (s), 1426 (w), 1382 (m), 173 (s), 997 (m), 974 (m), 932 (m), 900 (s), 841 (w), 790 (w), 705 (m). UV–vis (CH_3CN, nm): λ_{max} (log ε)=204 (4.16), 251 (3.92), 284 (4.27). MS (EI, 70 eV): *m*/*z* (%)=214 (M$^+$, 77), 160 (7), 146 (67), 137 (12), 129 (1), 120 (1), 108 (2), 105 (100), 77 (60). Anal. Calcd for $C_{14}H_{14}O_2$ (214.253): C 78.48, H 7.59; Found: C 78.33, H 7.63. *Z*-**5**: ^{1}H NMR (CDCl$_3$, 300 MHz): δ=1.85–2.01 (m, 2H, CH_2 at C-4), 2.59 (t, J=7.2 Hz, 2H, CH_2 at C-3), 4.18–4.25 (m, 1H, OCH at C-5), 5.14–5.31 (m, 2H, CH_2=CH), 5.84–5.95 (m, 1H, C*H*=CH_2), 6.20 (s, 1H, CH=C), 7.42–7.60 (m, 3H, 3×CH of Ph), 7.86–7.89 (m, 2H, 2×CH of Ph). ^{13}C NMR (CDCl$_3$, 75 MHz): δ_C=31.9 (C-3), 34.8 (C-4, CH_2), 71.3 (C-5, OCH), 95.8 (*C*H=C–O), 114.3 (*C*H_2=CH), 126.4 (2C), 128.1 (2C), 131.8 (CH of Ph), 134.0 (C of Ph), 140.2 (*C*H=CH_2), 181.8 (O–*C*=CH), 197.3 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=3069 (w), 2979 (w), 2927 (w), 1716 (w), 1604 (s), 1572 (s), 1490 (m), 1456 (m), 1424 (m), 1360 (w), 1320 (w), 1298 (m), 1271 (m), 1181 (w), 1160 (w), 1150 (w), 1118 (w), 1055 (w), 1025 (w), 994 (m), 927 (m), 767 (m), 694 (m). UV–vis (CH_3CN, nm): λ_{max} (log ε)=246 (3.85), 299 (4.00). MS (EI, 70 eV): *m*/*z* (%)=214 (M$^+$, 100), 160 (7), 147 (81), 137 (10), 129 (1), 120 (1), 105 (99), 77 (82). HRMS (ESI): calcd for $C_{14}H_{14}O_2$ [M$^+$]: 214.0988; found: 214.0983.

3.1.2. General procedure for the [3+2] cyclization of 1,3-bis-silyl enol ethers with epoxides. To a CH_2Cl_2 solution (10 mL/mmol) of 1,3-bis-silyl enol ether **7a**,**b** (1.0 equiv) and the epoxide (1.2 equiv) [in the presence of molecular sieves (4 Å)], was added $TiCl_4$ (2.4 equiv) at −78 °C. Subsequently, the temperature was allowed to rise to 20 °C during 14 h and the solution was stirred for 3 h at 20 °C. The molecular sieves were filtered-off and washed with CH_2Cl_2. To the solution was added a saturated aqueous solution of $NaHCO_3$, the organic layer was separated and the aqueous layer was repeatedly extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was

concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/EtOAc) to give the 2-alkylidenetetrahydrofurans **8a–e** and **9a**,**b**.

Compounds **8a**,**9a**: Starting with **7a** (6.131 g, 20 mmol), propeneoxide (1.7 mL, 24 mmol) and $TiCl_4$ (5.3 mL, 48 mmol) in CH_2Cl_2 (200 mL), **9a** (2.487 g, 62%) and **8a** (0.232 g, 6%) were isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as light brown oils.

3.1.2.1. (4-Methyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (9a).[15] ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.11 (d, J=6.6 Hz, 3H, CH_3), 2.45–2.56 (m, 1H, CH at C-4), 2.61–2.70 (m, 2H, CH_2 at C-3), 3.55–3.62 (m, 1H, OCH_2 at C-5), 4.07–4.15 (m, 1H, OCH_2 at C-5), 6.20 (d, J=1.8 Hz, 1H, CH=C), 7.43–7.54 (m, 3H, 3×CH of Ph), 7.87–7.91 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=17.4 (CH_3), 31.7 (C-4, CH), 39.4 (C-3, CH_2), 77.7 (C-5, OCH_2), 95.4 (*C*H=C-O), 127.6 (2C), 128.2 (2C), 131.6 (CH of Ph), 139.7 (C of Ph), 179.1 (O–*C*=CH), 190.2 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=2967 (w), 1607 (s), 1457 (m), 1418 (m), 1267 (m), 845 (w), 766 (m), 694 (m). MS (EI, 70 eV): *m*/*z* (%)=202 (M^+, 70), 187 (100), 162 (13), 147 (20), 125 (22), 105 (45). Anal. Calcd for $C_{13}H_{14}O_2$ (202.249): C 77.20, H 6.98. Found: C 77.03, H 7.16.

3.1.2.2. (5-Methyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (8a). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.57 (d, J=6.6 Hz, 3H, CH_3), 1.96–2.08 (m, 1H, CH_2 at C-4), 2.18–2.29 (m, 1H, CH_2 at C-4), 3.54–3.61 (m, 2H, CH_2 at C-3), 4.32–4.41 (m, 1H, OCH at C-5), 6.20 (d, J=1.8 Hz, 1H, CH=C), 7.38–7.54 (m, 3H, 3×CH of Ph), 7.87–7.91 (m, 2H, 2×CH of Ph). IR (neat, cm^{-1}): $\tilde{\nu}$=2967 (w), 1721 (w), 1681 (m), 1607 (s), 1453 (m), 1414 (m), 1269 (m), 1184 (w), 1079 (w), 1006 (w), 847 (w), 768 (m), 694 (m). MS (EI, 70 eV): *m*/*z* (%)=202 (M^+, 10), 187 (7), 162 (43), 147 (51), 105 (100), 91 (10), 77 (66), 69 (93). HRMS (ESI): calcd for $C_{13}H_{14}O_2$ [M^+]: 202.0988; found: 202.0994.

Compounds **8b**,**9b**: Starting with **7a** (6.131 g, 20 mmol), 1,2-epoxybutane (2.1 mL, 24 mmol) and $TiCl_4$ (5.3 mL, 48 mmol) in CH_2Cl_2 (200 mL), **9b** (2.792 g, 65%) and **8b** (0.402 g, 9%) were isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as light brown oils.

3.1.2.3. (4-Ethyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (9b). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.86 (t, J=7.5 Hz, 3H, CH_2CH_3), 1.29–1.43 (m, 2H, CH_2CH_3), 1.75–1.89 (m, 1H, CH at C-4), 2.31–2.37 (m, 1H, CH_2 at C-3), 2.44–2.52 (m, 1H, CH_2 at C-3), 3.63–4.01 (dm, 2H, OCH_2 at C-5), 6.08 (d, J=1.8 Hz, 1H, CH=C), 7.30–7.44 (m, 3H, 3×CH of Ph), 7.74–7.78 (m, 2H, 2×CH of Ph). IR (neat, cm^{-1}): $\tilde{\nu}$=2964 (m), 2880 (w), 1721 (w), 1607 (s), 1457 (s), 1266 (s), 1184 (m), 1150 (w), 1080 (m), 1040 (m), 1006 (w), 925 (w), 847 (m), 767 (m), 694 (m). MS (EI, 70 eV): *m*/*z* (%)=216 (M^+, 10), 187 (100), 105 (72), 77 (70), 69 (91). HRMS (ESI): calcd for $C_{14}H_{16}O_2$ [M^+]: 216.1145; found: 216.1142.

3.1.2.4. (5-Ethyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (8b).[15] ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.98 (t, J=7.5 Hz, 3H, CH_2CH_3), 1.43–1.55 (m, 2H, CH_2CH_3), 1.68–1.82 (m, 1H, CH_2 at C-4), 2.21–2.32 (m, 1H, CH_2 at C-4), 2.50 (dt, J=8.1, 0.6 Hz, 1H, CH_2 at C-3), 2.66–2.81 (m, 1H, CH_2 at C-3), 4.32–4.38 (m, 1H, OCH at C-5), 6.82 (d, J=1.8 Hz, 1H, CH=C), 7.34–7.50 (m, 3H, 3×CH of Ph), 7.81–7.85 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=9.7 (CH_2CH_3), 27.8 (CH_2CH_3), 28.6 (C-3), 31.8 (C-4, CH_2), 85.6 (C-5, OCH), 94.6 (*C*H=C-O), 127.4 (2C), 128.2 (2C), 131.5 (CH of Ph), 139.8 (C of Ph), 179.1 (O–*C*=CH), 190.2 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=3084 (w), 3061 (w), 3030 (w), 2966 (s), 2933 (s), 2879 (m), 2855 (m), 1654 (s), 1587 (s), 1571 (m), 1456 (m), 1447 (m), 1386 (s), 1167 (s). MS (EI, 70 eV): *m*/*z* (%)=216 (M^+, 70), 201 (100), 176 (13), 161 (20), 139 (22), 119 (45). The exact molecular mass *m*/*z*=216.1150±2 ppm [M^+] for $C_{14}H_{16}O_2$ was confirmed by HRMS (EI, 70 eV). Anal. Calcd for $C_{14}H_{16}O_2$ (216.276): C 77.75, H 7.46. Found: C 77.48, H 7.60.

3.1.2.5. (5-Chloromethyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (8c).[15] Starting with **7a** (6.131 g, 20 mmol), epichlorohydrin (1.9 mL, 24 mmol) and $TiCl_4$ (5.3 mL, 48 mmol) in CH_2Cl_2 (200 mL), **8c** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow oil (2.538 g, 54%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.00–2.12 (m, 1H, CH_2 at C-4), 2.30–2.41 (m, 1H, CH_2 at C-4), 3.19–3.31 (m, 1H, CH_2 at C-3), 3.43–3.55 (m, 1H, CH_2 at C-3), 3.69 (d, J=5.7 Hz, 2H, CH_2–Cl), 4.70–4.80 (m, 1H, OCH at C-5), 6.59 (t, J=1.5 Hz, 1H, CH=C), 7.40–7.53 (m, 3H, 3×CH of Ph), 7.89–7.92 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=26.6 (C-3), 31.2 (C-4, CH_2), 45.4 (CH_2–Cl), 82.1 (C-5, OCH), 95.3 (*C*H=C–O), 127.4 (2C), 128.2 (2C), 131.7 (CH of Ph), 139.4 (C of Ph), 177.7 (O–*C*=CH), 190.1 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=3099 (w), 3083 (w), 3055 (w), 2984 (w), 2919 (w), 1663 (s), 1598 (s), 1583 (s), 1566 (s), 1383 (m), 1374 (m), 1167 (s). MS (EI, 70 eV): *m*/*z* (%)=236 (M^+, 100), 159 (52), 105 (46), 77 (33), 69 (48). The exact molecular mass *m*/*z*=236.0604±2 ppm [M^+] for $C_{13}H_{13}ClO_2$ was confirmed by HRMS (EI, 70 eV). Anal. Calcd for $C_{13}H_{13}ClO_2$ (236.694): C 65.97, H 5.54. Found: C 66.12, H 5.43.

3.1.2.6. (5-Bromomethyltetrahydrofuran-2(3*H*)-ylidene)acetophenone (8d).[15] Starting with **7a** (6.131 g, 20 mmol), epibromohydrin (2.0 mL, 24 mmol) and $TiCl_4$ (5.3 mL, 48 mmol) in CH_2Cl_2 (200 mL), **8d** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow oil (3.149 g, 56%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.98–2.11 (m, 1H, CH_2 at C-4), 2.34–2.45 (m, 1H, CH_2 at C-4), 3.19–3.30 (m, 1H, CH_2 at C-3), 3.43–3.52 (m, 1H, CH_2 at C-3), 3.53–3.57 (m, 2H, CH_2–Br), 4.69–4.78 (m, 1H, OCH at C-5), 6.58 (t, J=1.5 Hz, 1H, CH=C), 7.40–7.52 (m, 3H, 3×CH of Ph), 7.88–7.92 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=27.8 (C-3), 31.2 (C-4, CH_2), 33.4 (CH_2–Br), 81.9 (C-5, OCH), 95.3 (*C*H=C–O), 127.4 (2C), 128.2 (2C), 131.7 (CH of Ph), 139.4 (C of Ph), 177.6 (O–*C*=CH), 190.1 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=3098 (w), 3054 (w), 3026 (w), 2981 (w), 2945 (w), 2917 (w), 1660 (s), 1596 (s), 1567 (s), 1457 (m), 1433 (m), 1380 (s), 1165 (s). MS (EI, 70 eV): *m*/*z* (%)=280 (M^+, 60), 203 (24), 180 (18), 147 (35), 122 (33), 105 (100). The exact molecular mass *m*/*z*=280.0099±2 ppm [M^+] for $C_{13}H_{13}BrO_2$ was

confirmed by HRMS (EI, 70 eV). Anal. Calcd for $C_{13}H_{13}BrO_2$ (281.145): C 55.54, H 4.66. Found: C 55.22, H 4.36.

3.1.2.7. 1-(5-Bromomethyldihydrofuran-2(3*H*)-ylidene)propan-2-one (8e). Starting with **7b** (7.334 g, 30 mmol), epibromohydrin (2.98 mL, 36 mmol) and $TiCl_4$ (7.91 mL, 72 mmol) in CH_2Cl_2 (150 mL), **8e** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a light brown oil (2.931 g, 45%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.92–2.05 (m, 1H, CH_2 at C-4), 2.14 (s, 3H, CH_3), 2.23–2.34 (m, 1H, CH_2 at C-4), 3.00–3.12 (m, 1H, CH_2 at C-3), 3.28–3.38 (m, 1H, CH_2 at C-3), 3.39–3.58 (m, 2H, CH_2–Br), 4.61–4.70 (m, 1H, OCH at C-5), 5.83 (t, J=1.5 Hz, 1H, CH=C). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=28.2 (C-3, CH_2), 31.3 (CH_3), 31.6 (C-4, CH_2), 34.0 (CH_2–Br), 82.1 (C-5, OCH), 99.4 (*C*H=C–O), 176.2 (O–*C*=CH), 198.4 (C=O). IR (neat, cm^{-1}): $\tilde{\nu}$=2960 (m), 2928 (m), 1704 (s), 1617 (s), 1591 (s), 1421 (s), 1365 (s), 1303 (s), 1252 (s), 1150 (s), 1086 (m), 1054 (m), 1020 (m), 979 (w), 951 (w), 662 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=260 (3.86). MS (EI, 70 eV): *m*/*z* (%)=220 (M^+ [^{81}Br], 9), 218 (M^+ [^{79}Br], 9), 205 (26), 203 (26), 139 (2), 123 (2), 95 (5), 85 (14), 69 (100). HRMS (ESI): calcd for $C_8H_{11}BrO_2$ [M^+]: 219.9916 (^{81}Br), 217.9937 (^{79}Br); found: 219.9921 (^{81}Br), 217.9942 (^{79}Br).

3.1.3. Cyclization of 1,3-dicarbonyl dianions with epibromohydrin: 1-(dihydro-5-(hydroxymethyl)furan-2(3*H*)-ylidene)propan-2-one (10). The synthesis of **10** has been previously reported.[16]

3.1.4. General procedure for synthesis of functionalized pyrimidines. To an ethanol (20 mL/mmol) solution of (tetrahydrofuran-2(3*H*)-ylidene)acetophenone (**2**, **5**, **8a**–**d**, **9a**,**b**, or **10**) (1 equiv) were added the amidine (**3a**–**c**) (10 equiv) and triethylamine (10 equiv) at 20 °C. The reaction mixture was heated and stirred for 12 h at 60 °C. After cooling, to the reaction mixture was added water (30 mL/mmol), and extracted with dichloromethane repeatedly. The combined organic extracts were dried (Na_2SO_4), filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (silica gel, *n*-hexane/EtOAc) to give (6-phenylpyrimidin-4-yl) alcohols (**4a**–**c**, **6a**–**c** or **11a**–**k**).

3.1.4.1. 3-(2,6-Diphenylpyrimidin-4-yl)propan-1-ol (4a). Starting with **2** (0.100 g, 0.53 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.928 g, 5.31 mmol) and NEt_3 (1.1 mL, 8.0 mmol) in ethanol (10 mL), **4a** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=20:1→1:1) as a pale yellow solid (0.92 g, 60%), mp=80.0 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.13 (quint, J=6.45 Hz, 2H, CH_2), 3.05 (t, J=6.9 Hz, 2H, CH_2), 3.20 (br s, 1H, OH), 3.80 (t, J=6.0 Hz, 2H, CH_2OH), 7.46–7.55 (m, 7H, CH, 6×CH of Ph), 8.20–8.24 (m, 2H, 2×CH of Ph), 8.54–8.57 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C=31.0, 35.0 (CH_2), 62.2 (*C*H_2OH), 113.9 (*C*H=C–N), 127.4 (2C), 128.5 (2C), 128.7 (2C), 129.0 (2C), 130.8, 131.0 (CH of Ph), 137.2, 138.0 (C of Ph), 164.3 (*C*–Ph), 164.4 (N=C–N), 170.8 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3372 (br), 3088 (m), 3064 (m), 3038 (m), 3006 (w), 2933 (s), 2873 (m, C–H), 1770 (w), 1731 (m), 1598 (s), 1571 (s), 1533 (s), 1497 (m), 1445 (m), 1425 (m), 1400 (m), 1374 (s), 1317 (m), 1290 (m), 1221 (m), 1177 (m), 1061 (s), 1031 (s), 934 (m), 911 (w), 879 (w), 837 (w), 778 (w), 751 (s), 695 (s), 667 (w), 636 (m), 582 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=206 (4.52), 256 (4.52). MS (EI, 70 eV): *m*/*z* (%)=290 (M^+, 1), 273 (6), 259 (11), 246 (100), 219 (1), 205 (15), 164 (14), 103 (45), 77 (29). The exact molecular mass *m*/*z*=290.1419±2 ppm [M^+] for $C_{19}H_{18}N_2O$ was confirmed by HRMS (EI, 70 eV).

3.1.4.2. 3-(2-Methyl-6-phenylpyrimidin-4-yl)propan-1-ol (4b). Starting with **2** (0.100 g, 0.53 mmol), acetamidine hydrochloride (**3b**) (0.527 g, 5.3 mmol) and NEt_3 (1.1 mL, 8.0 mmol) in ethanol (10 mL), **4b** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=50:1→1:1) as a pale yellow oil (0.049 g, 41%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.02 (quint, J=6.3 Hz, 2H, CH_2), 2.77 (s, 3H, CH_3), 2.95 (t, J=6.9 Hz, 2H, CH_2), 3.74 (t, J=5.7 Hz, 2H, OCH_2), 7.40 (s, 1H, CH), 7.48–7.51 (m, 3H, 3×CH of Ph), 8.04–8.07 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=26.0 (CH_3), 31.0, 35.1 (CH_2), 62.1 (OCH_2), 113.1 (*C*H=C–N), 127.2 (2C), 128.2, 128.9 (2C, CH of Ph), 137.0 (C of Ph), 164.5 (*C*–Ph), 167.7 (N=C–N), 170.2 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3340 (s), 3065 (m), 2933 (s), 2870 (m), 1585 (s), 1535 (s), 1441 (s), 1396 (s), 1339 (m), 1285 (m), 1221 (w), 1179 (w), 1118 (w), 1063 (s), 1005 (w), 921 (w), 874 (w), 753 (m), 695 (s), 650 (w), 622 (w), 588 (w), 541 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=203 (4.40), 247 (3.97), 275 (4.07), 322 (3.50). MS (EI, 70 eV): *m*/*z* (%)=228 (M^+, 1), 211 (4), 197 (8), 184 (100), 128 (3), 114 (2), 102 (8), 77 (6). HRMS (ESI): calcd for $C_{14}H_{16}N_2O$ ([M+1]$^+$): 229.13409; found: 229.13331.

3.1.4.3. 3-(2-Dimethylamino-6-phenylpyrimidin-4-yl)-propan-1-ol (4c). Starting with **2** (0.100 g, 0.53 mmol), 1,1-dimethylguanidine sulfate (**3c**) (1.489 g, 5.3 mmol) and NEt_3 (1.84 mL, 13.3 mmol) in ethanol (10 mL), **4c** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=50:1→1:1) as a yellow oil (0.076 g, 56%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.00 (quint, J=6.0 Hz, 2H, CH_2), 2.85 (t, J=6.6 Hz, 2H, CH_2), 3.26 (s, 6H, 2×CH_3), 3.75 (t, J=6.0 Hz, 2H, CH_2OH), 6.82 (s, 1H, CH=C), 7.43–7.48 (m, 3H, 3×CH of Ph), 8.04–8.08 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=30.3, 35.4 (CH_2), 37.3 (2C, CH_3), 62.5 (OCH_2), 104.1 (*C*H=C–N), 127.1 (2C), 128.6 (2C), 130.3 (CH of Ph), 137.9 (C of Ph), 162.2 (N=C–N), 164.7 (*C*–Ph), 170.6 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3365 (br), 2927 (w), 1670 (w), 1645 (w), 1563 (s), 1494 (w), 1448 (m), 1408 (m), 1368 (m), 1326 (w), 1246 (w), 1223 (w), 1181 (w), 1149 (w), 1117 (w), 1066 (m), 1027 (w), 770 (w), 695 (w). MS (EI, 70 eV): *m*/*z* (%)=257 (M^+, 8), 242 (2), 227 (4), 213 (100), 198 (6), 184 (4), 170 (9), 128 (4), 114 (2), 114 (2), 105 (12), 77 (10).

3.1.4.4. 5-(2,6-Diphenylpyrimidin-4-yl)pent-1-en-3-ol (6a). Starting with **5** (0.100 g, 0.467 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.815 g, 4.67 mmol), NEt_3 (0.7 mL, 4.67 mmol) in ethanol (10 mL), **6a** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a pale yellow solid (0.112 g, 76%), mp=85 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.01–2.20 (m, 2H, CH_2), 3.00–3.11 (m, 2H, CH_2), 3.59 (br s,

1H, OH), 4.30 (q, J=6.0 Hz, 1H, OCH), 5.16 (dt, J=10.4, 1.5 Hz, 1H, CH_2=CH), 5.33 (dt, J=17.2, 1.5 Hz, 1H, CH_2=CH), 5.91–6.01 (m, 1H, CH=CH_2), 7.41 (s, 1H, CH), 7.43–7.57 (m, 6H, 6×CH of Ph), 8.19–8.23 (m, 2H, 2×CH of Ph), 8.54–8.56 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=33.8, 34.9 (CH_2), 72.0 (OCH), 113.6 (*C*H=C–N), 114.6 (*C*H_2=CH), 127.1 (2C), 128.3 (2C), 128.4 (2C), 128.8 (2C), 130.5, 130.7 (CH of Ph), 136.9, 137.7 (C of Ph), 140.8 (*C*H=CH_2), 163.9 (*C*–Ph), 164.0 (N=C–N), 170.6 (N–*C*=CH). IR (KBr, cm^{-1}): $\tilde{\nu}$=3343 (br), 3064 (w), 2921 (w), 1571 (s), 1535 (s), 1373 (s), 926 (m), 752 (m), 697 (s). UV–vis (CH_3CN, nm): λ_{max} (log ε)=204 (4.56), 256 (4.54). MS (EI, 70 eV): *m*/*z* (%)=316 (M$^+$, 12), 299 (10), 285 (3), 271 (1), 259 (28), 246 (100), 103 (54), 77 (80). HRMS (ESI): calcd for $C_{21}H_{20}N_2O$ [M$^+$]: 315.1492; found: 315.1491. Anal. Calcd for $C_{21}H_{20}N_2O$ (316.402): C 79.72, H 6.37, N 8.85. Found: C 79.73, H 6.57, N 8.50.

3.1.4.5. 5-(2-Methyl-6-phenylpyrimidin-4-yl)pent-1-en-3-ol (6b). Starting with **5** (0.100 g, 0.467 g), acetamidine hydrochloride (**3b**) (0.465 g, 4.67 mmol) and NEt_3 (0.7 mL, 4.67 mmol) in ethanol (100 mL), **6b** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a pale yellow solid (0.061 g, 51%), mp=61.9 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.94–2.06 (m, 2H, CH_2), 2.77 (s, 3H, CH_3), 2.95 (dd, J=7.2, 1.5 Hz, 2H, CH_2), 4.19 (br s, 1H, OH), 4.21–4.27 (m, 1H, OCH), 5.13 (dt, J=10.5, 1.5 Hz, 1H, CH_2=CH), 5.30 (dt, J=17.1, 1.5 Hz, 1H, CH_2=CH), 5.87–5.99 (m, 1H, CH=CH_2), 7.39 (s, 1H, CH), 7.46–7.51 (m, 3H, 3×CH of Ph), 8.03–8.06 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=26.0 (CH_3), 33.9, 35.2 (CH_2), 72.0 (OCH), 113.1 (*C*H=C–N), 114.6 (*C*H_2=CH), 127.2 (2C), 128.9 (2 C), 130.7 (CH of Ph), 137.0 (C of Ph), 140.9 (*C*H=CH_2), 164.4 (*C*–Ph), 167.7 (N=C–N), 170.2 (N–*C*=CH). IR (KBr, cm^{-1}): $\tilde{\nu}$=3253 (br), 3091 (w), 3065 (w), 2921 (w), 2853 (w), 1583 (s), 1539 (s), 1497 (w), 1446 (m), 1404 (s), 1368 (m), 1326 (w), 1122 (m), 1055 (w), 998 (m), 925 (m), 862 (w), 785 (w), 743 (w), 692 (m), 624 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=204 (4.48), 249 (4.02), 255 (4.03), 275 (4.20). MS (EI, 70 eV): *m*/*z* (%)=254 (M$^+$, 9), 237 (6), 225 (2), 209 (5), 197 (25), 184 (100), 128 (5), 114 (3), 77 (6). Anal. Calcd for $C_{16}H_{18}N_2O$ (254.332): C 75.56, H 7.13, N 11.02. Found: C 75.61, H 7.18, N 10.89.

3.1.4.6. 5-(2-Dimethylamino-6-phenylpyrimidin-4-yl)-pent-1-en-3-ol (6c). Starting with **5** (0.100 g, 0.467 mmol), 1,1-dimethylguanidine sulfate (**3c**) (1.311 g, 4.67 mmol) and NEt_3 (0.7 mL, 4.67 mmol) in ethanol (10 mL), **6c** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a pale yellow oil (0.067 g, 51%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.89–1.98 (m, 1H, CH_2), 1.99–2.11 (m, 1H, CH_2), 2.79–2.93 (m, 2H, CH_2), 3.27 (s, 6H, 2×NCH_3), 4.25–4.28 (m, 1H, OCH), 5.13 (dt, J=10.5, 1.5 Hz, 1H, CH_2=CH), 5.31 (dt, J=17.1, 1.5 Hz, 1H, CH_2=CH), 5.87–5.98 (m, 1H, CH=CH_2), 6.82 (s, 1H, CH), 7.44–7.48 (m, 3H, 3×CH of Ph), 8.04–8.06 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=34.0, 34.6 (CH_2), 37.3 (NCH_3), 72.2 (OCH), 104.1 (*C*H=C–N), 114.3 (*C*H_2=CH), 127.1 (2C), 128.6 (2C), 130.3 (CH of Ph), 137.9 (C of Ph), 141.2 (*C*H=CH_2), 162.1 (N=C–N), 164.6 (*C*–Ph), 170.6 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3403 (br), 2926 (m), 2859 (w), 1680 (w), 1645 (w), 1562 (s), 1497 (w), 1445 (w), 1408 (m), 1368 (m), 1320 (w), 1245 (w), 1223 (w), 1180 (w), 1117 (w), 1067 (w), 1026 (w), 995 (w), 922 (w), 770 (w), 694 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=254 (4.27), 337 (3.45). MS (EI, 70 eV): *m*/*z* (%)=283 (M$^+$, 15), 226 (9), 213 (100), 198 (5), 184 (2), 105 (40), 77 (34). HRMS (ESI): calcd for $C_{17}H_{21}N_3O$ [M$^+$]: 283.16846; found: 283.16879.

3.1.4.7. 4-(2,6-Diphenylpyrimidin-4-yl)-butan-2-ol (11a). Starting with **8a** (0.100 g, 0.49 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.857 g, 4.9 mmol) and NEt_3 (0.67 mL, 4.9 mmol) in ethanol (5 mL), **11a** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow oil (0.067 g, 45%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.28 (d, J=6.6 Hz, 3H, CH_3), 1.92–2.03 (m, 2H, CH_2), 2.93–3.07 (m, 2H, CH_2), 3.90–3.99 (m, 1H, OCH), 7.41–7.55 (m, 7H, CH=C, 6×CH of Ph), 8.20–8.24 (m, 2H, 2×CH of Ph), 8.53–8.57 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=23.1 (CH_3), 34.0, 37.0 (CH_2), 66.6 (OCH), 113.1 (*C*H=C–N), 126.2 (2C), 127.9 (2C), 128.0 (2C), 128.3 (2C), 130.1, 130.5 (CH of Ph), 136.7, 137.7 (C of Ph), 163.2 (*C*–Ph), 163.7 (N=C–N), 169.2 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3336 (br), 3061 (w), 2956 (m), 2927 (m), 2873 (w), 1633 (w), 1575 (s), 1536 (s), 1498 (w), 1451 (m), 1374 (s), 1276 (w), 1177 (w), 1112 (m), 1077 (m), 1033 (m), 753 (m), 697 (s). UV–vis (CH_3CN, nm): λ_{max} (log ε)=207 (4.48), 256 (4.52). MS (EI, 70 eV): *m*/*z* (%)=304 (M$^+$, 1), 287 (5), 272 (4), 259 (15), 245 (100), 231 (19), 227 (1), 143 (7), 128 (6), 114 (7), 104 (26), 77 (14). Anal. Calcd for $C_{20}H_{20}N_2O$ (304.391): C 78.92, H 6.62, N 9.20. Found: C 78.83, H 6.49, N 9.12.

3.1.4.8. 4-(2-Methyl-6-phenylpyrimidin-4-yl)-butan-2-ol (11b). Starting with **8b** (0.100 g, 0.49 mmol), acetamidine hydrochloride (**3b**) (0.488 g, 4.9 mmol) and NEt_3 (0.67 mL, 4.9 mmol) in ethanol (5 mL), **11b** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=50:1→EtOAc), as a pale yellow oil (0.049 g, 41%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.24 (d, J=6.3 Hz, 3H, CH_3), 1.70–1.79 (m, 1H, CH_2), 1.97–2.10 (m, 1H, CH_2), 2.35–2.49 (m, 2H, CH_2), 2.77 (s, 3H, CH_3), 3.83–3.93 (m, 1H, OCH), 7.40 (s, 1H, CH=C), 7.48–7.51 (m, 3H, 3×CH of Ph), 8.04–8.08 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=20.4, 25.4 (CH_3), 32.5, 36.8 (CH_2), 66.0 (OCH), 112.6 (*C*H=C–N), 126.7 (2C), 127.7, 128.3 (2C, CH of Ph), 136.3 (C of Ph), 163.7 (*C*–Ph), 167.0 (N=C–N), 170.8 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3339 (br), 3063 (w), 2965 (m), 2932 (m), 2878 (w), 1606 (s), 1531 (s), 1452 (m), 1421 (m), 1377 (m), 1326 (s), 1287 (s), 1180 (w), 1119 (w), 1075 (m), 1041 (m), 741 (s), 698 (m), 649 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=242 (3.96), 325 (4.18). MS (EI, 70 eV): *m*/*z* (%)=242 (M$^+$, 98), 227 (1), 225 (2), 210 (2), 197 (5), 183 (100), 169 (17), 148 (5), 105 (14), 77 (19), 57 (22). Anal. Calcd for $C_{15}H_{18}N_2O$ (242.316): C 74.35, H 7.49, N 11.56. Found: C 73.95, H 7.21, N 11.16.

3.1.4.9. 1-(2,6-Diphenylpyrimidin-4-yl)pentan-3-ol (11c). Starting with **8b** (0.100 g, 0.46 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.808 g, 4.6 mmol) and NEt_3 (0.64 mL, 4.6 mmol) in ethanol (5 mL), **11c** was

isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow oil (0.081 g, 55%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.25 (t, J=7.2 Hz, 3H, CH_3), 1.51–1.63 (m, 2H, CH_2), 1.92–2.05 (m, 2H, CH_2), 2.92 (t, J=7.8 Hz, 2H, CH_2), 3.81–3.91 (m, 1H, OCH), 7.37–7.53 (m, 7H, CH=C, 6×CH of Ph), 8.19–8.22 (m, 2H, 2×CH of Ph), 8.57–8.60 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=23.5 (CH_3), 24.8, 37.8, 38.6 (CH_2), 67.7 (OCH), 113.4 (*C*H=C–N), 127.1 (2C), 128.3 (2C), 128.4 (2C), 128.8 (2C), 130.4, 130.6 (CH of Ph), 137.2, 138.1 (C of Ph), 163.7 (*C*–Ph), 164.2 (N=C–N), 171.1 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3382 (br), 3064 (w), 2974 (s), 2931 (m), 2865 (s), 2810 (w), 1721 (m), 1575 (s), 1532 (s), 1493 (m), 1451 (m), 1375 (s), 1314 (m), 1309 (m), 1278 (s), 1172 (m), 1118 (s), 1077 (m), 1029 (w), 921 (w), 841 (w), 746 (s), 697 (s), 637 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=256 (4.41). MS (EI, 70 eV): *m*/*z* (%)=318 (M^+, 1), 273 (16), 259 (14), 146 (100), 233 (2), 216 (31), 202 (5), 174 (28), 158 (5), 143 (6), 133 (16), 128 (5), 120 (17), 114 (9), 106 (14), 104 (83), 91 (6), 77 (41). Anal. Calcd for $C_{21}H_{22}N_2O$ (318.418): C 79.21, H 6.96, N 8.89. Found: C 78.99, H 7.01, N 8.64.

3.1.4.10. 1-Chloro-4-(2,6-diphenylpyrimidin-4-yl)-butan-2-ol (11d). Starting with **8c** (0.100 g, 0.42 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.725 g, 4.2 mmol) and NEt_3 (0.6 mL, 4.2 mmol) in ethanol (5 mL), **11d** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow solid (0.120 g, 85%), mp=62.6 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.28–2.36 (m, 2H, CH_2), 3.02–3.12 (m, 2H, CH_2), 3.81 (dd, J=14.7, 7.2 Hz, 1H, CH_2–Cl), 4.22 (dd, J=14.7, 9.6 Hz, 1H, CH_2–Cl), 4.87–4.94 (m, 1H, OCH), 7.37–7.54 (m, 5H, 5×CH), 7.93–7.96 (m, 2H, 2×CH), 8.19–8.22 (m, 2H, 2×CH), 8.58–8.62 (m, 2H, 2×CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=33.7, 34.0 (CH_2), 60.0 (CH_2–Cl), 79.2 (OCH_2), 113.5 (*C*H=C–N), 127.1 (2C), 128.1, 128.2 (2C), 128.3, 128.4 (2C), 128.8 (2C, CH of Ph), 137.1, 138.0 (C of Ph), 163.9 (*C*–Ph), 164.2 (N=C–N), 169.9 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3064 (w), 2970 (w), 2931 (w), 2865 (w), 1648 (m), 1575 (s), 1534 (s), 1495 (w), 1448 (w), 1374 (s), 1260 (w), 1174 (w), 1116 (m), 1072 (m), 1028 (w), 778 (m), 750 (m), 695 (s). UV–vis (CH_3CN, nm): λ_{max} (log ε)=256 (4.44). MS (EI, 70 eV): *m*/*z* (%)=302 ([M–Cl]$^+$, 15), 261 (30), 257 (14), 233 (4), 215 (100), 195 (5), 169 (3), 159 (24), 141 (4), 128 (13), 119 (3), 117 (21), 91 (22), 86 (20), 84 (37), 79 (4), 77 (16). Anal. Calcd for $C_{20}H_{19}ClN_2O$ (338.837): C 70.90, H 5.65, N 8.27. Found: C 71.17, H 6.11, N 8.25.

3.1.4.11. 1-Bromo-4-(2,6-diphenylpyrimidin-4-yl)-butan-2-ol (11e). Starting with **8d** (0.100 g, 0.36 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.629 g, 3.6 mmol) and NEt_3 (0.49 mL, 3.6 mmol in ethanol (5 mL), **11e** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow solid (0.109 g, 79%), mp=111.3 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=2.27–2.35 (m, 2H, CH_2), 3.01–3.14 (m, 2H, CH_2), 3.80 (dd, J=14.7, 7.2 Hz, 1H, CH_2–Br), 4.21 (dd, J=14.7, 9.6 Hz, 1H, CH_2–Br), 4.84–4.91 (m, 1H, OCH), 7.36–7.54 (m, 5H, 5×CH), 7.92–7.95 (m, 2H, 2×CH), 8.19–8.22 (m, 2H, 2×CH), 8.58–8.62 (m, 2H, 2×CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=33.7, 33.9 (CH_2), 60.0 (CH_2–Br), 79.2 (OCH), 113.5 (*C*H=C–N), 127.1 (2C), 128.0 (2C), 128.2 (2C), 128.27, 128.34, 128.7 (2C, CH of Ph), 137.1, 137.9 (C of Ph), 163.8 (*C*–Ph), 164.2 (N=C–N), 169.9 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3063 (w), 2944 (w), 1647 (s), 1572 (s), 1533 (s), 1495 (w), 1447 (m), 1424 (w), 1373 (s), 1327 (m), 1285 (w), 1258 (m), 1176 (w), 1082 (m), 1066 (m), 1027 (m), 991 (w), 920 (w), 909 (w), 868 (w), 779 (w), 748 (m), 694 (s). UV–vis (CH_3CN, nm): λ_{max} (log ε)=255 (4.54). MS (EI, 70 eV): *m*/*z* (%)=302 ([M–Br]$^+$, 3), 300 (11), 285 (18), 284 (68), 283 (27), 271 (21), 259 (30), 246 (100), 234 (2), 232 (1), 142 (7), 128 (4), 117 (15), 104 (30), 77 (30).

3.1.4.12. 3-(2,6-Diphenylpyrimidin-4-yl)-2-methyl-propan-1-ol (11f). Starting with **9a** (0.500 g, 2.5 mmol), benzamidine hydrochloride monohydrate (**3a**) (4.370 g, 25 mmol) and NEt_3 (3.4 mL, 25 mmol) in ethanol (25 mL), **11f** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow oil (0.381 g, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.03 (d, J=6.9 Hz, 3H, CH_3), 2.28–2.37 (m, 1H, CH_2), 2.82–2.89 (m, 1H, CH_2), 2.93–2.99 (m, 1H, CH_2), 3.48 (dd, J=11.1, 6.9 Hz, 1H, OCH_2), 3.63 (dd, J=11.1, 5.1 Hz, 1H, OCH_2), 7.35–7.53 (m, 7H, CH=C, 6×CH of Ph), 8.15–8.22 (m, 2H, 2×CH of Ph), 8.52–8.60 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=17.9 (CH_3), 35.4 (CH), 41.8 (CH_2), 66.9 (OCH_2), 114.4 (*C*H=C–N), 127.2 (2C), 128.2 (2C), 128.5 (2C), 128.8 (2C), 130.6, 130.8 (CH of Ph), 137.0, 137.7 (C of Ph), 163.7 (*C*–Ph), 164.0 (N=C–N), 169.6 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3338 (br), 3064 (w), 2966 (m), 2926 (m), 2872 (w), 1632 (w), 1574 (s), 1533 (s), 1498 (w), 1451 (m), 1374 (s), 1278 (w), 1176 (w), 1111 (m), 1075 (m), 1035 (m), 751 (m), 695 (s), 636 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=256 (4.47). MS (EI, 70 eV): *m*/*z* (%)=303 (M^+, 1), 289 (1), 287 (4), 273 (3), 259 (12), 246 (100), 143 (7), 128 (4), 114 (5), 104 (23). Anal. Calcd for $C_{20}H_{20}N_2O$ (304.391): C 78.92, H 6.62, N 9.20. Found: C 78.73, H 6.59, N 9.15.

3.1.4.13. 2-Methyl-3-(2-methyl-6-phenylpyrimidin-4-yl)propan-1-ol (11g). Starting with **9a** (0.708 g, 3.5 mmol), acetamidine hydrochloride (**3b**) (3.483 g, 35 mmol) and NEt_3 (4.9 mL, 35 mmol) in ethanol (35 mL), **11g** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→EtOAc), as a pale yellow oil (0.395 g, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.01 (d, J=6.6 Hz, 3H, CH_3), 2.01–2.14 (m, 1H, CH), 2.46 (dd, J=12.9, 5.7 Hz, 1H, CH_2), 2.76 (s, 3H, CH_3), 2.85 (dd, J=18.3, 5.7 Hz, 1H, CH_2), 3.48 (dd, J=10.5, 6.6 Hz, 1H, OCH_2), 3.61 (dd, J=10.8, 4.5 Hz, 1H, OCH_2), 7.38–7.44 (m, 4H, CH=C, 3×CH of Ph), 7.85–7.88 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=16.4, 25.8 (CH_3), 35.2 (CH), 40.3 (CH_2), 66.2 (OCH_2), 113.8 (*C*H=C–N), 126.8 (2C), 127.0, 128.0 (2C, CH of Ph), 136.6 (C of Ph), 164.1 (*C*–Ph), 167.3 (N=C–N), 169.3 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3346 (br), 3065 (w), 2963 (m), 2927 (m), 2874 (w), 1604 (s), 1529 (s), 1450 (m), 1419 (m), 1375 (m), 1325 (s), 1286 (s), 1182 (w), 1123 (w), 1074 (m), 1040 (m), 743 (s), 696 (m), 648 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=244 (3.99), 277 (3.89), 325 (4.03). MS (EI, 70 eV): *m*/*z* (%)=242 (M^+, 100), 197 (5), 184 (93), 173 (20), 148 (5), 105 (14), 77 (19), 57 (22). HRMS (ESI): calcd for $C_{15}H_{18}N_2O$ [M^+]: 241.1335; found: 241.1332.

3.1.4.14. 2-(2,6-Diphenylpyrimidin-4-ylmethyl)butan-1-ol (11h). Starting with **9b** (0.200 g, 0.92 mmol), benzamidine hydrochloride monohydrate (**3a**) (1.616 g, 9.2 mmol) and NEt_3 (1.3 mL, 9.2 mmol) in ethanol (10 mL), **11h** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→1:1) as a yellow oil (0.166 g, 75%). ^1H NMR ($CDCl_3$, 300 MHz): δ=1.01 (t, J=7.2 Hz, 3H, CH_3), 1.37–1.48 (m, 2H, CH_2), 1.51–1.61 (m, 1H, CH), 2.93–3.08 (m, 2H, CH_2), 3.55 (dd, J=11.4, 6.6 Hz, 1H, OCH_2), 3.71 (dd, J=11.4, 4.5 Hz, 1H, OCH_2), 7.44–7.56 (m, 7H, CH=C, 6×CH of Ph), 8.20–8.24 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=11.5 (CH_3), 24.0, 39.6 (CH_2), 42.0 (CH), 64.4 (OCH_2), 114.3 (*C*H=C–N), 127.1 (2C), 128.1 (2C), 128.4 (2C), 128.7 (2C), 130.5, 130.7 (CH of Ph), 136.8, 137.6 (C of Ph), 163.8 (*C*–Ph), 163.9 (N=C–N), 169.7 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3398 (br), 3064 (w), 2966 (m), 2928 (m), 2870 (m), 1627 (w), 1574 (s), 1534 (s), 1495 (w), 1452 (m), 1374 (s), 1175 (w), 1114 (m), 1072 (m), 1035 (w), 927 (w), 751 (m), 695 (s), 636 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=204 (4.53), 256 (4.52). MS (EI, 70 eV): *m*/*z* (%)=317 (M^+, 1), 301 (3), 300 (3), 289 (3), 287 (2), 285 (2), 273 (3), 272 (3), 271 (12), 259 (7), 246 (100), 167 (2), 143 (3), 128 (3), 114 (3), 104 (15), 77 (7). Anal. Calcd for $C_{21}H_{22}N_2O$ (318.418): C 79.21, H 6.96, N 8.80. Found: C 79.19, H 6.82, N 8.68.

3.1.4.15. 2-(2-Methyl-6-phenylpyrimidin-4-ylmethyl)-butan-1-ol (11i). Starting with **9b** (0.075 g, 0.347 mmol), acetamidine hydrochloride (**3b**) (0.328 g, 3.47 mmol) and NEt_3 (0.48 mL, 3.47 mmol) in ethanol (7 mL), **11i** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=50:1→1:1) as a pale yellow oil (0.037 g, 42%). ^1H NMR ($CDCl_3$, 300 MHz): δ=0.98 (t, J=7.5 Hz, 3H, CH_3), 1.33–1.45 (m, 2H, CH_2), 1.92–2.01 (m, 1H, CH), 2.76 (s, 3H, CH_3), 2.91 (dd, J=8.4, 8.4 Hz, 2H, CH_2), 3.50 (dd, J=11.1, 6.3 Hz, 1H, OCH_2), 3.66 (dd, J=11.1, 4.2 Hz, 1H, OCH_2), 7.39 (s, 1H, CH), 7.47–7.51 (m, 3H, 3×CH of Ph), 8.05–8.08 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=11.8 (CH_3), 24.4 (CH_2), 26.1 (CH_3), 40.1 (CH_2), 42.1 (CH), 64.8 (OCH_2), 113.9 (*C*H=C–N), 127.3 (2C), 128.9 (2C), 130.8 (CH of Ph), 137.0 (C of Ph), 164.6 (*C*–Ph), 167.6 (N=C–N), 169.4 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3376 (w), 2963 (m), 2928 (m), 2873 (m), 1654 (w), 1583 (s), 1540 (s), 1492 (w), 1449 (m), 1391 (m), 1382 (m), 1311 (w), 1250 (w), 1170 (m), 1116 (w), 1047 (m), 1000 (w), 974 (w), 789 (w), 756 (w), 696 (m). UV–vis (CH_3CN, nm): λ_{max} (log ε)=249 (3.94), 255 (3.93), 276 (4.07). MS (EI, 70 eV): *m*/*z* (%)=256 (M^+, 1), 241 (1), 239 (3), 225 (4), 211 (4), 197 (9), 184 (100). HRMS (ESI): calcd for $C_{16}H_{20}N_2O$ ($[M-H]^+$): 255.14879; found: 255.14974.

3.1.4.16. 1-Bromo-4-(6-methyl-2-phenylpyrimidin-4-yl)butan-2-ol (11j). Starting with **8e** (0.100 g, 0.46 mmol), benzamidine hydrochloride monohydrate (**3a**) (0.735 g, 4.6 mmol) and NEt_3 (0.64 mL, 4.6 mmol) in ethanol (5 mL), **11j** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=100:1→3:1) as a light brown oil (0.135 g, 91%). ^1H NMR ($CDCl_3$, 300 MHz): δ=2.17–2.31 (m, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.87–3.03 (m, 2H, CH_2), 3.77 (dd, J=14.7, 7.5 Hz, 1H, CH_2–Br), 4.19 (dd, J=14.7, 9.6 Hz, 1H, CH_2–Br), 4.80–4.88 (m, 1H, OCH), 6.94 (s, 1H, CH=C), 7.37–7.49 (m, 3H, 3×CH of Ph), 8.42–8.47 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=24.1 (CH_3), 33.2, 33.8 (CH_2), 59.7 (CH_2–Br), 79.2 (OCH), 117.4 (*C*H=C–N), 128.1 (2C), 128.3 (2C), 130.3 (CH of Ph), 137.9 (C of Ph), 164.0 (N=C–N), 167.0 (*C*–CH_3), 168.8 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3066 (br, w), 2935 (m), 2868 (w), 1646 (s), 1584 (s), 1543 (m), 1445 (m), 1371 (s), 1316 (w), 1260 (m), 1073 (m), 1030 (w), 696 (s). UV–vis (CH_3CN, nm): λ_{max} (log ε)=254 (4.17). MS (EI, 70 eV): *m*/*z* (%)=322 (M^+ [^{81}Br], 11), 320 (M^+ [^{79}Br], 11), 304 (3), 289 (3), 242 (35), 228 (41), 198 (19), 184 (100). HRMS (ESI): calcd for $C_{15}H_{17}BrN_2O$ [M^+]: 322.0504 (^{81}Br), 320.0524 (^{79}Br); found: 322.0509 (^{81}Br), 320.0529 (^{79}Br). Anal. Calcd for $C_{15}H_{17}BrN_2O$ (321.212): C 56.09, H 5.33, N 8.72. Found: C 55.95, H 5.62, N 8.68.

3.1.4.17. 4-(6-Methyl-2-phenylpyrimidin-4-yl)butane-1,2-diol (11k). Starting with **10** (0.100 g, 0.64 mmol), benzamidine hydrochloride monohydrate (**3a**) (1.023 g, 6.4 mmol) and NEt_3 (0.89 mL, 6.4 mmol) in ethanol (5 mL), **11k** was isolated after chromatography (silica gel, *n*-hexane/EtOAc=50:1→EtOAc) as a light brown oil (0.145 g, 88%). ^1H NMR ($CDCl_3$, 300 MHz): δ=1.87–1.98 (m, 2H, CH_2), 2.55 (s, 3H, CH_3), 2.94–2.98 (m, 2H, CH_2), 3.52 (dd, J=11.1, 6.6 Hz, 1H, CH_2–OH), 3.66 (dd, J=11.1, 3.3 Hz, 1H, CH_2–OH), 3.76–3.84 (m, 1H, OCH), 6.95 (s, 1H, CH=C), 7.43–7.49 (m, 3H, 3×CH of Ph), 8.35–8.39 (m, 2H, 2×CH of Ph). ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C=24.1 (CH_3), 31.2, 33.6 (CH_2), 66.5 (CH_2–OH), 71.5 (OCH), 117.7 (*C*H=C–N), 128.1 (2C), 128.5 (2C), 130.4 (CH of Ph), 137.6 (C of Ph), 163.9 (N=C–N), 167.4 (*C*–CH_3), 169.5 (N–*C*=CH). IR (neat, cm^{-1}): $\tilde{\nu}$=3375 (br, s), 2929 (m), 2867 (w), 1663 (m), 1586 (s), 1539 (s), 1442 (m), 1376 (s), 1099 (m), 1043 (w), 698 (w). UV–vis (CH_3CN, nm): λ_{max} (log ε)=256 (4.12). MS (EI, 70 eV): *m*/*z* (%)=258 (M^+, 12), 241 (3), 224 (5), 210 (37), 197 (39), 183 (45), 169 (100). HRMS (ESI): calcd for $C_{15}H_{18}N_2O_2$ [M^+]: 258.13683; found: 258.13678. Anal. Calcd for $C_{15}H_{18}N_2O_2$ (258.316): C 69.74, H 7.02, N 10.84. Found: C 69.91, H 6.83, N 10.68.

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Homogeneous electro-mediated reduction of unsaturated compounds using Ni and Fe as mediators in DMF

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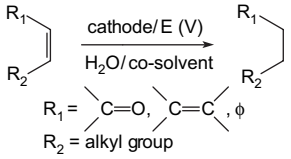
Abstract—The homogeneous electro-mediated reduction (HEMR) of several organic compounds (cyclohexene, cyclohexanone, acetophenone, benzaldehyde, styrene, linalool, 1,3-cyclohexadiene, citral, *trans*-4-phenyl-3-buten-2-one, and piperine) was carried out using Fe^{2+}, Ni^{2+}, and $[Ni^{II}(bpy)]Br_2$ (bpy=2,2′-bipyridine) as electron mediators. An electrochemical system composed of sacrificial anode (Fe, Ni or Zn), nickel cathode, NaI (0.2 M) as supporting electrolyte in DMF and an undivided cell, was used. A constant current $\leq$100 mA was applied with a maximum cell potential of 2.0 V. Non-conjugated olefins are not reactive, but ketones may be easily reduced to the respective alcohol. In the case of conjugated olefins and ketones, $[Ni^{II}(bpy)]Br_2$ or Ni^{2+} mediator presented good reactivity and selectivity in most cases. Fe^{2+} more efficiently mediates the reduction of carbonyl containing systems. Preliminary electroanalytical studies indicate the complexation of the organic substrate by Fe^{2+} and Ni^{2+} ions and $[Ni^{II}(bpy)]Br_2$ complex.

1. Introduction

Over the past several decades, a growing number of new synthetic methods has been forwarded. Most of these methods seek to increase the selectivity, efficiency, and simplicity of synthetic routes to organic molecules. One reaction that has been commonly employed involves the reduction of multiple bonds.[1,2] Catalytic hydrogenation is one among the many reactions available for the reduction of organic compounds, offering advantages of widespread applicability and experimental simplicity. However, the process may become complex when high hydrogen pressure, expensive noble metal catalysts, or specific complexes are involved in the process. Electrochemical methods may be applied as an alternative synthetic route,[3] presenting as principal advantages the simplicity of the system, mild conditions, and the use of electrons as the main reagent.

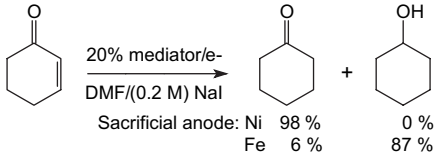
The electrocatalytic hydrogenation (ECH) of organic compounds, for instance, has been intensively studied on heterogeneous surface (Scheme 1), where adsorbed atomic hydrogen is generated in aqueous medium for a posterior catalytic hydrogenation step of the substrate on the cathode surface.[4–6]



Scheme 1. Heterogeneous electrocatalytic hydrogenation of olefins.

Direct electron transfer from the electrode to organic molecules normally occurs at elevated potentials and also produces unstable intermediates including radical anions, radicals, and dianions, often affecting the reaction specificity or selectivity. So the electron transfer by way of stable species (mediators or catalysts) may become a more advantageous route.[3]

Electron transfer mediators have provided the use of electrochemistry as a tool for specific reactions such as: aromatic homo- and hetero-coupling,[7–9] Reformatzky[10] and Barbier[11] type reactions, isoxazoline ring opening,[12] etc. Recently, a new synthetic route for π-bond reduction has been reported (Scheme 2).[13,14] 2-Cyclohexen-1-one was used as a standard



Scheme 2. Homogeneous electro-mediated reduction of 2-cyclohexen-1-one.

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substrate in aprotic solvent (DMF) and several metallic ions and complexes were tested as mediators. Selectivity has been achieved by proper choice of the sacrificial anode.

In this paper we report an extension of the scope of homogeneous electro-mediated reduction (HEMR) to different aliphatic and aromatic olefins and carbonyl compounds, as well as to some more complex conjugated systems. Several organic model compounds were used: cyclohexene, cyclohexanone, acetophenone, benzaldehyde, styrene, linalool, 1,3-cyclohexadiene, citral, and *trans*-4-phenyl-3-buten-2-one. The HEMR method also provided an interesting result for the reduction of a more complex conjugated system like piperine.

2. Results and discussion

The HEMR process was performed in an undivided cell as described previously for 2-cyclohexen-1-one reduction (Fig. 1).[14] A sacrificial anode was used to diminish the cell potential and to avoid undesired reactions on this electrode. A porous nickel cathode was employed in order to increase the surface contact and to allow a higher current without an increase of the cell potential (a stainless steel net also can be used). An inert atmosphere (N_2) is desirable to avoid the reduction of atmospheric oxygen. Conductivity was provided by addition of NaI (0.2 M) as the supporting electrolyte. Pre-electrolysis (30 min) in the presence of 1,2-dibromoethane is necessary to reduce the initial solution resistance.[14,15] Then the substrate was added together with 0.2 equiv of the mediator. In general, a constant current of 100 mA was maintained until complete consumption of the substrate was observed. In some cases, reduction of the current was necessary when the cell potential exceeded 2.0 V. The reaction products were identified by GC and GC–MS analysis and yields were determined by comparison with an added internal standard.

During the HEMR process, several reactions occur at the same time:[14] (1) oxidation of the (sacrificial) anode, (2) reduction of the mediator on the cathode surface, (3) reduction of the organic substrate by the mediator (reduced form), and (4) protonation of the reduced substrate by residual water in the solvent. If only these reactions occurred, the total electrochemical efficiency (E.E.) of the process ($Q_{theoretical}/Q_{passed}\times$total yield) should be 100%, i.e., all electrons transferred to the mediator, on the cathode surface, would be used to reduce the substrate. However, metallic ions generated from the sacrificial anode oxidation and dissolved in the reaction solution can also be reduced on the cathode surface, decreasing the E.E. of the process. In practice, this is not a serious problem since electrons are cheap.

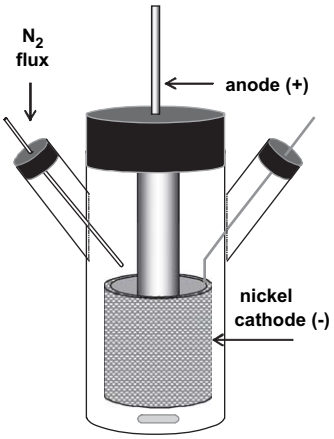


Figure 1. Undivided electrochemical cell used in the HEMR reactions using sacrificial anode. Cathode: Ni^0 foam; and anode: Fe, Zn or Ni.

Table 1 summarizes the results obtained from the reduction of a base set of substrates varying the HEMR method. Reduction of cyclohexene was unsuccessful with both mediators (Table 1, entry 1), but conjugation of the double bond with an aromatic ring as in styrene increased considerably the reactivity, especially with the $[Ni^{II}(bpy)]^{2+}$/Zn system, which gave ethylbenzene in nearly quantitative yield (entry 2). The presence of an allylic hydroxyl group also improved the selectivity of the hydrogenation reaction. In the case of linalool only the double bond between C-2 and C-3 was reduced (entry 3).

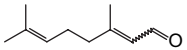
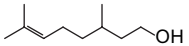
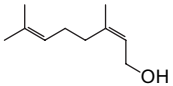
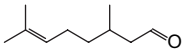
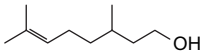
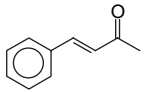
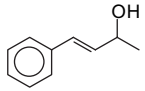
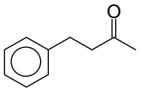
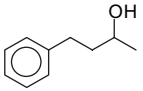
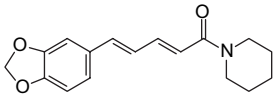
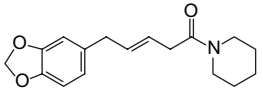
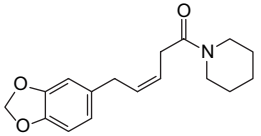
On the other hand, a typical aliphatic ketone such as cyclohexanone was reduced to the alcohol by both the metallic mediator systems (entry 4), but Fe^{2+}/Fe brought higher yield

Table 1. Homogeneous electro-mediated reduction of organic compounds (1.5 mmol), in 20 mL DMF+NaI (0.2 M), nickel cathode, using sacrificial anode (Fe or Zn) and 100 mA as initial constant current

Entry	Substrate	Mediator[a]/anode	Charge (C)	Product (%)	E.E.[b] (%)
1	Cyclohexene	$FeSO_4$/Fe	900	—	—
		$[Ni^{II}(bpy)]^{2+}$/Zn	900	—	—
2	Styrene	$FeSO_4$/Fe	1500	Ethylbenzene (09)	02
		$[Ni^{II}(bpy)]^{2+}$/Zn	2100	(99)	14
3	Linalool	$FeSO_4$/Fe	3000	3,7-Dimethyl-6-octen-3-ol (08)	01
		$[Ni^{II}(bpy)]^{2+}$/Zn	1500	(46)	09
4	Cyclohexanone	$FeSO_4$/Fe	900	Cyclohexanol (99)	32
		$[Ni^{II}(bpy)]^{2+}$/Zn	3000	(95)	09
5	Acetophenone	$FeSO_4$/Fe	2100	1-Phenyl-ethanol (70)	10
		$[Ni^{II}(bpy)]^{2+}$/Zn	2400	(71)	08
6	Benzaldehyde	$FeSO_4$/Fe	2400	Benzyl alcohol (87)	10
		$[Ni^{II}(bpy)]^{2+}$/Zn	1500	(72)	13

[a] The reactions used 20 mol % of the mediator.
[b] Electrochemical efficiency ($Q_{theoretical}/Q_{passed}\times$total yield).

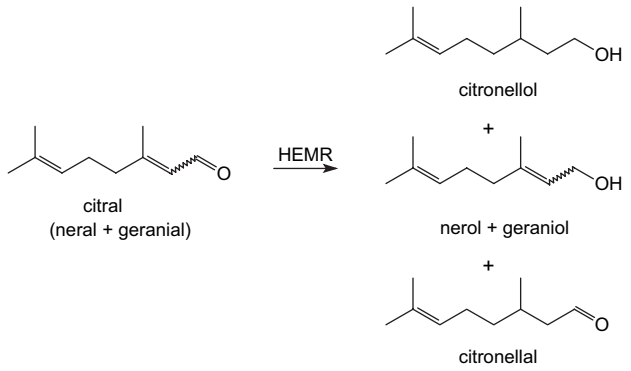
Table 2. Homogeneous electro-mediated reduction of organic compounds (1.5 mmol), in 20 mL DMF+NaI (0.2 M), nickel cathode, using sacrificial anode (Fe or Zn) and 100 mA as initial constant current

Entry	Substrate	Mediator[a]/anode	Charge (C)	Products (%)				E.E.[b] (%)
1	 1,3-cyclohexadiene	$FeSO_4$/Fe	900	 cyclohexene (38)	 cyclohexane (06)			16
		$[Ni^{II}(bpy)]^{2+}$/Zn	1500	(58)	(02)			12
		$NiBr_2$/Ni	1200	(81)	(18)			28
2	 citral (geranial/neral (6.5:3.5))	$FeSO_4$/Fe	1500	 geraniol (33)	 nerol (18)	 citronellal (02)	 citronellol (48)	27
		$[Ni^{II}(bpy)]^{2+}$/Zn	1200	(29)	(16)	(07)	(10)	17
		$NiBr_2$/Ni	1800	(04)	(04)	(14)	(77)	28
3	 *trans*-4-phenyl-3-buten-2-one	$FeSO_4$/Fe	900	 *trans*-4-phenyl-3-buten-2-ol (10)	 4-phenyl-2-butanone (84)	 4-phenyl-2-butanol (02)		38
		$[Ni^{II}(bpy)]^{2+}$/Zn	1500	(12)	(88)	(—)		20
		$NiBr_2$/Ni	1500	(03)	(57)	(13)		17
4	 piperine	$FeSO_4$/Fe	900	 *trans*-5-benzol[1,3]dioxol-5-yl-1-piperidin-1-yl-pent-3-en-1-one (75)	 *cis*-5-benzol[1,3]dioxol-5-yl-1-piperidin-1-yl-pent-3-en-1-one (25)			38
		$[Ni^{II}(bpy)]^{2+}$/Zn	900	(80)	(20)			38

[a] The reactions used 20 mol % of mediator.
[b] Electrochemical efficiency ($Q_{theoretical}/Q_{passed} \times$ total yield).

and electrochemical efficiency. Comparable preparative yields with both systems were also obtained in the case of the aromatic carbonyl compounds acetophenone and benzaldehyde (entries 5 and 6).

In the following experiments some conjugated compounds were examined (Table 2). 1,3-Cyclohexadiene produced mixtures resulting from simple and double hydrogenation (entry 1); the best yield, selectivity, and electrochemical efficiency were observed in the Ni^{2+}/Ni system. The same mediator combination was also able to reduce selectively citral (Scheme 3), a commercial mixture of geranial (trans) and neral (cis) (6.5:3.5); in contrast to the behavior of 2-cyclohexen-1-one,[14] the main product with Ni^{2+}/Ni was citronellol, a result of double hydrogenation of the conjugated carbonyl system (entry 2). Using the $[Ni^{II}(bpy)]^{2+}$ complex, a weak preference for the reduction of only the carbonyl bond to geraniol/nerol was observed, whereas Fe^{2+}/Fe produced comparable amounts of both types of product. It can be observed for Fe^{2+} and $[Ni^{II}(bpy)]^{2+}$ mediated reactions that the geraniol/nerol proportion remains the same as citral, while in the presence of Ni^{2+} as mediator an equal (1:1) trans/cis product was observed. In addition, minor amounts of citronellal, as a result of hydrogenation of the conjugated C=C bond, were also detected with all three mediator systems, but the isolated C=C bond was not reduced at all.



Scheme 3. Homogeneous electro-mediated reduction of the citral.

In the case of *trans*-4-phenyl-3-buten-2-one (an example of conjugation of an aromatic ring, a C=C double bond and a carbonyl group) the predominant reaction was the selective reduction of the central C=C bond (entry 3). Fe^{2+}/Fe and $[Ni^{II}(bpy)]^{2+}$/Zn gave similar yields, but the first mediator system was superior in electrochemical efficiency. Ni^{2+}/Ni proved to be less efficient and selective.

Finally, piperine, a natural diene conjugated to an aromatic ring and an amide moiety, was submitted to HEMR. Simple hydrogenation occurred selectively in a 1,4 manner to produce a mixture of trans and cis isomers (3:1 and 4:1, using Fe^{2+} and $[Ni^{II}(bpy)]^{2+}$ as mediators, respectively) of the mono-unsaturated compound dihydropiperine (entry 4). Preparative yields and good E.E. were obtained with both the mediator systems, comparable to the chemical reduction (Zn/HOAc) of the piperine.[16]

Analyzing the results described above, one can conclude that both metallic mediators are highly efficient for the hydrogenation of conjugated olefins. Non-conjugated olefins are only affected in the case of allylic alcohols. Unsaturated carbonyl compounds are selectively reduced at the C=C double bond in the case of ketones and amides. Unsaturated aldehydes like citral are more reactive and give double hydrogenation, especially when Ni^{2+}/Ni is used.

It is interesting to compare the results obtained from citral hydrogenation by ECH[4,6] and HEMR, described in Table 2. Although electrochemical efficiency obtained in the HEMR method is lower than those obtained in the ECH at variable current density (84%)[4] and static current density (38%),[6] the total hydrogenation product (citronellol) obtained from the HEMR method (77%) was superior to two other ECH methods (70%[4] and 60%,[6] respectively). Other substrates, described in Tables 1 and 2, corroborate the superiority of HEMR.

Similarly, the ECH reaction of *trans*-4-phenyl-3-buten-2-one was carried out using the same reaction conditions as described in the literature[6] (Q=222 C), producing 4-phenyl-2-butanone (45%) and 4-phenyl-2-butanol (15%), and E.E. of 75%. Also in this case, yield and selectivity of the HEMR method (Table 2, entry 3) showed better results.

The use of metallic ions as the catalyst has already been described in the reduction of organic substrates. The Zn/$NiCl_2$ system was used as a selective route for the reduction of α,β-unsaturated carbonyls.[17,18] Also, the reduction of alkenes and alkynes was tested with $LiAlH_4$ in the presence of several metallic ions ($TiCl_3$, VCl_3, $CrCl_3$, $FeCl_2$, $FeCl_3$, $CoCl_2$, and $NiCl_2$).[19] $NaBH_4$/$CoCl_2$ was applied in the reduction of disubstituted olefins[20] and benzonitrile.[21] The principal advantages of the HEMR over the above related processes is the ability to control the number of moles of electrons by following the charge passed, cell potential, and current density. The use of mild conditions and a clean source of electrons is another advantage.

2.1. Mechanism proposal

In the electro-mediated reduction of the 2-cyclohexen-1-one, described previously,[14] a reduction mechanism involving homogeneous electron transfer was proposed. A more detailed study of the electrochemical system by cyclic voltammetry revealed that Fe^{2+} (Fig. 2) and Ni^{2+} (Fig. 3) ions are reduced at more elevated potentials, than in aqueous medium, −1.74 and −1.28 V, respectively, and the corresponding oxidation peaks are observed at −0.38 and 0.24 V, respectively. The addition of small amount of substrate (2-cyclohexen-1-one) causes the displacement of the reduction peaks to more negative potentials and the disappearance of the oxidation peak, showing that the intermediation of the electron transfer process occurred, which may be explained by an intermediate complex formation before the reduction step (Scheme 4).

The cyclic voltamogram of the $[Ni^{II}(bpy)]Br_2$ complex shows a cathodic (Epc=−1.09 V) and anodic peak (Epa=−0.92 V) corresponding to a reversible reduction system $[Ni^{II}(bpy)]^{2+}$/$[Ni^{0}(bpy)]$[8] (Fig. 4). Differently to Fe^{2+} and Ni^{2+}, the intermediate complex is more evident when $[Ni^{II}(bpy)]Br_2$ complex is used as mediator. A second reversible system can be observed with addition of 2-cyclohexen-1-one

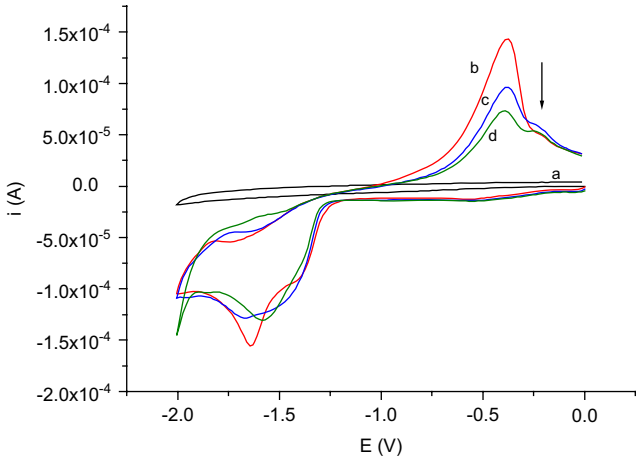


Figure 2. Cyclic voltamogram of: (a) DMF (0.1 M $TBABF_4$) with posterior addition of (b) $FeCl_2$ (0.01 M), and successive additions of (c) 10 μL and (d) 30 μL of 2-cyclohexen-1-one, using vitreous carbon as working electrode, Ag/AgCl (3.0 M KCl) as reference electrode, v=0.1 V s^{-1}.

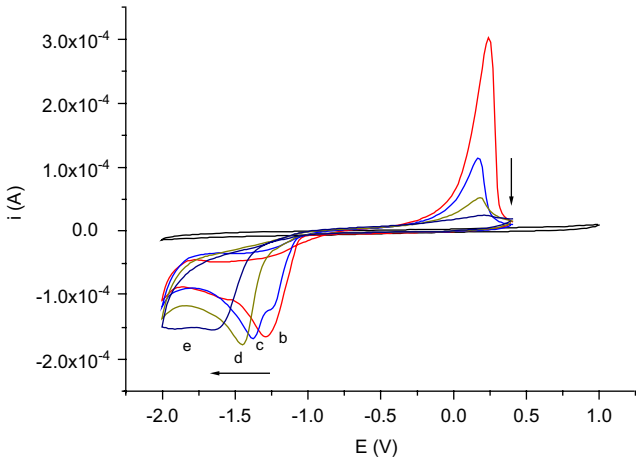
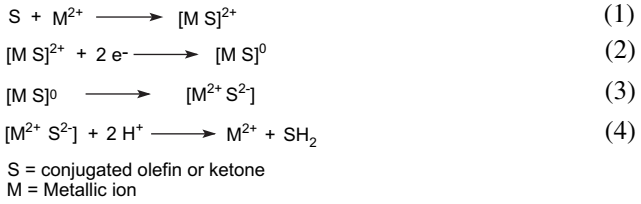


Figure 3. Cyclic voltamogram of: (a) DMF (0.1 M $TBABF_4$) with posterior addition of (b) $NiBr_2$ (0.01 M), and successive additions of (c, d, e) 10 μL of 2-cyclohexen-1-one, using vitreous carbon as working electrode, Ag/AgCl (3.0 M KCl) as reference electrode, v=0.1 V s^{-1}.



Scheme 4. HEMR of organic substrates by Fe^{2+} or Ni^{2+}.

(Epc=−1.58 V/Epa=−1.46 V) with a simultaneous displacement of the first oxidation peak (Epa=−0.69 V). Therefore, it is possible in this case that the electron transfer occurs before the interaction with the substrate (Scheme 5).

The results observed in electrosynthesis and electroanalytical experiments indicate a homogeneous electro-mediated mechanism for reduction of the organic substrates studied here. Additional work is necessary for a detailed mechanistic proposal.

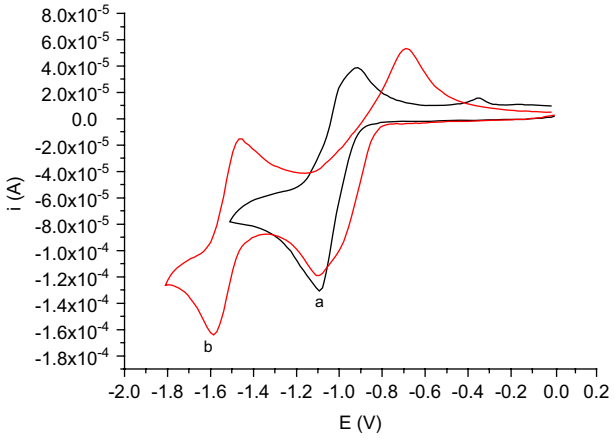
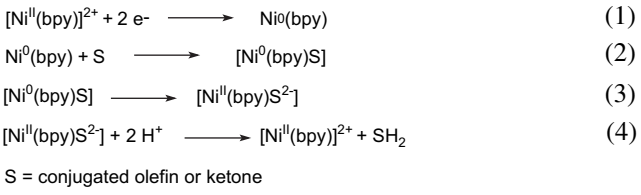


Figure 4. Cyclic voltamogram of: (a) $[Ni^{II}(bpy)]Br_2$ (1.0 mmol) in DMF (0.1 M $TBABF_4$) with posterior addition of (b) 2-cyclohexen-1-one (0.5 mmol), using vitreous carbon as working electrode, Ag/AgCl (3.0 M KCl) as reference electrode, v=0.1 V s^{-1}.



Scheme 5. HEMR of organic substrates by $[Ni^{II}(bpy)]Br_2$.

3. Conclusion

In conclusion, our results demonstrate that reduction of olefins and carbonyl compounds is possible using the HEMR approach. Non-conjugated olefins are unreactive, except allylic alcohols, which are partially reduced by the $NiBr_2$/Ni system. The same mediator showed good reactivity and selectivity for conjugated dienes. Carbonyl compounds can be reduced using both the metallic mediators; in the case of α,β-unsaturated aldehydes, ketones, and amides selective mono- or dihydrogenation is possible depending on the reactivity of the carbonyl group.

4. Experimental

All organic substrates and products, used as GC standards, were purchased from Aldrich or Acros. DMF, ethyl acetate, *n*-hexane, and toluene were used as received. NaI, $FeSO_4$, $FeCl_2 \cdot 6H_2O$, and $NiBr_2 \cdot xH_2O$ were purchased from Acros. All metal rods were purchased from Nitech and Ni foam cathode from Goodfellow.

The controlled current preparative electrolyses and cyclic voltammetry experiments were carried out with AUTOLAB/PGSTAT 30 potentiostat/galvanostat, using undivided cells of 20.0 mL capacity. A metal rod (Fe, Ni or Zn) of 0.8 cm diameter and nickel foam (10.0×4.0 cm, Nitech) were used as sacrificial anode (immersed >1 cm in solution) and working electrode, respectively, (Ni foil or bar can also be used as cathode). The Ni foam electrode can be reused about 20 times, after cleaning with a 6.0 M HCl solution

after each application. The anode should be polished before electrolysis. For experiments involving Ni^0bpy as mediator, the precursor $[Ni^{II}(bpy)]Br_2$ was prepared separately according to the literature.[22] The electrolytic cell was charged under nitrogen with 15.0 mL DMF containing NaI (4.0 mmol) and 1,2-dibromoethane (1.4 mmol). A pre-electrolysis was initiated under 150 mA constant current, during 30 min. The substrate (1.5 mmol) was mixed with mediator (0.3 mmol) ($FeSO_4$, $NiBr_2$, and $[Ni^{II}(bpy)]Br_2$) in 5.0 mL of solvent and then added to the cell. A 100 mA constant current was applied until full consumption of the starting reagent. The product yield was determined on a 0.5 mL aliquot of the reaction solution. Water (2.0 mL) was added to the aliquot and the products were extracted with 2.0 mL of ethyl acetate (hexane for cyclohexene and 1,3-cyclohexadiene substrates) containing 0.067 mmol of toluene, used as internal standard. The product yields were determined by GC analysis. Toluene was used as internal standard to quantify products and reagents. GC–MS analysis were obtained with a Varian 3380 GC or Finnigan MAT-GCQ instrument, fitted with a 30 m capillary CP-SPL5CB Chrompack column, using 60–200 °C temperature range (20 °C min^{-1}). Comparisons with authentic sample were performed to identify hydrogenation products and reagents and confirmed by GC–MS. Electrochemical efficiency of the process can be calculated by equations relating current (I), time (t), charge (Q), electrons (e^-) involved and mole number of hydrogen (n) promoted in the process: $Q=I$ (A)$\times t$ (s)$=e^-\times n\times F$, where $F=96{,}487$ C mol^{-1}.

The reaction product from HEMR of piperine was isolated from the reaction medium by using the same extraction procedure as described above; using 20.0 mL of distilled water and 3×10.0 mL of diethyl ether. It was identified by ^{1}H NMR and MS as a cis/trans isomers mixture. The crude product was purified by silica gel column chromatography.

4.1. *trans*-5-Benzol[1,3]dioxol-5-yl-1-piperidin-1-yl-pent-3-en-1-one [23512-55-2]

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (m, J=6.6 Hz, 4H), 1.62 (m, J=4.5 Hz, 2H), 3.20 (d, J=5.1 Hz, 2H), 3.35 (d, J=5.4 Hz, 2H), 3.30–3.60 (4H), 5.64 (m, 1H), 5.91 (s, 2H), 6.64 (m, 1H), 6.67–6.74 (m, 3H). *m/e* 287 (53), 204 (35), 174 (35), 152 (44), 135 (30), 112 (73), 84 (53), 69 (100).

4.2. *cis*-5-Benzol[1,3]dioxol-5-yl-1-piperidin-1-yl-pent-3-en-1-one

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.55 (m, J=6.6 Hz, 4H), 1.62 (m, J=4.5 Hz, 2H), 3.12 (d, J=6.3 Hz, 2H), 3.29 (d, J=3.9 Hz, 2H), 3.30–3.60 (4H), 5.63 (m, 1H), 5.91 (s, 2H), 6.64 (m, 1H), 6.66–6.74 (m, 3H). *m/e* 287 (53), 204 (35), 174 (35), 152 (44), 135 (30), 112 (73), 84 (53), 69 (100).

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Preparation of a new 1,2,3-trithiolane, *trans*-9,10,11-trithiabicyclo[6.3.0]undecane, and its oxidation reactions

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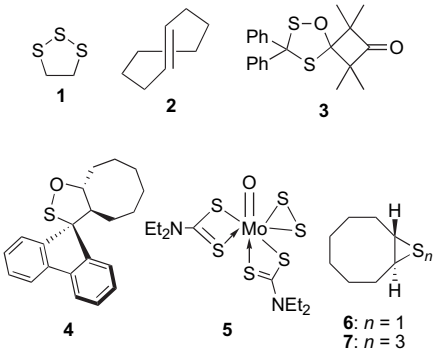
Abstract—The reaction of *trans*-cyclooctene with S_8O yielded a novel bicyclic 1,2,3-trithiolane and *trans*-9,10,11-trithiabicyclo[6.3.0]-undecane (**7**). Oxidation of the trithiolane with dimethyldioxirane yielded three monoxides, which are assigned to two isomeric 9-oxides, *rel*-(1*R*,8*R*,9*S*)-9-oxide (**15**) and *rel*-(1*R*,8*R*,9*R*)-9-oxide (**16**), and 10-oxide (**17**). Further oxidation of *rel*-(1*R*,8*R*,9*S*)-9-oxide (**15**) provided *rel*-(1*R*,8*R*,9*S*,11*S*)-9,11-dioxide (**18**) and *rel*-(1*R*,8*R*,9*R*,11*S*)-9,11-dioxide (**19**), while that of *rel*-(1*R*,8*R*,9*R*)-9-oxide (**16**) gave *rel*-(1*R*,8*R*,9*R*,11*S*)-9,11-dioxide (**19**) and *rel*-(1*R*,8*R*,9*R*,11*R*)-9,11-dioxide (**20**). The structures of **18** and **19** were determined by X-ray crystallography. The structures of other oxides were elucidated by the spectroscopic data and results of further chemical transformations. Two isomers, **15** and **16**, isomerized to one another. A 9,11-dioxide **20** isomerized to **19**, which is in equilibrium with **18**, where **18** is thermodynamically the most stable isomer.

1. Introduction

1,2,3-Trithiolane **1** is the trithio analog of molozonide, and several derivatives, including their oxides, have been reported so far.[1,2] Most of the derivatives were prepared by reaction of alkenes with sulfur allotropes under vigorous[3–12] or mild[13–15] conditions, and a few were obtained by dimerization of a thioketone *S*-oxide,[16] by reaction of 1,2-dithiols and their equivalents with sulfurating reagents,[17–19] or by reduction of a pentathiane followed by air oxidation.[20] On the other hand, oxidations of acyclic and cyclic di-,[21] tri-,[22] tetra-,[23] and higher polysulfanes[24,25] have attracted considerable attention from the viewpoints of regiochemistry, stereochemistry, stability, and reactivities of the oxides formed. We have been studying the synthesis and oxidation of cyclic di-, tetra-, and pentasulfanes.[24,25] *trans*-Cyclooctene (**2**) has high reactivity toward sulfurating reagents. Adam and his co-workers have reported that the reactions of **2** with **3**–**5** gave the corresponding *trans*-episulfide **6** in high yields.[26–30] In relation to our study on S_8O as an S_2O equivalent,[31] we examined the reaction of **2** with S_8O,[32] and found the formation of a novel 1,2,3-trithiolane derivative **7** together with **6**.



Concerning oxides of 1,2,3-trithiolanes, a limited number of 1- and 2-oxides[17,19,33,34] and 1,1-dioxides[16,34] have been reported. Ghosh and Bartlett reported on oxidation of trithiolane **8** with MCPBA giving *endo*- and *exo*-1-oxides and *endo*-2-oxide, and with ozone giving *endo*- and *exo*-2-oxides.[17] Oxidation of **9** with MCPBA yielded *endo*- and *exo*-3-oxides.[17] Satoh and Sato reported that oxidation of **10** with MCPBA gave 1-oxide **11**, while that with NCS or NBS provided 1,1-dioxide **12** through **11**.[34] Further oxidation of **11** with MCPBA gave disulfinic acid **13**, where intervention of 1,3-dioxide **14** was proposed.[34] In this paper we report on the preparation of a novel 1,2,3-trithiolane **7** and its oxidation giving three monoxides and three 1,3-dioxides together with isomerization between them.

Keywords: Trithiolane; Cyclooctene; Octasulfur monoxide; Oxidation; Isomerization.
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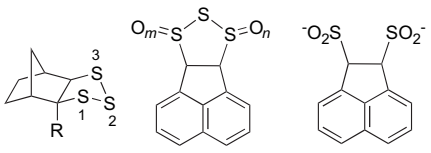


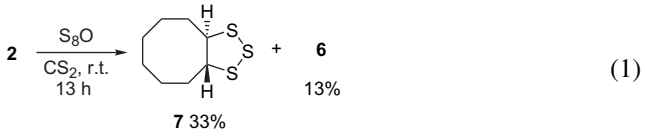
R = H (**8**)
Ph (**9**)

10: *m* = *n* = 0
11: *m* = 1, *n* = 0
12: *m* = 2, *n* = 0
14: *m* = *n* = 1

13

2. Results and discussion

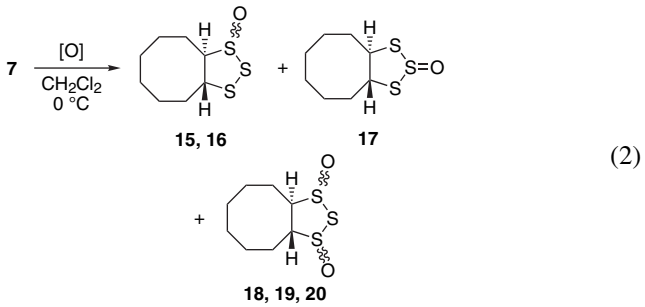
trans-Cyclooctene (**2**)[35] was treated with S_8O[32] in CS_2 at room temperature. After chromatographic purification, we isolated *trans*-9,10,11-trithiabicyclo[6.3.0]undecane **7**, a 1,2,3-trithiolane, as a yellow oil in 33% yield together with *trans*-episulfide **6** in 13% yield (Eq. 1).



2 → (S_8O, CS_2, r.t., 13 h) → **7** 33% + **6** 13% (1)

The ^{13}C NMR spectrum of **7** showed four signals due to sp^3 carbons at δ 24.6 (CH_2), 26.5 (CH_2), 34.8 (CH_2), and 63.5 (CH), and the ^1H NMR spectrum exhibited two methine protons at δ 3.78–3.84 as a multiplet. As we could not determine the stereochemistry of **7**, whether cis or trans, with these NMR data, we examined derivation of **7** into its oxides to make the structure unsymmetrical for observing the vicinal coupling constant between the methine protons.

Trithiolane **7** was treated with an acetone solution of dimethyldioxirane (DMD)[37,38] (1 equiv) in dichloromethane at 0 °C to give two 9-oxides **15** (16%) and **16** (33%), 10-oxide **17** (15%), and two 9,10-dioxides **18** (8%) with recovery of **7** (9%) (Eq. 2; Table 1, entry 1). When 2 equiv of DMD were used, 9,10-dioxides **18**, **19**, and **20** were formed in the ratio of 21:64:10 with the recovery of **7** (based on ^1H NMR integral ratio) (entry 2). Oxidation of **7** with MCPBA (1 equiv) provided a similar set of products. Except **20**, all the oxides were isolated.



7 → ([O], CH_2Cl_2, 0 °C) → **15, 16** + **17** + **18, 19, 20** (2)

2.1. Structure determination of the oxides of 7

While monoxides **15**–**17** were oily materials, we could obtain single crystals of dioxides **18** and **19** suitable for X-ray crystallography. The ORTEP drawings of **18** and **19** are depicted in Figures 1 and 2, respectively. The

Table 1. Yields of products in oxidation of trithiolane **7**

Entry	Oxidant (equiv)	Yields (%)						
		15	**16**	**17**	**18**	**19**	**20**	**7**
1	DMD[a] (1)	16	33	15	8	0	0	9
2	DMD[a] (2)	0	0	0	21[b]	64[b]	10[b]	5[b]
3	MCPBA (1)	20	53	7	4	[c]	0	0

[a] Dimethyldioxirane.
[b] ^1H NMR integral ratio.
[c] A small amount of **19** was detected by ^1H NMR.

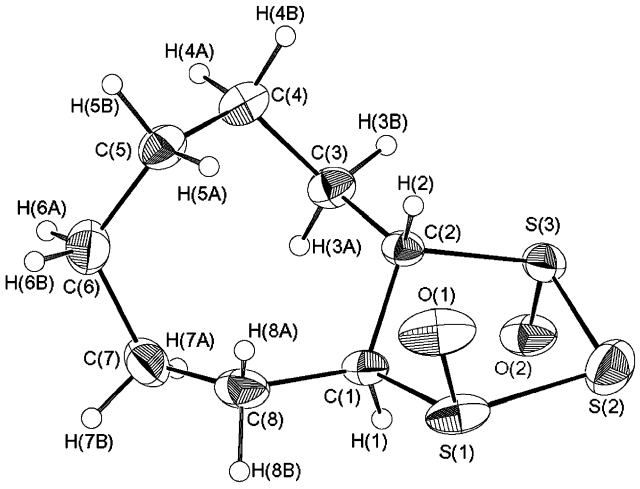


Figure 1. ORTEP drawing of *rel*-(1*R*,8*R*,9*S*,11*S*)-9,10,11-trithiabicyclo[6.3.0]undecane 9,11-dioxide (**18**) (30% ellipsoidal probability). Selected bond lengths (Å), bond angles (deg), and dihedral angles (deg): S1–O1 1.482(2); S1–C1 1.833(3); S1–S2 2.1249(16); S2–S3 2.1259(14); S3–O2 1.473(2); S3–C2 1.832(3); C1–C2 1.524(4); C1–S1–S2 93.58(10); S1–S2–S3 101.13(5); C2–S3–S2 94.09(10); C2–C1–S1 108.0(2); C1–C2–S3 107.29(18); H1–C1–C2–H2 −157(3); S3–C2–C1–S1 68.3(2); S1–S2–S3–C2 14.07(9); C1–S1–S2–S3 14.74(10); O1–S1–C1–C2 62.7(2); O2–S3–C2–C1 62.8(2).

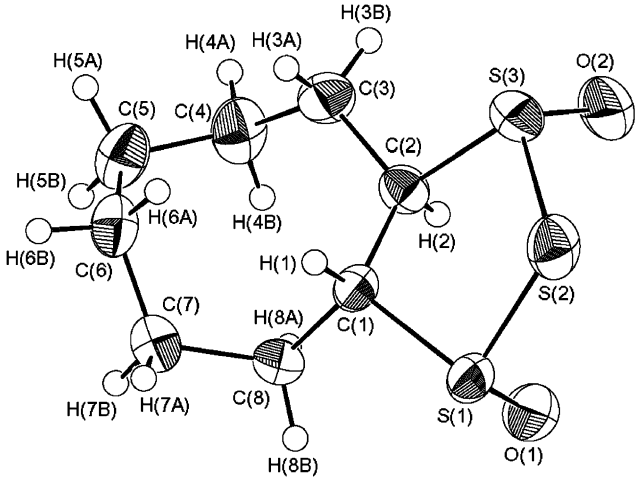
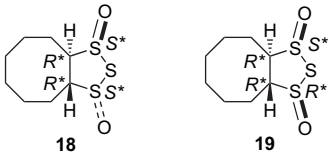


Figure 2. ORTEP drawing of *rel*-(1*R*,8*R*,9*R*,11*S*)-9,10,11-trithiabicyclo[6.3.0]undecane 9,11-dioxide (**19**) (30% ellipsoidal probability). Selected bond lengths (Å), bond angles (deg), and dihedral angles (deg): S1–O1 1.487(5); S1–C1 1.837(5); S1–S2 2.110(2); S2–S3 2.118(2); S3–O2 1.487(5); S3–C2 1.846(6); C1–C2 1.520(7); C1–S1–S2 92.49(19); S1–S2–S3 99.57(8); C2–S3–S2 96.12(18); C2–C1–S1 108.3(4); C1–C2–S3 114.8(4); H1–C1–C2–H2 −172.9(7); S3–C2–C1–S1 −51.0(3); S1–S2–S3–C2 15.4(2); C1–S1–S2–S3 −37.1(2); O1–S1–C1–C2 −58.8(4); O2–S3–C2–C1 129.9(4).

conjunction of the two rings in **18** and **19** was trans, and their relative stereochemistries including the configuration of the S═O groups were determined to be *rel*-(1*R*,8*R*,9*S*,11*S*)-9,11-dioxide and *rel*-(1*R*,8*R*,9*R*,11*S*)-9,11-dioxide, respectively. Of necessity, the stereochemistry of **7** was determined to be trans, showing that the stereochemistry of the starting *trans*-alkene was retained.

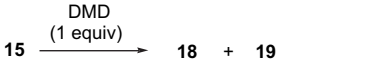


In the crystal, the trithiolane ring of **18** took a half-chair conformation, and the eight-membered ring took a boat–chair conformation, which is one of the most stable family of conformations of cyclooctane.[39] Each of the two oxygen atoms in **18** possessed the axial position and was trans to the vicinal hydrogen atom [O(1)–S(1)–C(1)–H(1) 178(2)°, O(2)–S(3)–C(2)–H(2) 177(2)°]. The ^{13}C NMR spectrum, measured at room temperature, showed only four signals due to sp^3 carbons, indicating that **18** has a symmetric structure in the NMR time scale in solution. In the ^{1}H NMR spectrum, H(1) and H(2) appeared at δ 4.23–4.25 as a multiplet.

In the case of dioxide **19**, the trithiolane and the cyclooctane rings took conformations similar to those of **18**. One of the two oxygen atoms [O(1)] possessed the axial position and the other [O(2)] possessed the equatorial one in the crystal, where the former is trans to the vicinal hydrogen [O(1)–S(1)–C(1)–H(1) 179.0(5)°] and the latter was cis to the vicinal hydrogen [O(2)–S(3)–C(2)–H(2) 9.9(4)°]. In the ^{1}H NMR spectrum, two methine protons resonated at δ 3.57 and 4.82 with the vicinal coupling constant of 12.0 Hz, indicating that the hydrogen atoms take a trans conformation also in solution.

The structure of 10-oxide **17** was elucidated as follows. In the ^{13}C NMR spectrum, all carbons were nonequivalent with each other and in the ^{1}H NMR spectrum the vicinal coupling constant between the methine protons (δ 4.19 and 4.60) was 11.3 Hz. Unlike the cases of **15** and **16** as discussed below, oxidation of **17** with DMD gave a mixture of unidentified compounds and not dioxides **18** or **19**, which indicates that **17** is not a 9-oxide but a 10-oxide of **7**.

^{1}H and ^{13}C NMR spectroscopies of **15** and **16** showed that all of their protons and carbons were nonequivalent. Oxidation reactions of **15** and **16** were investigated to obtain further information on regio- and stereochemistries of their S═O groups. When **15** was oxidized with DMD at 0 °C, dioxides **18** (43%) and **19** (47%) were formed (Eq. 3). On the other hand, oxidation of **16** yielded another dioxide **20** (54%) and **19** (35%) together with a small amount of **18** (2%) (Eq. 4). The formation of a small amount of **18** seems to be due to isomerization of **19** as described later. The ^{13}C NMR spectrum of the mixture of **19** and **20** (with a small amount of **18**), measured at −20 °C, showed only four sp^3 carbon signals due to **20** at δ 23.3, 25.5, 27.9, and 75.8. The methine protons of **20** were observed at δ 3.72–3.80 as a multiplet. Thus, dioxide **20** has a symmetric structure in the NMR time scale, and the structure was assigned to be *rel*-(1*R*,8*R*,9*R*,11*R*)-9,11-dioxide **20**.



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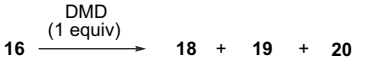

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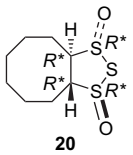
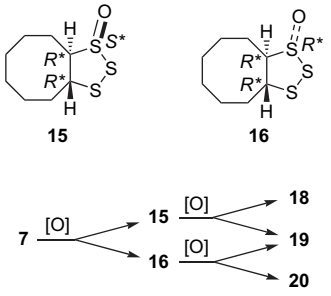


Three possible stereoisomers of 9,11-dioxides (**18**–**20**) were thus obtained and their structures were clearly determined. Based on the results of Eqs. 3 and 4, the structures of monoxides **15** and **16** are assigned to be *rel*-(1*R*,8*R*,9*S*)-9-oxide and *rel*-(1*R*,8*R*,9*R*)-9-oxide, respectively. As summarized in Scheme 1, oxidation of trithiolane **7** and 9-oxides **15** and **16** proceeded without stereoselectivity to give two possible stereoisomers, **15** and **16**, **18** and **19**, and **19** and **20**, respectively. Table 2 summarizes NMR data of the methine protons and carbons of **7** and its oxides, **15**–**20**.



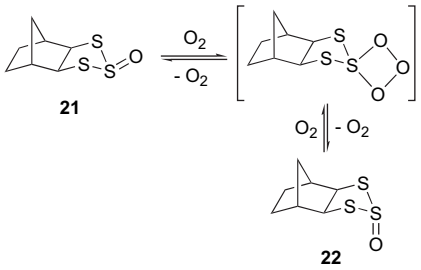
Scheme 1.

Table 2. NMR data of methine protons and methine carbons of **7** and **15**–**20**

	Methine protons (δ)	Methine carbons (δ)
7	3.78–3.84 (m)	63.5
15	3.39–3.44 (m), 3.92–3.98 (m)	57.2, 79.9
16	3.48–3.54 (m), 3.64–3.69 (m)	60.2, 86.6
17	4.19 (ddd), 4.60 (ddd) (J_{vic}=11.3 Hz)	60.8, 70.2
18	4.23–4.25 (m)	73.4
19	3.57 (ddd), 4.82 (ddd) (J_{vic}=12.0 Hz)	73.9, 81.2
20	3.72–3.80 (m)	75.8

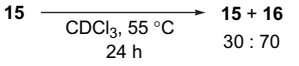
2.2. Isomerization

We observed mutual isomerization between monoxides **15** and **16**. When a solution of **15** in $CDCl_3$ was warmed at 55 °C, **16** appeared gradually and the ratio of **15** and **16** reached 30:70 after 24 h (Eq. 5). Similarly, a solution of pure **16** became a 29:71 mixture of **15** and **16** under same conditions (Eq. 6). Ghosh and Bartlett reported that mutual

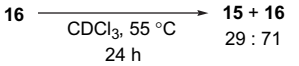


Scheme 2.

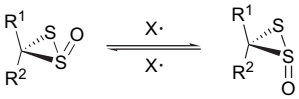
isomerization between **21** and **22** (2-oxides of **8**) took place not under argon but under O_2, and they proposed the adduct of **21** with $^3O_2$ as the intermediate (Scheme 2).[17] In contrast, the isomerization between **15** and **16** was not influenced by the presence or the absence of air. When 1,1-diphenyl-2-picryl hydrazyl (DPPH) was added to a solution of **15** or **16**, the isomerization was accelerated and the equilibrium was attained in 19 h. This acceleration by a radical scavenger was in quite contrast to our previous observations of remarkable retardation by DPPH in the epimerization of dithiirane 1-oxides (Eq. 7), where we proposed that a radical contaminant (X•) caused the epimerization.[40,41] In the present case, DPPH itself might act as a radical catalyst.



(5)

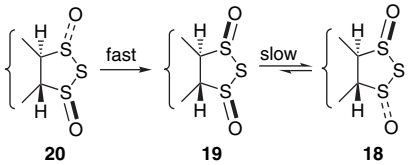


(6)



(7)

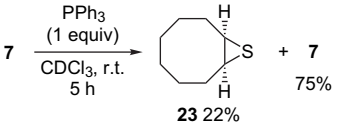
9,11-Dioxides **19** and **20** isomerized to **18**. When a solution of a mixture of **18** (2%), **19** (35%), and **20** (54%) in $CDCl_3$, obtained in Eq. 4, was stood at room temperature for one day, **20** disappeared completely and the amounts of **18** (24%) and **19** (59%) increased. After four days, **18** became the major component (69%) and **19** became the minor one (16%). These observations indicate that the isomerization of **20** to **18** takes place stepwise through **19** and that isomerization of **20** to **19** is faster than that of **19** to **18** (Scheme 3). The thermodynamic instability of **19** and **20** compared with **18** would be due to the repulsion between the oxygen atom(s) with the vicinal hydrogen atom.



Scheme 3. Isomerization of **20** to **18** through **19**.

2.3. Desulfuration of 7

Desulfuration of **7** was examined in expectation of the formation of the corresponding 1,2-dithietane.[42,43] Trithiolane **7** was treated with triphenylphosphine (1 equiv) in $CDCl_3$ at room temperature to give *cis*-episulfide **23**[26] (22%) with recovery of **7** (75%) (Eq. 7). Compound **23** would be formed by an intramolecular S_N2 reaction as shown in Scheme 4.



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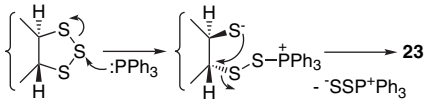

Scheme 4.

3. Conclusion

The reaction of *trans*-cyclooctene with S_8O at room temperature yielded *trans*-9,10,11-trithiabicyclo[6.3.0]undecane (**7**), a new 1,2,3-trithiolane, stereospecifically, together with the corresponding *trans*-episulfide. In this reaction S_8O behaved like elemental sulfur activated under vigorous conditions and worked towards *trans*-cyclooctene as an S_n-transfer reagent under mild conditions, which should be contrasted with S_8O acting as an S_2O-transfer reagent towards diazomethanes,[31] 1,3-butadienes,[31] and cycloheptatriene.[44] Oxidation of **7** with DMD provided two 9-oxides (**15** and **16**), one 10-oxide (**17**), and three 9,11-dioxides (**18**, **19**, and **20**). The 9,11-dioxides are the first example of stable 1,2,3-trithiolane 1,3-dioxides. Configuration of the sulfinyl groups in **15**, **16**, **19**, and **20** was relatively unstable and we observed mutual isomerization between **15** and **16** and stepwise isomerization of **20** to **18** through **19**.

4. Experimental

4.1. General

The melting points were determined on a Mel-Temp capillary tube apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were determined on Bruker AM400 or DRX400 (400 and 100.7 MHz, respectively) spectrometers using $CDCl_3$ as the solvent at 25 °C, unless otherwise noted. IR spectra were taken on a Perkin–Elmer System 2000 FT-IR spectrometer. Mass spectra were determined on a JEOL JMS-700AM spectrometer operating at 70 eV in the EI mode. Elemental analysis was performed by the Chemical Analysis Center of Saitama University. Column chromatography was performed with silica gel (70–230 mesh), and high-pressure liquid chromatography (HPLC) with a packed SiO_2 column (INERTSIL PREP-SIL: 10 or 20 mm i.d., GL Science Inc.); the eluent is shown in parentheses. *trans*-Cyclooctene was prepared by photoisomerization of commercially available *cis*-cyclooctene.[35,36] S_8O was prepared

by oxidation of S_8 with trifluoroperacetic acid.[32] An acetone solution of dimethyldioxirane (DMD) was prepared by oxidation of acetone with Oxone® (Sigma–Aldrich).[37,38]

4.2. Reaction of *trans*-cyclooctene with S_8O

trans-Cyclooctene (305 mg, 2.77 mmol) was added to a solution of S_8O (4.10 g, 15 mmol) in CS_2 (70 mL) under argon. The mixture was stirred for 13 h at room temperature. After evaporation of the solvent under reduced pressure, the residue was subjected to column chromatography (hexane/dichloromethane 4/1) to remove elemental sulfur and then subjected to HPLC (hexane/dichloromethane 4/1) to give *trans*-9,10,11-trithiabicyclo[6.3.0]undecane (**7**) (188 mg, 33%) and *trans*-episulfide **6** (52 mg, 13%) in this order. Trithiolane **7** was purified by bulb-to-bulb distillation (1.2 mmHg, 115 °C).

4.2.1. *trans*-9,10,11-Trithiabicyclo[6.3.0]undecane (7). Yellow oil. ^{1}H NMR δ 1.54–1.84 (m, 10H), 2.14–2.22 (m, 2H), 3.78–3.84 (m, 2H); ^{13}C NMR δ 24.6 (CH_2), 26.5 (CH_2), 34.8 (CH_2), 63.5 (CH); MS *m*/*z* 206 (M^+). HRMS: calcd for $C_8H_{14}S_3$: M, 206.0258. Found: M^+, 206.0258. Anal. Calcd for $C_8H_{14}S_3$: C, 46.55; H, 6.84. Found: C, 46.70; H, 6.86.

4.3. Oxidation of trithiolane 7

4.3.1. DMD (1 equiv). DMD (0.0753 M, 2.70 mL, 0.20 mmol) was added dropwise to a solution of **7** (42 mg, 0.20 mmol) in dichloromethane (2 mL) under argon at 0 °C. The mixture was stirred for 2 h at 0 °C. The mixture was transferred to a round-bottom flask and the solvent was removed under reduced pressure with the flask being dipped in an ice-water bath. The residue was subjected to column chromatography (dichloromethane) to give trithiolane **7** (4.0 mg, 9%), 10-oxide **17** (6.4 mg, 15%), a mixture of 9-oxides **15** and **16**, and 9,11-dioxide **18** (3.7 mg, 8%) in this order. The mixture of **15** and **16** was separated with HPLC (hexane/ether 2/1) to give 9-oxide **15** (6.8 mg, 16%) and 9-oxide **16** (14.5 mg, 33%).

4.3.2. DMD (2 equiv). DMD (0.0753 M, 2.63 mL, 0.20 mmol) was added dropwise to a solution of **7** (20.4 mg, 0.10 mmol) in dichloromethane (1 mL) under argon at −35 °C. The mixture was stirred for 2 h at −35 °C. At this temperature the solvent was removed under reduced pressure. The ^{1}H NMR spectrum of the mixture showed that the mixture consisted of **18**, **19**, **20**, and **7** in the ratio of 21:64:10:5. Compound **20** could not be isolated because of its instability.

4.3.3. MCPBA (1 equiv). A solution of MCPBA (88.6%, 40.4 mg, 0.21 mmol) in dichloromethane (3 mL) was added dropwise to a solution of **7** (43.8 mg, 0.21 mmol) in dichloromethane (2 mL) under argon at 0 °C. The mixture was stirred for 2 h at 0 °C. To the mixture were added aqueous Na_2SO_3 and then aqueous $NaHCO_3$. The mixture was extracted with dichloromethane, and the organic layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated to dryness. The residue was subjected to column chromatography (dichloromethane) to give 10-oxide **17** (3.3 mg, 7%), a mixture of **15** and **16**, and 9,10-dioxide **18** (2.1 mg, 4%) in this order. The mixture of **15** and **16** was separated with HPLC (hexane/ether 2/1) to give 9-oxide **15** (9.4 mg, 20%) and 7-oxide **16** (24.9 mg, 53%).

4.3.4. *rel*-(1*R*,8*R*,9*S*)-9,10,11-Trithiabicyclo[6.3.0]undecane 9-oxide (15). Colorless oil. ^{1}H NMR δ 1.55–1.88 (m, 9H), 1.91–2.00 (m, 1H), 2.16–2.26 (m, 2H), 3.39–3.44 (m, 1H), 3.92–3.98 (m, 1H); ^{13}C NMR δ 23.9 (CH_2), 24.7 (CH_2), 26.2 (CH_2), 26.4 (CH_2), 27.5 (CH_2), 34.9 (CH_2), 57.2 (CH), 79.9 (CH); IR (neat) 1094 cm^{-1} (S=O); MS *m*/*z* 222 (M^+). HRMS: calcd for $C_8H_{14}OS_3$: M, 222.0207. Found: M^+, 222.0201.

4.3.5. *rel*-(1*R*,8*R*,9*R*)-9,10,11-Trithiabicyclo[6.3.0]undecane 9-oxide (16). Colorless oil. ^{1}H NMR δ 1.38–1.47 (m, 1H), 1.50–1.88 (m, 9H), 2.22–2.37 (m, 2H), 3.48–3.54 (m, 1H), 3.64–3.69 (m, 1H); ^{13}C NMR δ 22.6 (CH_2), 24.7 (CH_2), 25.9 (CH_2), 26.5 (CH_2), 29.4 (CH_2), 33.5 (CH_2), 60.2 (CH), 86.6 (CH); IR (neat) 1088 cm^{-1} (S=O); MS *m*/*z* 222 (M^+). HRMS: calcd for $C_8H_{14}OS_3$: M, 222.0207. Found: M^+, 222.0209.

4.3.6. 9,10,11-Trithiabicyclo[6.3.0]undecane 10-oxide (17). Colorless oil. ^{1}H NMR δ 1.53–1.74 (m, 5H), 1.79–1.90 (m, 3H), 1.98–2.15 (m, 2H), 2.22–2.37 (m, 2H), 4.19 (ddd, *J*=11.3, 8.1, 3.5 Hz, 1H), 4.60 (ddd, *J*=11.3, 8.6, 3.0 Hz, 1H); ^{13}C NMR δ 24.3 (CH_2), 25.2 (CH_2), 25.3 (CH_2), 26.4 (CH_2), 26.5 (CH_2), 27.6 (CH_2), 60.8 (CH), 70.2 (CH); IR (neat) 1107 cm^{-1} (S=O); MS *m*/*z* 222 (M^+). HRMS: calcd for $C_8H_{14}OS_3$: M, 222.0201. Found: M^+, 222.0200.

4.3.7. *rel*-(1*R*,8*R*,9*S*,11*S*)-9,10,11-Trithiabicyclo[6.3.0]undecane 9,11-dioxide (18). Colorless plates, mp 114–116 °C (dichloromethane/ethanol). ^{1}H NMR δ 1.50–1.60 (m, 2H), 1.65–1.82 (m, 4H), 2.03–2.08 (m, 2H), 2.26–2.35 (m, 2H), 2.57–2.65 (m, 2H), 4.23–4.25 (m, 2H); ^{13}C NMR δ 24.6 (CH_2), 25.2 (CH_2), 26.1 (CH_2), 73.4 (CH); IR (KBr) 1066 cm^{-1} (S=O); MS *m*/*z* 238 (M^+). HRMS: calcd for $C_8H_{14}O_2S_3$: M, 238.0156. Found: M^+, 238.0140.

4.3.7.1. Crystallographic data for 18. $C_8H_{14}O_2S_3$, M_w=238.39, colorless plate, 0.34×0.24×0.08 mm^3, monoclinic, $P2_1/c$, a=7.3540(3), b=16.4810(8), c=9.5776(7) Å, β=115.430(2)°, V=1048.34(10) $Å^3$, ρ_{calcd}=1.510 g cm^{-3}, Z=4, μ (Mo Kα)=0.672 cm^{-1}. Mac Science DIP3000 diffractometer with a graphite-monochromated Mo Kα radiation (λ=0.71073 Å). The data reduction was made by the maXus program system. Intensity data of 2045 unique reflections were collected in the range of $-9\leq h\leq 9$, $-20\leq k\leq 20$, and $-11\leq l\leq 12$. Absorption corrections were done by a multi-scan method (SORTAV[45]). The structure was solved with a direct method (SIR97[46]) and refined with full-matrix least-squares (SHELXL-97[47]) using all independent reflections, where nonhydrogen atoms were refined anisotropically and hydrogen atoms were refined isotropically. $R1$=0.0524 ($I\geq 2\sigma I$, 1858 reflections), $wR2$=0.1394 (for all), and GOF=1.038, 175 parameters; max/min residual electron density=0.771/−0.604 e$Å^{-3}$.

4.3.8. *rel*-(1*R*,8*R*,9*R*,11*S*)-9,10,11-Trithiabicyclo[6.3.0]undecane 9,11-dioxide (19). Colorless plates, mp 109–111 °C (dichloromethane/ethanol). ^{1}H NMR δ 1.51–1.80 (m, 5H), 1.81–1.87 (m, 2H), 1.93–2.02 (m, 1H), 2.09–2.19

(m, 1H), 2.30–2.39 (m, 1H), 2.41–2.48 (m, 1H), 2.49–2.58 (m, 1H), 3.57 (ddd, J=12.1, 9.4, 2.5 Hz, 1H), 4.82 (ddd, J=12.0, 7.7, 3.9 Hz, 1H); ^{13}C NMR δ 23.9, 24.2, 24.9, 26.3, 26.5, 28.5, 73.9, 81.2; IR (KBr) 1083, 1059 cm^{-1} (S=O); MS m/z 238 (M^+). HRMS: calcd for $C_8H_{14}O_2S_3$: M, 238.0156. Found: M^+, 238.0146.

4.3.8.1. Crystallographic data for 19. $C_8H_{14}O_2S_3$, M_w=238.39, colorless plate, 0.34×0.22×0.08 mm^3, orthorhombic, *Pbca*, a=13.285(3), b=16.608(3), c=9.411(2) Å, V=2067.4(7) Å^3, ρ_{calcd}=1.525 g cm^{-3}, Z=8, μ(Cu Kα)=6.259 cm^{-1}. Mac Science MXC3KHF diffractometer with graphite-monochromated Cu Kα radiation (λ=1.54178 Å), $\theta/2\theta$ scans method in the range $3°<2\theta<140°$ ($0<h<16, 0<k<20, 0<l<11$), 1938 independent reflections. Absorption correction was done by the psi-scan method.[48] The structure was solved with a direct method (SIR97[46]) and refined with full-matrix least-squares (SHELXL-97[47]) using all independent reflections, where nonhydrogen atoms were refined anisotropically. Hydrogen atoms were placed by calculation. $R1$=0.0862 ($I>2\sigma I$, 1907 reflections), $wR2$=0.2264 (for all), GOF=1.146, 119 parameters; max/min residual electron density=1.947/−0.709 eÅ^{-3}.

CCDC-299893 (**18**) and CCDC-299892 (**19**) contain the supplementary crystallographic data. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk].

4.3.9. *rel*-(1*R*,8*R*,9*R*,11*R*)-9,10,11-Trithiabicyclo[6.3.0]-undecane 9,11-dioxide (20). ^{1}H NMR (−20 °C) δ 3.72–3.80 [m, 2H, C(1)–H and C(8)–H]; ^{13}C NMR (−20 °C) δ 23.3, 25.5, 27.9, 75.8.

4.4. Oxidation of 9-oxide 15

DMD (0.0803 M, 0.50 mL, 0.040 mmol) was added to a solution of 9-oxide **15** (9.1 mg, 0.041 mmol) in $CDCl_3$ (1 mL) under argon at 0 °C. The mixture was stirred for 2 h at 0 °C. To 0.5 mL of the mixture, was added 6.5 mg (0.018 mmol) of 1,2,3,4-tetraphenyl-1,3-cyclopentadiene as the internal standard. The ^{1}H NMR spectrum of the mixture showed the formation of 0.018 mmol (43%) of 9,11-dioxide **18** and 0.019 mmol (47%) of 9,11-dioxide **19**.

4.5. Oxidation of 9-oxide 16

DMD (0.0803 M, 0.85 mL, 0.068 mmol) was added to a solution of 9-oxide **16** (14.6 mg, 0.0656 mmol) in $CDCl_3$ (1 mL) under argon at 0 °C. The mixture was stirred for 2 h at 0 °C. To 0.5 mL of the mixture, was added 7.2 mg (0.019 mmol) of 1,2,3,4-tetraphenyl-1,3-cyclopentadiene as the internal standard. The ^{1}H NMR spectrum of the mixture showed the formation of 0.0041 mmol (2%) of 9,11-dioxide **18**, 0.023 mmol (35%) of 9,11-dioxide **19**, and 0.035 mmol (54%) of 9,11-dioxide **20**.

4.6. Isomerization

4.6.1. Isomerization of 9-oxide 15. In an NMR tube, a solution of 9-oxide **15** (2.1 mg, 0.094 mmol) in $CDCl_3$ (0.4 mL) was warmed at 55 °C for 24 h (during heating, indoor light was not shaded in particular). The mixture consisted of 30% of 9-oxide **15** and 70% of 9-oxide **16** based on the ^{1}H NMR integral ratio.

4.6.2. Isomerization of 9-oxide 16. In an NMR tube, a solution of 9-oxide **16** (2.3 mg, 0.010 mmol) in $CDCl_3$ (0.4 mL) was warmed at 55 °C for 24 h (during heating, indoor light was not shaded in particular). The mixture consisted of 29% of 9-oxide **15** and 71% of 9-oxide **16** based on the ^{1}H NMR integral ratio.

4.7. Reaction of trithiolane 7 with triphenylphosphine

A solution of triphenylphosphine (26.2 mg, 0.10 mmol) in $CDCl_3$ (1.5 mL) was added dropwise to a solution of trithiolane **7** (21.6 mg, 0.10 mmol) in $CDCl_3$ (1 mL) under argon at room temperature. The mixture was stirred for 5 h at room temperature. To 0.5 mL of the mixture, was added 10.6 mg (0.029 mmol) of 1,2,3,4-tetraphenyl-1,3-cyclopentadiene as the internal standard. The ^{1}H NMR spectrum of the mixture showed the formation of 0.022 mmol (22%) of *cis*-episulfide **23**[27] together with 0.075 mmol (75%) of **7**.

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Mechanism of catalytic asymmetric hydrogenation of 2-formyl-1-methylene-1,2,3,4-tetrahydroisoquinoline using $Ru(CH_3COO)_2$[(*S*)-binap]

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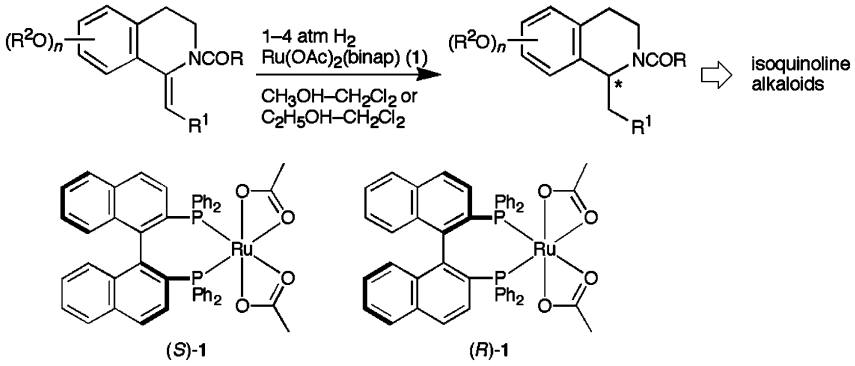
Abstract—The mechanism of the asymmetric hydrogenation of 2-acyl-1-alkylidene-1,2,3,4-tetrahydroisoquinolines, the first reported reaction with the Noyori–Takaya $Ru(CH_3COO)_2$(binap) complex, has been investigated by means of deuterium labeling, kinetics, and NMR analysis. A series of experiments has revealed that (1) a monohydride-unsaturated mechanism operates involving the initial formation of RuH followed by reaction with the enamide substrate, (2) the hydride transfer from RuH to the olefinic double bond is endothermic and reversible, and (3) the rate is determined in the hydrogenolysis step. This view is consistent with that of proposed for the BINAP–Ru catalyzed Kagan reaction.

1. Introduction

The Ru(II) dicarboxylate complex **1** with 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) is a milestone catalyst in asymmetric hydrogenation chemistry. The complex was reported in 1986 in the hydrogenation of 2-acyl-1-alkylidene-1,2,3,4-tetrahydroisoquinolines, opening a new route to the general asymmetric synthesis of biologically active isoquinoline alkaloid derivatives such as morphine, morphinane, benzomorphane, and salsolidine (Scheme 1).[1] This initial reaction has prompted the asymmetric hydrogenation of a variety of functionalized olefins including unsaturated alcohols, carboxylic acids, and phosphonic acid derivatives, making a great impact in both industry and academia.[2] However, in deference to Kagan's 1971 report,[3] in which α-(acylamino)acrylic acids or esters were first used successfully in combination with a DIOP–Rh(I) complex, we have studied the mechanisms of the BINAP–Ru analogues of his system.[4] In response to many inquiries,[1] we now describe in full the details of the mechanism of our original reaction system using **1**.

Based on the results obtained in the mechanistic study of BINAP–Ru catalyzed hydrogenation in the Kagan system,[4] the reaction in Scheme 1 is thought to proceed via a monohydride-unsaturated route as shown in Scheme 2. First, (*S*)-**1**



Scheme 1. BINAP–Ru catalyzed hydrogenation toward the general asymmetric synthesis of isoquinoline alkaloids.

Keywords: Asymmetric hydrogenation; BINAP–Ru; 2-Acyl-1-alkylidene-1,2,3,4-tetrahydroisoquinoline; Monohydride-unsaturated mechanism.
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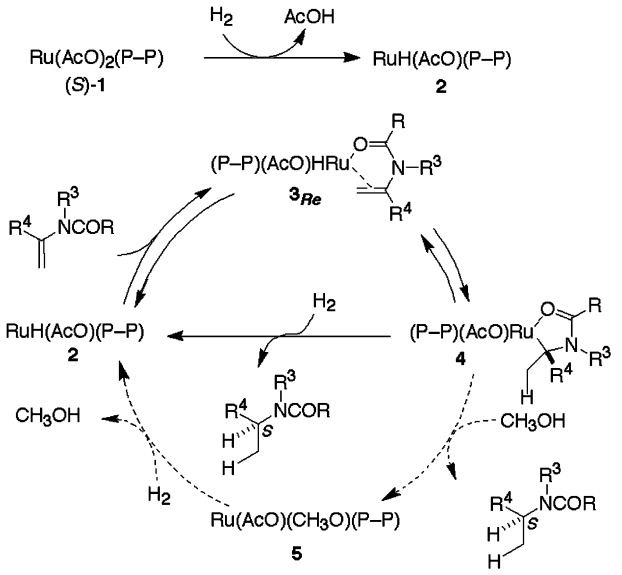
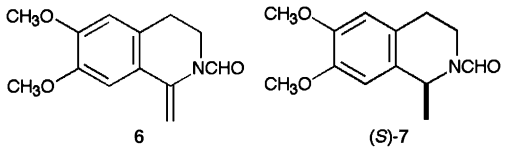
reacts with molecular hydrogen to generate the monohydride complex **2**, which then interacts with an enamide. In a short-lived substrate–catalyst complex **3**, the olefin inserts into the Ru–H bond to generate the five-membered metallacycle **4** in a reversible manner. The Ru–C bond in **4** is preferentially cleaved by H_2 rather than CH_3OH to regenerate **2** together with release of the saturated product, completing the catalytic cycle. Protonolysis of the Ru–C linkage by a coordinated methanol occurs to a lesser extent, giving the product and the Ru(II) species **5**. The Ru–OCH_3 bond in **5** is hydrogenolyzed to regenerate the catalyst **2**.



Scheme 2. Supposed catalytic cycle of (*S*)-BINAP–Ru catalyzed hydrogenation of 1-methylene tetrahydroisoquinoline in methanol. Tetrahydroisoquinoline aromatic rings in the substrates are omitted for clarity. P–P=(*S*)-BINAP.

To confirm the present mechanistic view, the simple 2-formyl-1-methylene-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**6**), for which there is no problem of *Z*/*E* isomerism, was selected as a standard substrate to do a series of experiments: (1) examination of the isotope incorporation patterns of the product obtained under H_2/CH_3OD, HD/CH_3OD, and D_2/CH_3OD conditions, (2) kinetic study to deduce the order of the reaction, and (3) rate law analysis to determine the energy profile of the catalytic cycle.



2. Results and discussion

The optimized reaction conditions ([(*S*)-**1**]$_0$=0.075 mM, [**6**]$_0$=15 mM (substrate/catalyst ratio (S/C)=200:1), temperature=30 °C, 5:1 CH_3OH/CH_2Cl_2) in the original 1986 report quantitatively gives (*S*)-2-formyl-1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline [(*S*)-**7**] in 97% ee.[1a,1c] As the initial concentration of **6** is too dilute to conduct an effective rate measurement by monitoring the total pressure decrease in the closed reaction vessel (vide infra), the concentration of **6** was set to 100 mM in a 1:1 CH_3OH/CH_2Cl_2 solvent system. The increase in the CH_2Cl_2 ratio solves the low solubility problem at the cost of enantioselectivity. Thus, under the following conditions, [(*S*)-**1**]$_0$= 0.5 mM, [**6**]$_0$=100 mM (S/C=200:1), temperature=30 °C, H_2 pressure=2–16 atm, reaction time=12–24 h, (*S*)-**7** was obtained quantitatively in 89–94% ee. A significant decrease in ee (67%) was observed at 100 atm. The pressure effect is similar to that observed in the reactions of methyl (*Z*)-α-(acetamido)cinnamate[4] and dehydro amino phosphonic acids.[5]

2.1. Isotopomer ratios

By detailed analysis of the isotopomeric products, **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d*, which are obtained by the reactions in a 1:1 mixture of CH_3OD and CH_2Cl_2 using HD, H_2, or D_2 it is possible to discriminate between monohydride/dihydride mechanisms as well as hydrogenolysis/protonolysis routes. The ratio of these four isotopomers can be determined by spectral analysis of phase-sensitive $^{13}C\{^1H,^2H\}$–^1H correlation NMR and $^{13}C\{^1H\}$ NMR. Figure 1 shows the β-carbon region of the correlation spectrum of a mixture of **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d* in a 19:30:17:33 ratio. The ^{13}C signals of these four isotopomers appear at δ 20.86 (s), 20.75 (s), 20.58 (s), and 20.47 (s), respectively, which are correlated with β-proton signals at δ 1.448 (d, *J*=6.3 Hz), 1.440 (s), 1.432 (d, *J*=6.3 Hz), and 1.424 (s), respectively. Considering the coupling patterns of ^1H-decoupled ^{13}C and ^1H signals, the four carbon signals are unequivocally assigned to **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d* from low to high field. This is consistent with the empirical rule that carbon

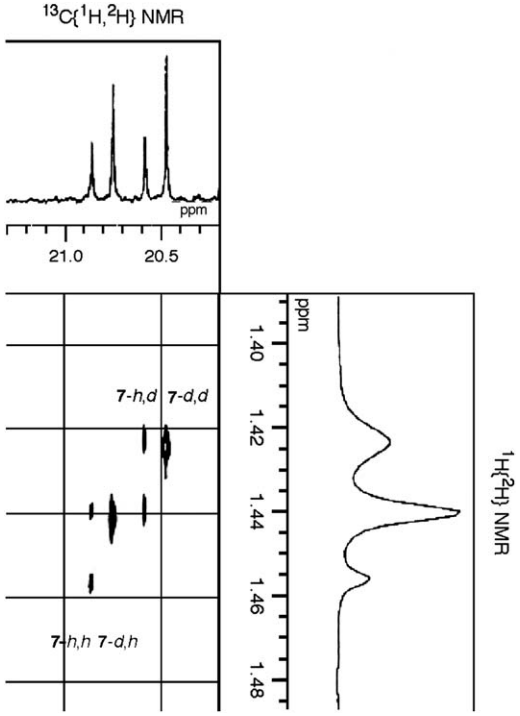
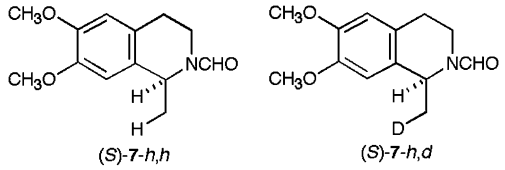


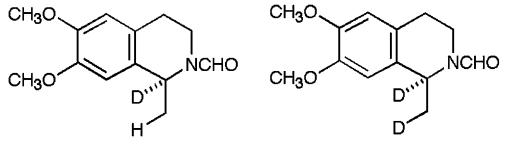
Figure 1. Phase-sensitive $^{13}C\{^1H,^2H\}$–^1H correlation spectrum (β position) of a 19:30:17:33 mixture of **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d* and **7**-*d*,*d* in $CDCl_3$ at 23 °C.

atoms bearing deuterium resonate at a higher field in proportion to the number of deuterium.[6] Another set of four ^{13}C signals around δ 23.5 with similar correlation patterns to above is assignable to the amide conformers.[7]



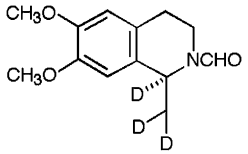
(*S*)-**7**-*h*,*h*

(*S*)-**7**-*h*,*d*



(*S*)-**7**-*d*,*h*

(*S*)-**7**-*d*,*d*



(*S*)-**7**-*d*,d_2

The deuterium labeling experiments at 4 atm under the above standard conditions gave the *S*-enriched isotopomers in 93% ee. The results of the product analysis are summarized in Table 1. The reactions using HD and H_2 were stopped at an early stage of conversion (7–16%) to avoid the complication caused by gas/solvent and gas/gas isotope exchange.[8] The initial proportion of the isotopes in gas and solvent was confirmed to be virtually unchanged by careful GC analysis of the unreacted hydrogen[9] and GC–MS analysis of the recovered solvent.[4a] The use of HD/CH_3OD (H_2/HD/D_2=1:98:1) afforded, after 7% conversion, **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d* in a 25:30:14:30 ratio with an average ee of 93%. The approximately even distribution amongst the four isotopomers indicates that RuH but not RuH_2 is operating as the catalytic species. If the dihydride mechanism were predominant,[10,11] **7**-*d*,*h* and **7**-*h*,*d* would be the major isotopomers. Replacement of HD with H_2 gave, after 16% conversion, a mixture of **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d* in an 82:13:5:0 ratio. This distribution indicates that the RuH species generated from (*S*)-**1** and H_2 delivers its H to the β carbon of enamide **6**, giving a five-membered metallacycle intermediate **4**. The Ru–C bond is then cleaved by H_2 and CH_3OD in an 82:13 ratio. Preference for the hydrogenolysis route is also supported by the 5:0 ratio of **7**-*h*,*d* and **7**-*d*,*d*. The enamide bond C_α=C_β is inserted into the RuD species, which is probably formed by H/D exchange between the RuH chain carrier and CH_3OD,[8] to form Ru–C_α–C_β–D. Cleavage of the Ru–C_α bond with H_2 produces **7**-*h*,*d*. Use of D_2 and CH_3OD under the standard conditions requires 49 h to complete the reaction. As would be expected, the isotopomeric product is **7**-*d*,*d*. However, a significant amount of **7**-*d*,d_2 (6%) was obtained, and the amount of **7**-*d*,d_2 was increased to 16% at 1 atm. The incorporation of multiple deuterium atoms at the β carbon suggests that the migratory insertion of RuH in C_α=C_β is reversible.[4a,b]

Table 1. Analysis of *S*-enriched product **7** obtained by isotope labeling experiments using **6** and (*S*)-**1**[a] in a 1:1 mixture of CH_3OD and CH_2Cl_2

	Gas		
	HD	H_2	D_2
Time	15 min	16 min	49 h
Conversion, %	7	16	>99
% ee[b]	93	93	93
Product distribution, %			
7-*h*,*h*	25	82	<1
7-*d*,*h*	30	13	<1
7-*h*,*d*	14	5	<1
7-*d*,*d*	30	0	94
7-*d*,d_2	1	0	6

[a] Reactions were carried out at 4 atm under the standard conditions. The detailed procedures for the reaction and analysis are described in Section 4. Solvent CH_3OD contains 0.5% of CH_3OH. HD gas contains 1% each of H_2 and D_2. D_2 gas contains 0.4% of HD.

[b] The ee was determined by HPLC analysis.

2.2. Rate measurements

The rate of hydrogenation of **6** with (*S*)-**1** in a 1:1 mixture of methanol and CH_2Cl_2 under 4 atm of H_2 at 30 °C was determined by monitoring the decrease in hydrogen pressure in a closed Teflon-coated hydrogenation vessel. The relationship between ln[**6**]$_t$ and time was reasonably linear for the initial concentrations, [**6**]$_0$=100 mM and [(*S*)-**1**]$_0$=0.50 mM, indicating a pseudo first-order dependence on the enamide concentration in the reaction system. Therefore, the rate is described simply by the rate law $-d[\mathbf{6}]/dt=k_{obs}[\mathbf{6}]$, where k_{obs} is 6.0×10^{-3} min^{-1}. In order to deduce the rate law and to gain insight into the catalytic cycle, the reaction rates were measured at varying concentrations of (*S*)-**1** and hydrogen pressures. As shown in Figure 2a and b, the reaction follows first-order kinetics both in [(*S*)-**1**] and in H_2 in a boundary region of the standard conditions ([(*S*)-**1**]$_0$=0.25–1.0 mM; 2–16 atm H_2). On the basis of this kinetic study, the present catalysis can be simply viewed as a bis-substrate/uni-product system. The catalyst **2** reversibly binds **6** to form a catalyst–substrate complex, which then transfers a hydride to **6**. The resulting intermediary Ru–alkyl complex **4** reacts with H_2, releasing the product by regenerating the catalyst **2**. As the monohydride formation step from (*S*)-**1** to **2** is independent of hydrogen pressure and controlled only by acid/base thermodynamics under the standard conditions,[4a] the total concentration of the Ru hydride species, either active or inactive, is constant ([**2**]=a[(*S*)-**1**]$_0$).[12] Thus, the turnover rate is limited by the hydrogenolysis step but not by the hydride-transfer step or by the hydride formation step.

2.3. Rate law analysis

Since the rate of conversion of enamide is estimated by that of the turnover-limiting step **4**→**2**, by assuming a steady-state for [**4**] together with the relation [**2**]=a[(*S*)-**1**]$_0$, the rate law can be expressed as Eq. 1.

$$-d[\mathbf{6}]/dt = ak_1[(S)\text{-}\mathbf{1}]_0[\mathbf{6}](k_2[H_2]+k_3[CH_3OH]) \times (k_{-1}+k_2[H_2]+k_3[CH_3OH])^{-1} \quad (1)$$

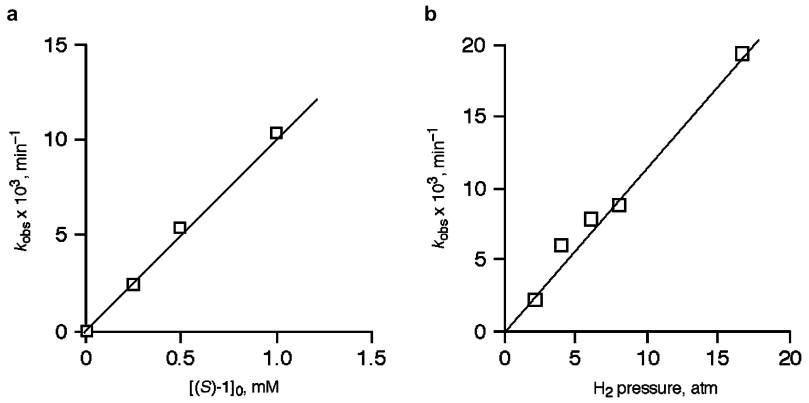


Figure 2. Dependence of $[(S)\text{-}\mathbf{1}]_0$, and H_2 pressure on the rate constant k_{obs} in hydrogenation of **6** ($[\mathbf{6}]_0$=100 mM) in a 1:1 mixture of CH_3OH and dichloromethane at 30 °C. a: Plots of k_{obs} as a function of $[(S)\text{-}\mathbf{1}]_0$ (4 atm H_2). b: Plots of k_{obs} as a function of H_2 pressure ($[(S)\text{-}\mathbf{1}]_0$=0.50 mM).

Here, k_1 is for the intramolecular hydride transfer, **2**→**4**, k_{-1} for the β-elimination, **4**→**2**, k_2 for the hydrogenolysis, **4**→**2**, and k_3 for the methanolysis, **4**→**5**. In order to satisfy the experimental results (the first-order kinetics in $[(S)\text{-}\mathbf{1}]_0$ and $[H_2]$), the $k_2[H_2]+k_3[CH_3OH]$ term in the denominator of the rate law should be negligible in comparison to k_{-1}. The inequality $k_{-1} \gg k_2[H_2]+k_3[CH_3OH]$ indicates that the rate of β-elimination is significant in comparison to the overall rate of reaction expressed as Eq. 2, and that the hydride-transfer step is endothermic. The energy profile obtained by the rate law analysis is consistent with formation of **7**-d,d_2 under D_2/CH_3OD conditions.

$$-\mathrm{d}[\mathbf{6}]/\mathrm{d}t = ak_1k_{-1}^{-1}[(S)\text{-}\mathbf{1}]_0[\mathbf{6}](k_2[H_2] + k_3[CH_3OH]) \qquad (2)$$

3. Conclusion

Deuterium labeling experiments, kinetic studies as well as rate law analysis have suggested that the (*S*)-BINAP–Ru catalyzed hydrogenation of 2-formyl-1-methylene-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline proceeds via a monohydride-unsaturated mechanism in a similar way as α-(acylamino)acrylic esters. An RuH species, which is first generated from the diacetate and H_2 with the liberation of acetic acid, forms a short-lived catalyst–substrate complex. A reversible and endothermic intramolecular hydride transfer from RuH to the β carbon of the coordinating enamide substrate occurs to generate the five-membered metallacycle, the Ru–C bond of which is then cleaved largely by H_2 and partly by protic CH_3OH. Consequently, the two protiums at the α and β positions of the enamide substrate are derived from two different hydrogen molecules. The overall rate is limited by the hydrogenolysis step. Although the present results can be used only for the mechanism in the formation of the major enantiomeric product, the minor product may also be expected to be generated via the same monohydride-unsaturated pathway but diastereomorphic to the major cycle.[13] Higher enantioselectivity with **6** than with α-(acylamino)acrylic esters can be ascribed to a bulkier substituent at the α-position and to a restricted ring system. The structural features would enhance the stereo-complementarity in the major RuH/enamide complexes, resulting in the higher $\Delta\Delta G^{\ddagger}$. Consistent with the model, dehydro amino phosphonic acids[5] and *N*-acyl-4-methylene-1,3-oxazolidin-2-ones[14] show high enantioselectivities in our catalyst system.

4. Experimental

4.1. General

Nuclear magnetic resonance (NMR) spectra were measured on a JEOL JNM-A400 equipped with a pulsed field gradient unit and a triple-resonance probe or a JEOL JNM-ECP500 instrument. The chemical shifts are expressed in parts per million (ppm) downfield from $Si(CH_3)_4$ or in ppm relative to $CHCl_3$ (δ 7.26 in ^{1}H NMR and δ 77.0 in ^{13}C NMR). Signal patterns of ^{1}H NMR as well as ^{13}C NMR are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal. GC–MS analyses were conducted using a Shimadzu QP-5000. All melting points were determined on a Yanaco melting point apparatus and were uncorrected. Analytical thin-layer chromatography (TLC) was performed using Merck 5715 indicating plates precoated with silica-gel 60 F254 (layer thickness 0.25 mm). Liquid chromatographic purifications were performed by flash column chromatography, using glass columns packed with Merck 9385 (230–400 mesh). All manipulations in the BINAP–Ru catalyzed hydrogenation were carried out by using standard Schlenk techniques on a dual manifold vacuum/Ar system. The conversions of **6** to **7** were determined by comparing ratios of the formyl ^{1}H NMR signals at δ 8.64 (sickle isomer of **6**), δ 8.38 (U isomer of **6**), δ 8.28 (**7**), and δ 8.12 (**7**).[7] The % ee of **7**, after deprotection of the formyl group, was determined by the GITC (2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl isothiocyanate) method employing a reversed-phase C_{18} silica-gel column (column, Nomura Chemical Co. Develosil ODS-5; eluent, 1:2 CH_3CN/H_2O containing ammonium phosphate (1.4 g/L); flow rate, 1.0 mL/min; detection, 254-nm light, retention times (t_R) of the thiourea of (*R*)-**7** and (*S*)-**7**, 71.6 min and 74.9 min).[1c] $H_2/HD/D_2$ and CH_3OH/CH_3OD analyses were conducted by the reported methods.[4a,13a]

4.2. Materials

CH_3OH and CH_3OD for hydrogenation were degassed at refluxing temperature in the presence of Mg (250 mg/

100 mL) under an argon stream for 6 h and distilled into Schlenk flasks. CH_3OD contained 0.5% of OH species. CH_2Cl_2 was distilled from CaH_2. The solvent was degassed by three freeze–thaw cycles before hydrogenation. $CDCl_3$ was purchased from Aldrich and used without further purification. Argon gas was purified by passing through a column of the BASF catalyst R3-11 at 80 °C and then a column of $CaSO_4$ at room temperature. H_2 gas of 99.99999% grade and D_2 gas containing 0.4% HD were purchased from Nippon Sanso, and HD gas containing 1% H_2 and 1% D_2 was obtained from Isotec. These gases were used for hydrogenation without purification. $Ru(CH_3COO)_2$[(*S*)-binap] [(*S*)-**1**][15] and 2-formyl-6,7-dimethoxy-l-methylene-1,2,3,4-tetrahydroisoquinoline (**6**)[1c] were prepared by the reported methods.

4.3. Isotope labeling experiments

4.3.1. Structural determination of isotopomers. A mixture of **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d* in a 19:30:17:33 ratio containing 1% of **7**-*d*,d_2 was prepared by the following conditions (for the detailed procedure, see Sections 4.3.2 and 4.3.3): (*S*)-**1** (13.5 mg, 0.016 mmol), **6** (350 mg, 1.50 mmol), CH_3OD (7.5 mL), CH_2Cl_2 (7.5 mL), 4 atm HD, 30 °C, 329 h. The structures of the four isotopomers, **7**-*h*,*h*, **7**-*d*,*h*, **7**-*h*,*d*, and **7**-*d*,*d*, were determined by analysis of the phase-sensitive $^{13}C\{^1H,^2H\}$–^1H correlation spectrum taken in $CDCl_3$ at 24 °C using a JEOL JNM-A400 spectrometer. To minimize the magnetic field instability associated with the lack of a D-spin lock, measuring time was shortened by use of a high concentration of the sample (>100 mg/0.6 mL) under the following conditions: acquisition time=0.70371 s, pulse delay=1.2963 s, scan number=8, and measurement time=90 min.

4.3.2. H_2/CH_3OD conditions. The substrate **6** (1.7229 g, 7.3858 mmol), CH_3OD (36.9 mL), and CH_2Cl_2 (36.9 mL) were placed into a dry, argon-filled Schlenk tube, and the whole mixture was degassed by three freeze–thaw cycles. The solution was transferred via stainless cannula to another Schlenk tube containing a pale brown solid $Ru(CH_3COO)_2$[(*S*)-binap] [(*S*)-**1**] (31.1 mg, 36.9 μmol) under a slightly positive argon pressure. After being further degassed by two freeze–thaw cycles, the mixture was transferred to a 420-mL glass autoclave. Argon gas in the whole system was replaced three times by H_2, and the reaction vessel was pressurized to 4 atm. The yellowish solution was vigorously stirred at 30 °C. After 16 min, a portion of the unreacted gas in the reaction vessel was transferred to an 80-mL evacuated Schlenk tube equipped with a Young's tap, and then ca. 20 mL of the reaction mixture was collected in an argon-filled 80 mL Schlenk tube. The solvent was recovered by distillation. The $H_2/HD/D_2$ and CH_3OH/CH_3OD ratios were analyzed and found to be 99.5:0.5:0 and 1:99, respectively. The residue was subjected to ^1H NMR and HPLC analyses, by which the conversion and ee were determined to be 16% and 93%, respectively, on the basis of the methods described in Section 4.1.

The crude reaction mixture was separated into **6** and **7** by silica-gel chromatography (40 g; eluent; 2:1 hexane/ethyl acetate and then 1:3 hexane/ethyl acetate). The isotopomer ratio was determined by measurements of the $^{13}C_\beta$ signal area in the $^{13}C\{^1H,^2H\}$ NMR spectrum, which was taken using a JEOL JNM-A400 spectrometer under the following conditions: flip angle, 45°; acquisition time (AT), 2.1823 s; pulse delay (PD), 7.8177 s. ^1H and ^2H decoupling was affected only during acquisition time.

4.3.3. HD/CH_3OD conditions. The reaction was conducted similar to that for the H_2/CH_3OD system except for the size of the reactor (130-mL glass autoclave) and the use of HD instead of H_2. The whole reaction mixture was collected for further analysis. Reaction scale: 598 mg of **6**; $[(S)\text{-}\mathbf{1}]_0$= 0.5 mM; $[\mathbf{6}]_0$=100 mM; 4 atm of HD; CH_3OD; 30 °C; 15 min. The conditions converted 7% of **6** to give (*S*)-**7** in 93% ee (HPLC analysis). The $H_2/HD/D_2$ ratio was 3:95:2.

4.3.4. D_2/CH_3OD conditions. The reaction was conducted similar to that for the HD/CH_3OD system except for the use of D_2 instead of HD. Reaction scale, 202 mg of **6**; $[(S)\text{-}\mathbf{1}]_0$=0.5 mM; $[\mathbf{6}]_0$=100 mM; 4 atm of D_2; 1:1 CH_3OD/CH_2Cl_2; 30 °C; 49 h. The conditions converted >99% of **6** to give (*S*)-**7** in 93% ee (HPLC analysis). The $^{13}C\{^1H,^2H\}$ NMR signal of tri-deuterated isotopomer **7**-*d*,d_2 was assigned at δ 20.36 by the empirical rule.[9] A reaction under 1 atm of D_2 was conducted by use of a 1-L Schlenk tube with a Young's tap: reaction scale, 178 mg of **6**; $[(S)\text{-}\mathbf{1}]_0$=0.5 mM; $[\mathbf{6}]_0$=100 mM; 1 atm of D_2; 1:1 CH_3OD/CH_2Cl_2; 30 °C; 49 h, >99% conversion.

4.4. Kinetics

Kinetics investigations were conducted using a Teflon-coated stainless autoclave fitted with a variable temperature jacket and a magnetically controlled mechanical stirring system. A typical procedure is represented as follows. A yellow solution of a 1:1 mixture of CH_3OH (7.5 mL) and CH_2Cl_2 (7.5 mL) containing **6** (598 mg, 2.56 mmol) and (*S*)-**1** (6.3 mg, 7.5 μmol) in an Ar-filled 80-mL Schlenk tube was introduced into the Ar-filled autoclave by use of Ar pressure. The temperature of the autoclave was set to 30 °C, and the whole system was allowed to equilibrate for 10 min. The inner Ar gas was replaced with H_2 by three pressurization–release cycles, and then the pressure was adjusted to 4 atm. The reaction was started by closing the connection of the autoclave to the H_2 cylinder and then stirring the reaction mixture at 1200 rpm. The total H_2 pressure, which gradually decreased to 3.7 atm over the course of the reaction, was recorded by the pressure sensor for a total of 240 min. The observed rate constant k_{obs} was calculated to be 6.0×10^{-3} min^{-1} on the basis of percentage of conversion and the change in total pressure. The conversion was calculated to be 78% by ^1H NMR analysis. The ee of (*S*)-**7** was determined to be 89% by the GITC method. Likewise, the effects of catalyst concentration and H_2 pressure were examined under the standard conditions ($[\mathbf{6}]_0$=100 mM, solvent=a 1:1 mixture of CH_3OH and CH_2Cl_2, 30 °C). The conditions and the rate constant k_{obs} are as followings. $[(S)\text{-}\mathbf{1}]_0$=*0.25 mM*: (*S*)-**1** (3.2 mg, 3.8 μmol), 4.0 atm H_2, 360 min, 45% convn, 2.4×10^{-3} min^{-1}. After stirring for ca. 2 h, hydrogen pressure started to decrease. $[(S)\text{-}\mathbf{1}]_0$=*1.0 mM*: (*S*)-**1** (12.6 mg, 0.015 mmol), 4.0 atm H_2, 210 min, 90% convn, 1.0×10^{-2} min^{-1}; *2.1 atm* H_2: (*S*)-**1** (6.3 mg, 7.5 μmol), 300 min, 52% convn, 2.2×10^{-3} min^{-1}; *6.1 atm* H_2: (*S*)-**1** (6.3 mg, 7.5 mmol), 240 min, 85% convn, 7.9×10^{-3} min^{-1}; *8.1 atm*

H_2: (*S*)-**1** (6.3 mg, 7.5 μmol), 180 min, 83% convn, 8.8×10^{-3} min^{-1}; *16.6 atm* H_2: (*S*)-**1** (6.3 mg, 7.5 μmol), 120 min, 94% convn, 1.9×10^{-2} min^{-1}.

The reaction at 100 atm in a stainless autoclave[15b] (78 mg of **6**; $[(S)\text{-}\mathbf{1}]_0$=0.7 mM; $[\mathbf{6}]_0$=100 mM) at 30 °C for 48 h in a 1:1 mixture of CH_3OH (1.7 mL) and CH_2Cl_2 (1.7 mL) gave (*S*)-**7** in 67% ee with 91% conversion.

Acknowledgements

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Synthesis of highly-functionalised pyridines via hetero-Diels–Alder methodology: reaction of 3-siloxy-1-aza-1,3-butadienes with electron deficient acetylenes

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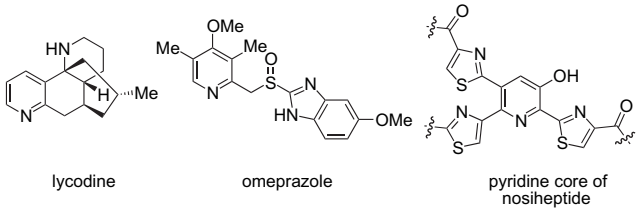
Abstract—The hetero-Diels–Alder reaction between 1-aza-3-siloxy-1,3-butadienes and electron deficient acetylenes is described. The reactivity of a range of α,β-unsaturated oximes and hydrazones is assessed in the synthesis of tri- and tetra-substituted pyridines bearing an oxygen functionality at C-3. Microwave irradiation has been employed to decrease the extended reaction times and increase the poor yields often associated with this reaction.

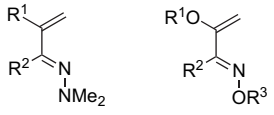
1. Introduction

The pyridine ring appears in a range of bioactive compounds, both naturally occurring and synthetic, often in highly substituted form; examples include the lycopodium alkaloids such as lycodine,[1] and the well-known proton-pump inhibitor omeprazole. Our own particular interest lies in the thiopeptide antibiotics, a class of sulfur containing highly modified cyclic peptides characterised by the presence of a heterocyclic centrepiece consisting of a tri- or tetra-substituted pyridine embedded in a macrocyclic array.[2] Examples of these types of natural products are amythiamicin D,[3] recently synthesised in our laboratory,[4] and nosiheptide.[5] In our synthesis of amythiamicin D, the pyridine core was successfully constructed via a biomimetic hetero-Diels–Alder reaction of a 2-azabutadiene. However, the presence of a hydroxyl group at C-3 (or C-5) of the pyridine ring, as in nosiheptide, presents a different challenge.[6] Therefore, as part of our ongoing work towards the synthesis of nosiheptide, we decided to investigate a complementary cycloaddition route to highly-functionalised pyridines, namely the hetero-Diels–Alder reaction of 1-aza-3-siloxy-1,3-butadienes with acetylenes.



lycodine omeprazole pyridine core of nosiheptide

Since the discovery by Ghosez and co-workers that *N*,*N*-dimethylhydrazones **1**, readily available from condensation of α,β-unsaturated aldehydes and *N*,*N*-dimethylhydrazine, participate readily in [4+2] cycloadditions,[7] the hetero-Diels–Alder reaction of 1-azabutadienes has proved to be a versatile method for the preparation of a large range of pyridines and dihydropyridines.[8–16] The high reactivity of these dienes towards electron deficient dienophiles has been attributed to the strong electron-donating effect of the dimethylamino substituent. Introduction of an additional electron-releasing substituent such as an alkyl (**1a**) or siloxy (**1b**) group into the C-3 position was also found to be beneficial.[9] In contrast, hetero-Diels–Alder reaction of analogous α,β-unsaturated oximes **2** have received relatively little attention in the literature.[17–23]



1a, R^1 = alkyl
1b, R^1 = $OSiR_3$

2

Keywords: Hetero-Diels–Alder reaction; Pyridines; Microwave irradiation.
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The scope of the hetero-Diels–Alder reaction of 1-azabutadienes has been extended by the discovery of Boger and Blagg that *N*-sulfonyl-2-(ethoxycarbonyl)-1-aza-1,3-butadienes participate in the [4+2] cycloadditions with electron rich dienophiles.[24] Fowler and co-workers have demonstrated that *N*-acyl-2-cyano-1-aza-1,3-butadienes may also be used.[25,26] In both cases cycloaddition takes place with inverse electron demand, in contrast to the hydrazones, which are considered as 'normal' electron rich dienes.

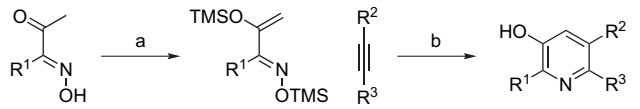
Herein we report our findings on the synthesis of highly-functionalised pyridines from 3-siloxy-1-aza-1,3-butadienes and electron deficient acetylenes. In particular, application of microwave irradiation has been investigated to decrease the relatively long reaction times commonly observed under standard thermal conditions. In accordance with Ghosez's initial observations, electron-donating substituents on nitrogen were found to accelerate the rate of cycloaddition.[9] Less electron rich dienes may also be employed, although higher temperatures and longer reaction times are often necessary.

2. Results and discussion

2.1. Synthesis and cycloadditions of α,β-unsaturated oximes

A range of 3-siloxy-1-azadienes **4a–d** was prepared from the corresponding free oximes **3a–d**[27–28] according to known or modified procedures,[17] and their reactivity in the hetero-Diels–Alder reaction was evaluated (Scheme 1). Furukawa and co-workers have reported that the cycloaddition of 1-azadiene **4a** and methyl ester **4b** with dimethyl acetylenedicarboxylate (DMAD, **5**) proceeds in 60% and 62% yield, respectively, after heating under reflux in benzene for 8 h.[17] However, attempts to repeat these results in our laboratory proved less successful, with the 3-hydroxypyridines **9a** and **9b** isolated in 38% and 42% yield, respectively, only after heating under reflux in benzene for several days (Table 1, entries 1 and 2). The trimethylsilyl group is presumably lost on work-up. Similar results were obtained on reaction of ester analogues **4c** and **4d** with DMAD (**5**) in refluxing toluene (Table 1, entries 3 and 4). Poor yields may be explained by the recovery of a large amount (up to 45%) of an *O*-trimethylsilyl keto-oxime side-product due to silyl enol ether hydrolysis under these conditions. As may be expected, changing the dienophile for the bulkier di-*tert*-butyl acetylenedicarboxylate (**6**) increases the reaction time and lowers the yield (Table 1, entry 5). Moderate yields were also obtained from the less reactive unsymmetrical dienophiles methyl propiolate (**7**, Table 1, entry 6) and 3-butyn-2-one (**8**, Table 1, entry 7). A single regioisomer was obtained in both cases, the regiochemistry being that expected on the basis of the likely coefficients of the relevant frontier orbitals ($HOMO_{diene}/LUMO_{dienophile}$).

The application of microwave irradiation has been shown to accelerate the rate of many organic reactions.[29,30] Indeed, several examples of hetero-Diels–Alder reactions have been reported under microwave conditions, including both 1- and 2-azadienes.[4,31–34] We envisaged that the long reaction times previously observed might be reduced by performing the reaction under these conditions. Unfortunately,



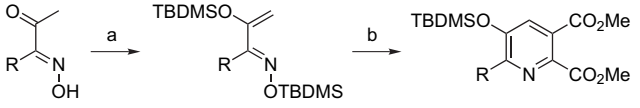
Scheme 1. a: R^1=Me, R^2=R^3=CO_2Me; **b**: R^1=R^2=R^3=CO_2Me; **c**: R^1=$CO_2{}^tBu$, R^2=R^3=CO_2Me; **d**: R^1=CO_2Bn, R^2=R^3=CO_2Me; reagents and conditions: (a) TMSCl, Et_3N, NaI, MeCN, rt, 18 h; (b) benzene or toluene, reflux, 4–14 days.

Table 1. Cycloaddition of α,β-unsaturated oximes with acetylenes under thermal conditions

Entry	R^1	R^2	R^3	Product	Time (days)	Yield (%)[a]
1	Me	CO_2Me	CO_2Me	**9a**	4	38
2	CO_2Me	CO_2Me	CO_2Me	**9b**	4	42
3	$CO_2{}^tBu$	CO_2Me	CO_2Me	**9c**	14	39
4	CO_2Bn	CO_2Me	CO_2Me	**9d**	6	48
5	CO_2Me	CO_2Me	$CO_2{}^tBu$	**10**	7	32
6[b]	CO_2Bn	H	CO_2Me	**11**	4	21
7[c]	CO_2Me	H	COMe	**12**	0.8	39

[a] Isolated yield after chromatography on silica.
[b] Methyl propiolate (2 equiv), toluene, sealed tube and 120 °C.
[c] 3-Butyn-2-one (5 equiv), toluene, sealed tube and 110 °C.

microwave irradiation of 1-azadiene **4b** with DMAD (**5**) in toluene in a sealed vessel at 150 °C afforded only degradation products. A more hydrolytically stable silyl protecting group was therefore investigated; we chose the bulkier *tert*-butyldimethylsilyl (TBDMS) derivatives **13a** and **13b**, prepared from the free oximes **3a** and **3b**, respectively, using TBDMS triflate and Hünigs base (Scheme 2). Treatment of **13a** and **13b** with either 1 or 2 equiv of DMAD (**5**) in a sealed tube at 150 °C under microwave irradiation (300 W) proceeded smoothly to afford the protected pyridines in moderate yields after only a few hours (Table 2, entries 1, 3 and 5). Increasing the temperature to 180 °C shortens the reaction time even further (Table 2, entries 2, 4, 6 and 7). A control



Scheme 2. a: R=Me; **b**: R=CO_2Me; reagents and conditions: (a) TBDMSOTf, Et^iPr_2N, CH_2Cl_2, 0 °C, 5–18 h; (b) DMAD, toluene or toluene/THF, MW (300 W).

Table 2. Cycloaddition of α,β-unsaturated oximes with acetylenes under microwave conditions[a]

Entry	R	DMAD (equiv)	Temp (°C)	Time (h)	Product	Yield (%)[b]
1	Me	2.0	150	6	**14a**	56
2	Me	2.0	180	2	**14a**	56
3	Me	1.0	150	8	**14a**	50
4	Me	1.0	180	3	**14a**	50
5	CO_2Me	2.0	150	10	**14b**	32
6	CO_2Me	2.0	180	6	**14b**	31
7	CO_2Me	1.0	180	8	**14b**	45

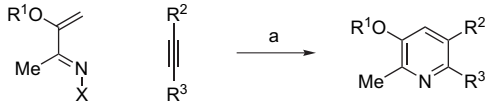
[a] Reactions were carried out in a CEM Discover™ microwave reactor operating at 300 W with simultaneous cooling.
[b] Isolated yield after chromatography on silica.

reaction performed in a sealed tube at 150 °C gave the appropriate pyridine **14a** in 57% yield after 6 h. However, the use of microwave irradiation remains as a safe, clean and efficient means for performing high temperature reactions and was used in the following studies on hydrazone derived 1-azadienes.

2.2. Synthesis and cycloadditions of α,β-unsaturated hydrazones

As discussed above, the most commonly used 1-azadienes in hetero-Diels–Alder reactions are the *N*,*N*-dimethylhydrazones **1a**, and a number of reactions with electron deficient alkenes and benzoquinones as dienophiles have been reported.[15] Reactions with alkynes are less common. The C-3 oxygenated 1-azadienes, hydrazones **1b**, are also known,[9,14,15,35] although to our knowledge, no Diels–Alder reactions of these dienes with alkynes have been reported. Hence, our initial work on hydrazones focussed on the cycloaddition of the previously unknown 1-dimethylamino-2-methyl-3-trimethylsiloxy-1-aza-1,3-butadiene with DMAD (**5**). However, only 30% of the desired cycloadduct was obtained after heating under reflux in toluene for 5 days. As has been shown with the oximes, a more hydrolytically stable diene is obtained by employing the TBDMS derivative. Thus, treatment of the known[35] 1-azadiene **17a** with DMAD (**5**) gave the desired cycloadduct **14a** in 53% yield after 20 h (Table 3, entry 3). Once again we turned our attention to the use of microwave irradiation in an attempt to decrease the reaction time and improve the yield.

Irradiation of equimolar amounts of 1-azadiene **17a** and DMAD (**5**) at 150 °C in toluene in a sealed tube for 2 h afforded the desired pyridine, still protected as the TBDMS ether and in poor yield, due to the competing formation of Michael adducts between the dienophile and liberated dimethylamine (Table 3, entry 4), a problem commonly observed with *N*,*N*-dimethylhydrazones that is not found with oximes.[14] Thus, 2 equiv of the dienophile were necessary to achieve complete consumption of the 1-azadiene, allowing the product to be isolated in comparable yield to the thermal reaction in only 2 h (Table 3, entry 5). Once again, increasing the temperature to 180 °C shortens the reaction time (Table 3, entry 6). A control reaction performed in a sealed tube at 150 °C under thermal conditions gave the appropriate pyridine **14a** in 46% yield after 2 h.



15-16, 17a-d **5, 7-8** **14a, 18-19**

Scheme 3. **a**: X=NMe_2, R^2=R^3=CO_2Me; **b**: X=piperidinyl, R^2=R^3=CO_2Me; **c**: X=NMeCbz, R^2=R^3=CO_2Me; **d**: X=phthalimido, R^2=R^3=CO_2Me; reagents and conditions: (a) toluene or toluene/THF, MW (300 W).

1-Azadienes **15** and **16** were prepared from butane-2,3-dione monohydrazone[35] via analogous procedures to the oximes in order to evaluate the effect of different silyl protecting groups on diene reactivity. Cycloaddition of triethylsilyl (TES) analogue **15** with DMAD (**5**) gave, after acidic work-up, the deprotected pyridine **9a** in 34% yield (Table 3, entry 1). The *tert*-butyldiphenylsilyl (TBDPS) analogue **16** however, could not be induced to undergo cycloaddition, even at elevated temperatures (Table 3, entry 2). This confirmed our choice of the TBDMS ether **17a** as the most suitable 3-siloxy-1-azadiene.

To date, the only comparative study on the effect of the N-1 nitrogen substituents of hydrazones on 1-azadiene reactivity has been reported by Gilchrist and co-workers.[36,37] Therefore, we prepared the 1-aza-1,3-butadienes **17b–d** from 2,3-butanedione in two steps by condensation with the appropriate hydrazine and silyl enol ether formation, in order to probe the effect on diene reactivity and reaction by-product profile. As expected, the piperidinyl derivative **17b** possessed similar reactivity to **17a** (Table 3, entry 7), including the formation of unwanted Michael adducts. Introduction of a single electron withdrawing substituent onto the leaving group (**17c**) also failed to prevent by-product formation (Table 3, entry 8). However, the introduction of a second electron withdrawing group in hydrazone **17d**, derived from *N*-aminophthalimide,[38] completely suppressed Michael addition of the nitrogen leaving group into the dienophile, leading to increased yield, although higher temperature is necessary to effect the cyclisation (Table 3, entry 9). Only a single equivalent of DMAD (**5**) is needed with this diene (Table 3, entry 10).

Table 3. Cycloaddition of α,β-unsaturated hydrazones with acetylenes under microwave conditions,[a] using 2 equiv of dienophile

Entry	1-Azadiene	R^1	R^2	R^3	Temp (°C)	Time (h)	Product	Yield (%)[b]
1	**15**	TES	CO_2Me	CO_2Me	150	2	**9a**	34
2	**16**	TBDPS	CO_2Me	CO_2Me	180	6	—	—
3[c]	**17a**	TBDMS	CO_2Me	CO_2Me	110	20	**14a**	53
4[d]	**17a**	TBDMS	CO_2Me	CO_2Me	150	2	**14a**	24
5	**17a**	TBDMS	CO_2Me	CO_2Me	150	2	**14a**	52
6	**17a**	TBDMS	CO_2Me	CO_2Me	180	0.75	**14a**	44
7	**17b**	TBDMS	CO_2Me	CO_2Me	150	2	**14a**	47
8	**17c**	TBDMS	CO_2Me	CO_2Me	150	4	**14a**	46
9	**17d**	TBDMS	CO_2Me	CO_2Me	180	3	**14a**	58
10[d]	**17d**	TBDMS	CO_2Me	CO_2Me	180	4	**14a**	54
11	**17a**	TBDMS	H	CO_2Me	180	6	**18**	29
12	**17a**	TBDMS	H	COMe	180	6	**19**	28

[a] Reactions were carried out in a CEM Discover™ microwave reactor operating at 300 W with simultaneous cooling.
[b] Isolated yield after chromatography on silica.
[c] Reaction carried out under thermal conditions in refluxing toluene.
[d] DMAD (1 equiv).

Unsymmetrical dienophiles **7** and **8** were also investigated. The most reactive 1-azadiene **17a** was chosen for this study as longer reaction times were expected with the less electron deficient (and hence less reactive) dienophiles. Indeed, increasing the reaction temperature to 180 °C for 6 h was necessary to achieve complete consumption of the diene. Poor yields of a single regioisomer were obtained, in an analogous fashion to the oximes (Table 3, entries 11and 12; Scheme 3).

3. Conclusions

The hetero-Diels–Alder reaction between 1-aza-3-siloxy-1,3-butadienes and electron deficient acetylenes has been investigated. We have expanded the scope of 3-siloxy-α,β-unsaturated oximes as heterodienes, and utilised microwave irradiation to reduce the often extended reaction times and improve the poor yields associated with this process.

A series of 3-siloxy-α,β-unsaturated hydrazones were also prepared and their reactivity in the hetero-Diels–Alder reaction was evaluated. More electron rich 1-azadienes proved to be most reactive, although side reactions were observed. Introduction of electron withdrawing groups into the hydrazone suppressed these side reactions, but higher temperatures were required for cycloaddition.

4. Experimental

Commercially available reagents were used throughout without further purification unless otherwise stated; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C and ether refers to diethyl ether. Reactions were routinely carried out under a nitrogen atmosphere. Microwave reactions were carried out in a CEM Discover™ 300 W focussed microwave reactor. Fully characterised compounds were chromatographically homogeneous. IR spectra were recorded in the range 4000–600 cm^{-1} using a Nicolet Magna FT-550 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 300 or 400 MHz (^{1}H frequencies, corresponding ^{13}C frequencies are recorded at 75 and 100 MHz). In the ^{13}C NMR spectra, signals corresponding to CH, CH_2 or CH_3 groups are assigned from DEPT. High and low-resolution mass spectra were recorded on a Micromass GCT time of flight high-resolution mass spectrometer, or at the EPSRC Mass Spectrometry Service (Swansea). Elemental analysis was carried out on a Perkin Elmer 2400 CHN analyser within ±0.3% of the theoretical values. Melting points were measured on a Gallenkamp electrothermal digital melting point apparatus or on a Reichert–Kofler hot stage apparatus. Compounds **3b**,[27] **3c**,[28] **3d**,[27] *N*,*N*-dimethyl butane-2,3-dione monohydrazone[35] and **17a**[35] were prepared according to literature procedure.

4.1. General procedure 1 for the silylation of α-keto-oximes

To a stirred mixture of the α-keto-oxime (13.5 mmol) and sodium iodide (2.02 g, 13.5 mmol) in dry acetonitrile (40 mL) was added dropwise triethylamine (5.46 g, 54.0 mmol) and chlorotrimethylsilane (5.87 g, 54.0 mmol). The mixture was stirred at room temperature overnight and then the solvent was removed in vacuo. Ether (60 mL) was added to the residue, which was then filtered. The filtrate was concentrated in vacuo and distilled (Kugelrohr) to afford the title compound.

4.2. General procedure 2 for the silylation of α-keto-oximes

To a stirred solution of the α-keto-oxime (5.0 mmol) in dry dichloromethane (10 mL) at 0 °C was added dropwise ethyldiisopropylamine (1.68 g, 13.0 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (3.30 g, 12.5 mmol). Stirring was continued at 0 °C for 2–4 h and then the solvent was evaporated. The residue was diluted with *n*-pentane (25 mL), stirred at 0 °C for 1 h, then filtered and evaporated to afford the title compound, which was used without further purification.

4.3. General procedure 3 for the preparation of α-keto-hydrazones

To a stirred solution of 2,3-butanedione (0.861 g, 10.0 mmol) in ethanol (10 mL) at 0 °C was added the hydrazine (11.0 mmol) in dropwise. Stirring was continued at 0 °C until the reaction was judged to be complete by TLC. The solution was then dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:9) to afford the title compound.

4.4. General procedure 4 for the silylation of α-keto-hydrazones

To a stirred solution of the α-ketohydrazone (10.0 mmol) in dry dichloromethane (10 mL) at 0 °C was added dropwise ethyldiisopropylamine (1.68 g, 13.0 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (3.30 g, 12.5 mmol). Stirring was continued at 0 °C for 2 h and then the solvent was evaporated. The residue was diluted with *n*-pentane (25 mL), stirred at 0 °C for 1 h, then filtered and evaporated to afford the title compound, which was used without further purification.

4.5. General procedure 5 for hetero-Diels–Alder reaction under thermal conditions

Equimolar amounts of the 1-aza-1,3-butadiene and the dienophile were heated under reflux in the appropriate solvent (2 mL per mmol of 1-azadiene) for the time indicated. The resulting mixture was concentrated in vacuo, and the crude product was purified by flash chromatography on silica, eluting with methanol–chloroform (0.5:99.5–2:98) to afford the title compound.

4.6. General procedure 6 for hetero-Diels–Alder reaction under microwave irradiation

A solution of the 1-aza-1,3-butadiene (1.0 mmol) and the dienophile (1.0–2.0 mmol) in toluene (2 mL) in a sealed microwave tube (10 mL capacity) was irradiated at 300 W with simultaneous cooling and held at 150 °C for the time indicated. The resulting mixture was concentrated in vacuo,

and the crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:19) to afford the title compound.

4.7. General procedure 7 for hetero-Diels–Alder reaction under microwave irradiation

A solution of the 1-aza-1,3-butadiene (1.0 mmol) and the dienophile (1.0–2.0 mmol) in toluene (2 mL) and THF (0.25 mL) in a sealed microwave tube (10 mL capacity) was irradiated at 300 W with simultaneous cooling and held at 180 °C for the time indicated. The resulting mixture was concentrated in vacuo, and the crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:19) to afford the title compound.

4.7.1. 2-Methyl-1,3-bis(trimethylsiloxy)-1-aza-1,3-butadiene 4a. Following general procedure 1 using similar molar amounts, the title compound was obtained from 2,3-butanedione monoxime in 37% yield as a colourless oil, bp 80–100 °C (oven temperature) at 3 mmHg; δ_H (400 MHz, $CDCl_3$) 4.81 (1H, d, J=1.4 Hz, C=CH), 4.55 (1H, d, J=1.4 Hz, C=CH), 1.96 (3H, s, Me), 0.23 (9H, s, $SiMe_3$), 0.22 (9H, s, $SiMe_3$). This compound has been prepared previously.[17]

4.7.2. 2-Methoxycarbonyl-1,3-bis(trimethylsiloxy)-1-aza-1,3-butadiene 4b. Following general procedure 1 using similar molar amounts, the title compound was obtained from **3b** in 85% yield as a colourless oil, bp 130–150 °C (oven temperature) at 0.3 mmHg; δ_H (400 MHz, $CDCl_3$) 4.71 (1H, d, J=2.0 Hz, C=CH), 4.68 (1H, d, J=2.0 Hz, C=CH), 3.84 (3H, s, OMe), 0.22 (18H, s, 2×$SiMe_3$). This compound has been prepared previously.[17]

4.7.3. 2-*tert*-Butoxycarbonyl-1,3-bis(trimethylsiloxy)-1-aza-1,3-butadiene 4c. Following general procedure 1 using similar molar amounts, the *title compound* was obtained from **3c** in 82% yield as a colourless oil, bp 140–155 °C (oven temperature) at 0.3 mmHg; (Found: M^+, 331.1636. $C_{14}H_{29}NO_4Si_2$ requires 331.1636); ν_{max} (film)/cm^{-1} 2965, 1740, 1615, 1370, 1255, 1175, 1105, 1025, 965, 845, 755; δ_H (400 MHz, $CDCl_3$) 4.72 (1H, d, J=1.9 Hz, C=CH), 4.70 (1H, d, J=1.9 Hz, C=CH), 1.53 (9H, s, CMe_3), 0.23 (9H, s, $SiMe_3$), 0.22 (9H, s, $SiMe_3$); δ_C (100 MHz, $CDCl_3$) 162.6 (C), 155.5 (C), 148.6 (C), 100.7 (CH_2), 83.0 (CMe_3), 28.0 (CMe_3), 0.0 ($SiMe_3$), −0.9 ($SiMe_3$); m/z (EI) 331 (M^+, 17%), 275 (17), 274 (10), 259 (10), 147 (27), 115 (16), 113 (25), 75 (44), 74 (20), 73 (81), 57 (100).

4.7.4. 2-Benzyloxycarbonyl-1,3-bis(trimethylsiloxy)-1-aza-1,3-butadiene 4d. Following general procedure 1 using similar molar amounts, the *title compound* was obtained from **3d** in 66% yield as a colourless oil, bp 200–225 °C (oven temperature) at 0.3 mmHg; (Found: M^+, 365.1476. $C_{17}H_{27}NO_4Si_2$ requires 365.1479); ν_{max} (film)/cm^{-1} 3035, 2960, 2900, 1750, 1700, 1615, 1500, 1455, 1380, 1350, 1315, 1255, 1100, 1020; δ_H (300 MHz, $CDCl_3$) 7.42–7.30 (5H, m, ArH), 5.32 (2H, s, CH_2), 4.70 (1H, d, J=2.0 Hz, C=CH), 4.65 (1H, d, J=2.0 Hz, C=CH), 0.22 (9H, s, $SiMe_3$), 0.20 (9H, s, $SiMe_3$); δ_C (75 MHz, $CDCl_3$) 163.3 (C), 154.4 (C), 148.4 (C), 135.3 (C), 128.5 (CH), 128.3 (CH), 128.3 (CH), 101.1 (CH_2), 66.9 (CH_2), 0.0 ($SiMe_3$), −0.8 ($SiMe_3$); m/z (EI) 365 (M^+, 1%), 350 (2), 274 (19), 91 (100), 73 (56).

4.7.5. 2-Methyl-1,3-bis(*tert*-butyldimethylsiloxy)-1-aza-1,3-butadiene 13a. Following general procedure 2, the *title compound* (1.44 g, 87%) was obtained as a pale oil from 2,3-butanedione monoxime (0.435 g, 5.0 mmol); (Found: MH^+, 330.2281. $C_{16}H_{35}NO_2Si_2$+H requires 330.2284); ν_{max} (film)/cm^{-1} 2957, 2931, 2888, 2859, 1615, 1580, 1472, 1464, 1391, 1362, 1340, 1254, 1168; δ_H (300 MHz, $CDCl_3$) 4.78 (1H, d, J=1.1 Hz, C=CH), 4.48 (1H, d, J=1.1 Hz, C=CH), 1.96 (3H, s, Me), 0.94 (9H, s, CMe_3), 0.92 (9H, s, CMe_3), 0.16 (6H, s, $SiMe_2$), 0.14 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 158.2 (C), 154.1 (C), 96.6 (CH_2), 26.5 (Me), 26.1 (Me), 18.6 (CMe_3), 18.4 (CMe_3), 11.6 (Me), −4.1 ($SiMe_2$), −4.3 ($SiMe_2$); m/z (CI) 358 (15%), 330 (MH^+, 90), 314 (100), 272 (85), 231 (37), 216 (13), 200 (18), 189 (30), 156 (12), 115 (21).

4.7.6. 2-Methoxycarbonyl-1,3-bis(*tert*-butyldimethylsiloxy)-1-aza-1,3-butadiene 13b. Following general procedure 2, the *title compound* (1.73 g, 93%) was obtained as a pale oil from **3b** (0.716 g, 5.0 mmol); (Found: MH^+, 374.2182. $C_{17}H_{35}NO_4Si_2$+H requires 374.2183); ν_{max} (film)/cm^{-1} 2956, 2931, 2887, 2859, 1751, 1614, 1473, 1464, 1435, 1391, 1362, 1254, 1102; δ_H (300 MHz, $CDCl_3$) 4.68 (1H, d, J=2.1 Hz, C=CH), 4.64 (1H, d, J=2.1 Hz, C=CH), 3.83 (3H, s, OMe), 0.94 (9H, s, CMe_3), 0.89 (9H, s, CMe_3), 0.16 (12H, s, 2×$SiMe_2$); δ_C (75 MHz, $CDCl_3$) 164.3 (C), 155.2 (C), 149.1 (C), 100.3 (CH_2), 52.4 (Me), 26.1 (Me), 26.0 (Me), 18.5 (CMe_3), 18.3 (CMe_3), −4.3 ($SiMe_2$), −5.0 ($SiMe_2$); m/z (CI) 402 (8%), 374 (MH^+, 32), 358 (50), 316 (60), 286 (10), 247 (18), 231 (90), 200 (10), 189 (100), 184 (20), 157 (40), 147 (50), 115 (70), 86 (100), 73 (28).

4.7.7. 1-(Dimethylamino)-2-methyl-3-(trimethylsiloxy)-1-aza-1,3-butadiene. To a solution of *N*,*N*-dimethyl butane-2,3-dione monohydrazone (1.60 g, 12.5 mmol) and sodium iodide (0.934 g, 6.20 mmol) in acetonitrile (30 mL) was added triethylamine (2.52 g, 25.0 mmol) and chlorotrimethylsilane (2.71 g, 25.0 mmol). The reaction mixture was stirred for 18 h. The reaction mixture was then concentrated in vacuo and the residue was diluted with ether (50 mL), filtered under nitrogen and concentrated in vacuo. The crude product was distilled to afford the *title compound* as a brown oil (1.69 g, 68%), bp 115–130 °C (oven temperature) at 9 mmHg; (Found: M^+, 200.1343. $C_9H_{20}N_2OSi$ requires 200.1345); ν_{max} (film)/cm^{-1} 2990, 2955, 2900, 2860, 2820, 2775, 1685, 1615, 1590, 1470, 1440, 1335, 1280; δ_H (300 MHz, $CDCl_3$) 4.85 (1H, d, J=1.1 Hz, C=CH), 4.81 (1H, d, J=1.1 Hz, C=CH), 2.54 (6H, s, NMe_2), 2.02 (3H, s, Me), 0.22 (9H, s, $SiMe_3$); δ_C (75 MHz, $CDCl_3$) 159.5 (C), 155.3 (C), 96.8 (CH_2), 47.1 (Me), 14.2 (Me), 0.35 (Me); m/z (EI) 311 (25%), 271 (14), 200 (17), 185 (56), 151 (48), 135 (48), 109 (78), 86 (45), 73 (91), 58 (100).

4.7.8. 1-(Dimethylamino)-2-methyl-3-(triethylsiloxy)-1-aza-1,3-butadiene 15. To a solution of *N*,*N*-dimethyl butane-2,3-dione monohydrazone (1.28 g, 10.0 mmol) and sodium iodide (0.750 g, 5.00 mmol) in acetonitrile (20 mL) was added triethylamine (2.02 g, 20.0 mmol) and

chlorotriethylsilane (3.01 g, 20.0 mmol). The reaction mixture was stirred for 24 h, then further sodium iodide (0.375 g, 2.50 mmol), triethylamine (1.01 g, 10.0 mmol) and chlorotriethylsilane (1.50 g, 10.0 mmol) was added, and the reaction mixture was stirred for further 18 h. The reaction mixture was then concentrated in vacuo and the residue was diluted with ether (35 mL), filtered under nitrogen and concentrated in vacuo. The crude product was distilled in vacuo at room temperature to afford the *title compound* as a brown oil (1.62 g, 67%); (Found: M^+, 242.1808. $C_{12}H_{26}N_2OSi$ requires 242.1814); ν_{max} (film)/cm^{-1} 3428, 2953, 2821, 2777, 1684, 1611, 1589, 1459, 1415, 1379, 1337, 1238, 1210, 1147; δ_H (300 MHz, $CDCl_3$) 4.77 (1H, d, J=0.8 Hz, C═CH), 4.40 (1H, d, J=0.8 Hz, C═CH), 2.47 (6H, s, NMe_2), 1.97 (3H, s, Me), 0.93 (9H, t, J=7.9 Hz, 3×CH_2*Me*), 0.67 (6H, q, J=7.9 Hz, 3×CH_2Me); δ_C (75 MHz, $CDCl_3$) 160.2 (C), 155.9 (C), 96.0 (CH_2), 47.5 (Me), 14.6 (Me), 7.1 (Si(CH_2*Me*)$_3$), 7.0 (Si(CH_2*Me*)$_3$), 6.8 (Si(*C*H_2Me)$_3$), 5.6 (Si(*C*H_2Me)$_3$); *m*/*z* (EI) 242 (M^+, 18%), 227 (48), 217 (92), 213 (20), 189 (100), 170 (10), 161 (51), 133 (17), 115 (28), 105 (14), 87 (50), 83 (33), 75 (13), 58 (34).

4.7.9. 3-(*tert*-Butyldiphenylsiloxy)-1-(dimethylamino)-2-methyl-1-aza-1,3-butadiene 16. To a solution of *N*,*N*-dimethyl butane-2,3-dione monohydrazone (0.256 g, 2.00 mmol) and sodium iodide (0.150 g, 1.00 mmol) in acetonitrile (2 mL) was added triethylamine (0.405 g, 4.00 mmol) and *tert*-butyldiphenylsilyl chloride (1.10 g, 4.00 mmol). The reaction mixture was stirred for 3 days, then further sodium iodide (0.150 g, 1.00 mmol), triethylamine (0.405 g, 4.00 mmol) and *tert*-butyldiphenylsilyl chloride (1.10 g, 4.00 mmol) was added and stirring was continued for further 24 h. The reaction mixture was then concentrated in vacuo and the residue was diluted with ether (15 mL), cooled in an ice-bath for 30 min, filtered and concentrated in vacuo. The crude product was purified by flash chromatography on silica to afford the *title compound* as a brown oil (0.471 g, 64%); (Found: M^+, 366.2124. $C_{22}H_{30}N_2OSi$ requires 366.2127); ν_{max} ($CHCl_3$)/cm^{-1} 3392, 3072, 3050, 2958, 2931, 2892, 2858, 1611, 1590, 1472, 1428, 1336, 1317, 1157, 1114; δ_H (300 MHz, $CDCl_3$) 7.76–7.70 (4H, m, ArH), 7.42–7.34 (6H, m, ArH), 4.77 (1H, d, J=1.5 Hz, C═CH), 4.30 (1H, d, J=1.5 Hz, C═CH), 2.35 (6H, s, NMe_2), 2.06 (3H, s, Me), 1.05 (9H, s, CMe_3); δ_C (75 MHz, $CDCl_3$) 159.3 (C), 155.5 (C), 135.9 (CH), 135.4 (CH), 133.9 (C), 128.2 (CH), 96.8 (CH_2), 47.3 (NMe_2), 27.1 (Me), 20.2 (*C*Me_3), 14.9 (Me); *m*/*z* (EI) 366 (M^+, 19%), 351 (17), 309 (46), 273 (15), 266 (19), 200 (100), 181 (49), 135 (35), 77 (43).

4.7.10. 3-(Piperidin-1-ylimino)-butan-2-one. Following general procedure 3, the *title compound* (1.02 g, 62%) was obtained as a pale oil from 1-aminopiperidine (1.10 g, 11.0 mmol); (Found: MH^+, 169.1363. $C_9H_{16}N_2O$+H requires 169.1341); ν_{max} (film)/cm^{-1} 2939, 2856, 2822, 1687, 1581, 1443, 1354, 1294, 1261, 1156, 1119, 1103, 1074, 1014; δ_H (300 MHz, $CDCl_3$) 3.12–3.08 (4H, m, 2×CH_2), 2.35 (3H, s, Me), 1.98 (3H, s, Me), 1.74–1.67 (4H, m, 2×CH_2), 1.59–1.54 (2H, m, CH_2); δ_C (75 MHz, $CDCl_3$) 199.7 (C), 152.4 (C), 55.9 (CH_2), 25.7 (CH_2), 24.9 (Me), 24.4 (CH_2), 13.6 (Me); *m*/*z* (CI) 197 (8%), 169 (MH^+, 100), 84 (15).

4.7.11. 3-(*tert*-Butyldimethylsiloxy)-2-methyl-1-(piperidinyl)-1-aza-1,3-butadiene 17b. Following general procedure 4, the *title compound* (0.715 g, 84%) was obtained as a pale oil from 3-(piperidin-1-ylimino)-butan-2-one (0.504 g, 3.0 mmol); (Found: MH^+, 283.2205. $C_{15}H_{30}N_2O$-Si+H requires 283.2205); ν_{max} ($CHCl_3$)/cm^{-1} 3386, 2934, 2857, 2817, 1615, 1593, 1472, 1442, 1360, 1334, 1253, 1160, 1036; δ_H (300 MHz, $CDCl_3$) 4.86 (1H, d, J=0.9 Hz, C═CH), 4.52 (1H, d, J=0.9 Hz, C═CH), 2.75–2.71 (4H, m, 2×CH_2), 2.04 (3H, s, Me), 1.72–1.65 (4H, m, 2×CH_2), 1.48–1.44 (2H, m, CH_2), 0.96 (9H, s, CMe_3), 0.17 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 160.4 (C), 156.2 (C), 96.9 (CH_2), 56.5 (CH_2), 26.2 (CH_2), 25.7 (CH_2), 24.3 (CH_2), 18.8 (*C*Me_3), 14.8 (Me), −2.5 ($SiMe_2$); *m*/*z* 311 (8%), 283 (MH^+, 100), 267 (45), 225 (30), 200 (5), 169 (10), 159 (5).

4.7.12. 1-Benzyloxycarbonyl-1-methyl-hydrazine. To a stirred solution of methylhydrazine (0.461 g, 10.0 mmol) in dry dichloromethane (20 mL) at 0 °C was added triethylamine (1.21 g, 12.0 mmol) and benzyl chloroformate (1.88 g, 11.0 mmol). The reaction was allowed to warm to room temperature and stirred for 2 h. Water (15 mL) was added and the organic layer was separated. The aqueous layer was further extracted with dichloromethane (3×25 mL). The combined organics were dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:1) to afford the title compound as a pale oil (1.04 g, 58%), lit. bp 104–134 °C, 0.2 mmHg; ν_{max} ($CHCl_3$)/cm^{-1} 3026, 3014, 1697, 1627, 1498, 1455, 1395, 1351, 1310, 1230, 1169; δ_H (300 MHz, $CDCl_3$) 7.38–7.31 (5H, m, ArH), 5.15 (2H, s, CH_2), 3.82 (2H, br s, NH_2), 3.14 (3H, s, Me). This compound has been prepared previously.[39]

4.7.13. *N*-Benzyloxycarbonyl-*N*-methyl butane-2,3-dione monohydrazone. Following general procedure 3, the *title compound* (0.963 g, 78%) was obtained as a colourless solid from 1-methyl-1-benzyloxycarbonylhydrazine (0.991 g, 5.5 mmol), mp 64–65 °C (from light petroleum); (Found: MH^+, 249.1236. $C_{13}H_{16}N_2O_3$+H requires 249.1239); ν_{max} ($CHCl_3$)/cm^{-1} 3442, 3031, 2990, 2930, 1703, 1613, 1469, 1456, 1428, 1382, 1359, 1320, 1191, 1120; δ_H (300 MHz, $CDCl_3$) 7.39–7.32 (5H, m, ArH), 5.21 (2H, s, CH_2), 3.35 (3H, s, NMe), 2.44 (3H, s, Me), 1.95 (3H, s, Me); δ_C (75 MHz, $CDCl_3$) 199.2 (C), 164.8 (C), 153.6 (C), 136.3 (C), 129.0 (CH), 128.8 (CH), 128.5 (CH), 68.5 (CH_2), 39.3 (Me), 25.6 (Me), 14.6 (Me); *m*/*z* (CI) 339 (30%), 249 (MH^+, 100), 205 (25), 181 (10), 137 (10), 91 (48).

4.7.14. 1-(Benzyloxycarbonylmethylamino)-3-(*tert*-butyldimethylsiloxy)-2-methyl-1-aza-1,3-butadiene 17c. Following general procedure 4, the *title compound* (1.01 g, 93%) was obtained as a pale oil from *N*-benzyloxycarbonyl-*N*-methyl butane-2,3-dione monohydrazone (0.745 g, 3.0 mmol); (Found: MH^+, 363.2097. $C_{19}H_{30}N_2O_3$-Si+H requires 363.2104); ν_{max} ($CHCl_3$)/cm^{-1} 3024, 3016, 2958, 2932, 2887, 2859, 1698, 1595, 1472, 1427, 1389, 1336, 1256, 1228, 1168; δ_H (300 MHz, $CDCl_3$) 7.35–7.32 (5H, m, ArH), 5.15 (2H, s, CH_2), 5.04 (1H, d, J=1.3 Hz, C═CH), 4.60 (1H, d, J=1.3 Hz, C═CH), 3.20 (3H, s, NMe), 1.93 (3H, s, Me), 0.95 (9H, s, CMe_3), 0.16 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 178.0 (C), 163.5 (C), 154.7

(C), 136.8 (C), 128.9 (CH), 128.5 (CH), 128.4 (CH), 98.7 (CH_2), 67.9 (CH_2), 38.1 (Me), 26.1 (Me), 16.0 (CMe_3), 14.5 (Me), −2.5 ($SiMe_2$); *m/z* (CI) 453 (8%), 363 (MH^+, 25), 339 (8), 311 (10), 283 (80), 271 (22), 249 (12), 221 (20), 193 (57), 181 (25), 149 (27), 137 (18), 91 (100).

4.7.15. *N*-Phthaloyl butane-2,3-dione monohydrazone. To a stirred solution of 2,3-butanedione (8.61 g, 0.100 mol) in chloroform (200 mL) was added *N*-aminophthalimide (17.8 g, 0.110 mol). The reaction mixture was heated under reflux for 3 days, allowed to cool to room temperature, filtered and concentrated in vacuo to afford the title compound as a colourless solid (20.1 g, 87%), mp 157–158 °C (from chloroform), (lit.[38] mp 165 °C), which was used without further purification; δ_H (300 MHz, $CDCl_3$) 7.95–7.92 (2H, m, ArH), 7.82–7.79 (2H, m, ArH), 2.60 (3H, s, Me), 2.11 (3H, s, Me).

4.7.16. 3-(*tert*-Butyldimethylsiloxy)-2-methyl-1-(phthalimido)-1-aza-1,3-butadiene 17d. Following general procedure 4, the *title compound* (1.38 g, 79%) was obtained as a colourless solid from *N*-phthaloyl butane-2,3-dione monohydrazone (1.15 g, 5.0 mmol), mp 106–107 °C (from ethanol); (Found: MH^+, 345.1632. $C_{18}H_{24}N_2O_3Si$+H requires 345.1634); ν_{max} ($CHCl_3$)/cm^{-1} 2955, 2930, 2894, 2857, 1787, 1717, 1618, 1596, 1467, 1373, 1354, 1339, 1311, 1256, 1170, 1115; δ_H (300 MHz, $CDCl_3$) 7.89–7.86 (2H, m, ArH), 7.76–7.73 (2H, m, ArH), 5.30 (1H, d, *J*=1.3 Hz, C=CH), 4.75 (1H, d, *J*=1.3 Hz, C=CH), 2.07 (3H, s, Me), 1.00 (9H, s, CMe_3), 0.23 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 174.0 (C), 164.3 (C), 153.8 (C), 134.6 (CH), 131.6 (C), 124.0 (CH), 100.3 (CH_2), 26.1 (Me), 18.7 (CMe_3), 17.5 (Me), −4.7 ($SiMe_2$); *m/z* (CI) 373 (10%), 345 (MH^+, 95), 287 (50), 200 (32), 148 (100).

4.7.17. Dimethyl 5-hydroxy-6-methylpyridine-2,3-dicarboxylate 9a

(a) Following general procedure 5 from **4a** (0.466 g, 1.9 mmol) and DMAD (**5**, 0.270 g, 1.9 mmol) the title compound (0.162 g, 38%) was obtained after 4 days in toluene as yellow crystals, mp 163–166 °C (from ethyl acetate–light petroleum), (lit.[17] mp 157–159 °C); δ_H (300 MHz, $CDCl_3$) 9.50 (1H, br s, OH), 7.41 (1H, s, H-4), 3.90 (6H, s, 2×OMe), 2.54 (3H, s, Me); δ_C (75 MHz, $CDCl_3$) 166.9 (C), 166.4 (C), 153.2 (C), 150.5 (C), 138.7 (C), 127.6 (C), 121.3 (CH), 53.1 (2×Me), 18.8 (Me).

(b) Following general procedure 5 from 3-(trimethylsiloxy)-1-(dimethylamino)-2-methyl-1-aza-1,3-butadiene (0.721 g, 3.6 mmol) and DMAD (**5**, 0.512 g, 3.6 mmol) the title compound (0.246 g, 30%) was obtained after 5 days in toluene as yellow crystals; data as above.

(c) A solution of the 1-aza-1,3-butadiene **15** (0.242 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol) in toluene (2 mL) in a sealed microwave tube (10 mL capacity) was irradiated at 300 W with simultaneous cooling and held at 150 °C for 2 h. The resulting mixture was concentrated in vacuo. The residue was taken up in methanol (1 mL), 2 M HCl (1 mL) was added and the mixture was stirred for 5 min. The mixture was then neutralised with saturated $NaHCO_3$, extracted with ethyl acetate (3×15 mL) and the combined organics were dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:4) to afford the title compound as yellow crystals (0.076 g, 34%); data as above.

(d) To a stirred solution of **14a** (0.125 g, 0.401 mmol) in THF (4 mL) was added dropwise TBAF (1.0 M in THF, 1 mL, 1.00 mmol). Stirring was continued for 2 h, and the reaction was quenched with water (10 mL) and extracted with ethyl acetate (4×10 mL). The combined organics were washed with brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:1) to afford the title compound as a pale oil (0.064 g, 71%); data as above.

4.7.18. Dimethyl 5-(*tert*-butyldimethylsiloxy)-6-methylpyridine-2,3-dicarboxylate 14a

(a) Following general procedure 6 from **13a** (0.330 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the *title compound* (0.190 g, 56%) was obtained in 6 h as a pale oil; (Found: M^+, 339.1507. $C_{16}H_{25}NO_5Si$ requires 339.1502); ν_{max} (film)/cm^{-1} 2955, 2933, 2888, 2860, 1732, 1588, 1461, 1431, 1406, 1367, 1325, 1258, 1178, 1145, 1055, 1004; δ_H (300 MHz, $CDCl_3$) 7.35 (1H, s, H-4), 3.95 (3H, s, OMe), 3.90 (3H, s, OMe), 2.52 (3H, s, Me), 1.00 (9H, s, CMe_3), 0.25 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 167.1 (C), 166.5 (C), 155.0 (C), 151.8 (C), 142.0 (C), 126.4 (C), 125.0 (CH), 53.4 (Me), 53.3 (Me), 26.2 (Me), 20.5 (Me), 18.6 (CMe_3), −3.9 ($SiMe_2$); *m/z* (EI) 339 (M^+, 12%), 308 (8), 282 (36), 250 (100), 222 (18), 192 (28), 164 (20).

(b) Following general procedure 6 from **13a** (0.330 g, 1.0 mmol) and DMAD (**5**, 0.142 g, 1.0 mmol), the title compound (0.170 g, 50%) was obtained in 8 h as a pale oil; data as above.

(c) Following general procedure 7 from **13a** (0.330 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.190 g, 56%) was obtained in 2 h as a pale oil; data as above.

(d) Following general procedure 7 from **13a** (0.330 g, 1.0 mmol) and DMAD (**5**, 0.142 g, 1.0 mmol), the title compound (0.170 g, 50%) was obtained in 3 h as a pale oil; data as above.

(e) A solution of 1-azadiene **13a** (0.330 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol) in toluene (2 mL) in a sealed tube was heated to 150 °C for 6 h. The resulting mixture was concentrated in vacuo and the crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:19) to afford the title compound (0.194 g, 57%) as a pale oil; data as above.

(f) Following general procedure 6 from **17a** (0.242 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.176 g, 52%) was obtained in 2 h as a pale oil; data as above.

(g) Following general procedure 7 from **17a** (0.242 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.150 g, 44%) was obtained in 45 min as a pale oil; data as above.

(h) Following general procedure 6 from **17b** (0.283 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.160 g, 47%) was obtained in 2 h as a pale oil; data as above.
(i) Following general procedure 6 from **17c** (0.363 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.157 g, 46%) was obtained in 4 h as a pale oil; data as above.
(j) Following general procedure 7 from **17d** (0.344 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.197 g, 58%) was obtained in 3 h as a pale oil; data as above.
(k) Following general procedure 7 from **17d** (0.344 g, 1.0 mmol) and DMAD (**5**, 0.142 g, 1.0 mmol), the title compound (0.183 g, 54%) was obtained in 4 h as a pale oil; data as above.
(l) A solution of 1-azadiene **17a** (0.242 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol) in toluene (2 mL) in a sealed tube was heated to 150 °C for 2 h. The resulting mixture was concentrated in vacuo and the crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:19) to afford the title compound (0.157 g, 46%) as a pale oil; data as above.

4.7.19. Trimethyl 5-hydroxypyridine-2,3,6-tricarboxylate 9b. Following general procedure 5 from **4b** (0.839 g, 2.9 mmol) and DMAD (**5**, 0.412 g, 2.9 mmol) the title compound (0.327 g, 42%) was obtained after 4 days in benzene as yellow crystals, mp 121–124 °C (from ethanol), (lit.[17] mp 118–119 °C); δ_H (400 MHz, $CDCl_3$) 11.05 (1H, s, OH), 7.66 (1H, s, H-4), 4.06 (3H, s, OMe), 3.96 (3H, s, OMe), 3.95 (3H, s, OMe); δ_C (75 MHz, $CDCl_3$) 168.8 (C), 165.3 (C), 159.4 (C), 159.4 (C), 139.8 (C), 134.0 (C), 127.0 (CH), 54.7 (Me), 53.3 (Me), 53.2 (Me).

4.7.20. 6-*tert*-Butyl-2,3-dimethyl 5-hydroxypyridine-2,3,6-tricarboxylate 9c. Following general procedure 5 from **4c** (0.431 g, 1.3 mmol) and DMAD (**5**, 0.185 g, 1.3 mmol) the *title compound* (0.157 g, 39%) was obtained after 14 days in toluene as yellow crystals, mp 64–70 °C (from ether); (Found: C, 54.0; H, 5.47; N, 4.50. $C_{14}H_{17}NO_7$ requires C, 54.0; H, 5.50; N, 5.50%); (Found: M^+, 311.1013. $C_{14}H_{17}NO_7$ requires 311.1005); ν_{max} (film)/cm^{-1} 3300–2800, 1740, 1675, 1570, 1430, 1370, 1320, 1265, 1220, 1150, 1125, 1050, 970; δ_H (400 MHz, $CDCl_3$) 11.34 (1H, s, OH), 7.62 (1H, s, H-4), 3.94 (3H, s, OMe), 3.92 (3H, s, OMe), 1.66 (9H, s, CMe_3); δ_C (100 MHz, $CDCl_3$) 168.0 (C), 165.5 (C), 165.3 (C), 159.4 (C), 139.7 (C), 133.2 (C), 132.0 (C), 126.7 (CH), 85.6 (*C*Me_3), 53.1 (Me), 52.9 (Me), 28.0 (C*Me*$_3$); *m*/*z* (EI) 311 (M^+, 2%), 296 (5), 280 (10), 257 (19), 256 (74), 255 (35), 238 (34), 225 (22), 224 (81), 211 (10), 210 (63), 206 (48), 181 (14), 180 (26), 179 (85), 178 (29), 167 (17), 150 (15), 139 (32), 123 (18), 122 (10), 121 (36), 95 (11), 94 (17), 93 (22), 69 (16), 59 (39), 58 (14), 57 (100) 56 (48).

4.7.21. 6-Benzyl-2,3-dimethyl 5-hydroxypyridine-2,3,6-tricarboxylate 9d. Following general procedure 5 from **4d** (0.585 g, 1.6 mmol) and DMAD (**5**, 0.227 g, 1.6 mmol) the *title compound* (0.267 g, 48%) was obtained after 6 days in toluene as yellow crystals, mp 116–118 °C (from ethanol); (Found: C, 58.9; H, 4.21; N, 3.95. $C_{17}H_{15}NO_7$ requires C, 59.1; H, 4.38; N, 4.06%); (Found: M^+, 345.0850. $C_{17}H_{15}NO_7$ requires 345.0849); ν_{max} (Nujol)/cm^{-1} 3500–3100, 1735, 1725, 1685, 1560, 1460, 1430, 1345, 1315, 1265, 1200, 1150, 1130; δ_H (300 MHz, $CDCl_3$) 11.00 (1H, s, OH), 7.66 (1H, s, H-4), 7.49–7.30 (5H, m, ArH), 5.50 (2H, s, CH_2), 3.93 (3H, s, OMe), 3.92 (3H, s, OMe); δ_C (75 MHz, $CDCl_3$) 168.3 (C), 165.2 (C), 165.1 (C), 159.4 (C), 140.4 (C), 134.6 (C), 133.5 (C), 130.8 (C), 128.8 (CH), 128.7 (CH), 128.7 (CH), 127.0 (CH), 68.4 (CH_2), 53.2 (Me), 53.1 (Me); *m*/*z* (EI) 345 (M^+, 3%), 314 (17), 239 (70), 211 (89), 179 (3), 91 (100).

4.7.22. 2,3-Di-*tert*-butyl-6-methyl 5-hydroxypyridine-2,3,6-tricarboxylate 10. Following general procedure 5 from **4b** (0.811 g, 2.8 mmol) and DBAD (**6**, 0.634 g, 2.8 mmol) the *title compound* (0.320 g, 32%) was obtained after 7 days in toluene as yellow crystals, mp 119–122 °C (from ethanol); (Found: C, 57.7; H, 6.51; N, 3.87. $C_{17}H_{23}NO_7$ requires C, 57.8; H, 6.56; N, 3.96%); (Found: M^+, 353.1472. $C_{17}H_{23}NO_7$ requires 353.1475); ν_{max} (Nujol)/cm^{-1} 3230, 1735, 1685, 1560, 1455, 1370, 1315, 1200, 1145, 1120; δ_H (400 MHz, $CDCl_3$) 10.92 (1H, s, OH), 7.60 (1H, s, H-4), 4.05 (3H, s, OMe), 1.62 (9H, s, CMe_3), 1.60 (9H, s, CMe_3); δ_C (100 MHz, $CDCl_3$) 168.9 (C), 164.0 (C), 163.7 (C), 158.8 (C), 142.5 (C), 134.6 (C), 129.9 (C), 126.9 (H-4), 83.4 (*C*Me_3), 83.1 (*C*Me_3), 53.3 (Me), 27.9 (2×C*Me*$_3$); *m*/*z* (EI) 353 (M^+, 0.6%), 297 (1), 253 (5), 252 (5), 242 (65), 224 (56), 198 (44), 197 (21), 192 (13), 179 (10), 167 (21), 139 (34), 57 (100).

4.7.23. Trimethyl 5-(*tert*-butyldimethylsiloxy)pyridine-2,3,6-tricarboxylate 14b

(a) Following general procedure 6 from **13b** (0.374 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the *title compound* (0.121 g, 32%) was obtained in 10 h as a pale oil; (Found: M^+, 384.1473. $C_{17}H_{25}NO_7Si$+H requires 384.1478); ν_{max} ($CHCl_3$)/cm^{-1} 2955, 2934, 2888, 2862, 2254, 1742, 1589, 1555, 1430, 1409, 1363, 1336, 1253, 1215, 1170, 1148, 1119, 1047; δ_H (300 MHz, $CDCl_3$) 7.48 (1H, s, H-4), 3.92 (3H, s, OMe), 3.91 (3H, s, OMe), 3.90 (3H, s, OMe), 0.96 (9H, s, CMe_3), 0.25 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 165.9 (C), 165.7 (C), 164.7 (C), 152.7 (C), 144.0 (C), 141.4 (C), 131.3 (C), 128.7 (CH), 53.6 (Me), 53.5 (Me), 53.2 (Me), 25.7 (Me), 18.6 (*C*Me_3), −3.8 ($SiMe_2$); *m*/*z* (CI) 384 (MH^+, 6%), 270 (100), 238 (23).
(b) Following general procedure 7 from **13b** (0.374 g, 1.0 mmol) and DMAD (**5**, 0.284 g, 2.0 mmol), the title compound (0.119 g, 31%) was obtained in 6 h as a pale oil; data as above.
(c) Following general procedure 7 from **13b** (0.374 g, 1.0 mmol) and DMAD (**5**, 0.142 g, 1.0 mmol), the title compound (0.171 g, 45%) was obtained in 8 h as a pale oil; data as above.

4.7.24. 2-Benzyl-6-methyl 3-hydroxypyridine-2,6-dicarboxylate 11. A solution of 1-aza-1,3-butadiene **4d** (0.621 g, 1.7 mmol) and methyl propiolate (**7**, 0.294 g, 3.5 mmol) in toluene (2 mL) in a sealed tube was heated to 120 °C for 4 days. The resulting mixture was concentrated in vacuo and the crude product was purified by flash

chromatography on silica, eluting with methanol–chloroform (0.5:99.5–2:98) to afford the *title compound* (0.103 g, 21%) as yellow crystals, mp 102–106 °C (from ethanol); (Found: M^+, 287.0788. $C_{15}H_{13}NO_5$ requires 287.0794); ν_{max} (Nujol)/cm^{-1} 1725, 1665, 1595, 1580, 1430, 1425, 1350, 1310, 1220, 1180, 1135, 1095; δ_H (400 MHz, $CDCl_3$) 11.10 (1H, s, OH), 8.22 (1H, d, J=8.8 Hz, ArH), 7.52–7.47 (2H, m, ArH), 7.44 (1H, d, J=8.8 Hz, ArH), 7.41–7.31 (3H, m, ArH), 5.52 (2H, s, CH_2), 3.96 (3H, s, OMe); δ_C (100 MHz, $CDCl_3$) 169.0 (C), 164.6 (C), 161.0 (C), 139.8 (C), 134.8 (C), 131.0 (CH), 130.0 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 126.7 (CH), 68.2 (CH_2), 52.8 (Me); *m/z* (EI) 287 (M^+, 0.4%), 256 (1), 181 (22), 153 (26), 92 (19), 91 (100), 65 (20), 57 (29), 55 (18).

4.7.25. Methyl 6-acetyl-3-hydroxypyridine-2-carboxylate 12. A solution 1-aza-1,3-butadiene **4b** (0.345 g, 1.19 mmol) and 3-butyn-2-one (**8**, 0.406 g, 5.96 mmol) in toluene (5 mL) in a sealed tube was heated to 110 °C for 20 h. The resulting mixture was concentrated in vacuo and the crude product was purified by flash chromatography on silica, eluting with ethyl acetate–light petroleum (1:3) to afford the *title compound* (0.090 g, 39%) as yellow crystals, mp 125–127 °C (from ethyl acetate–light petroleum); (Found: C, 55.1; H, 4.65; N, 7.18. $C_9H_9NO_4$ requires C, 55.4; H, 4.45; N, 7.04%); (Found: M^+, 195.0528. $C_9H_9NO_4$ requires 195.0532); ν_{max} (Nujol)/cm^{-1} 3120, 1700, 1685, 1655, 1575, 1560, 1540, 1290, 1275, 1210, 1175, 1130, 1105, 1090; δ_H (300 MHz, $CDCl_3$) 11.08 (1H, s, OH), 8.15 (1H, d, J=8.8 Hz, ArH), 7.40 (1H, d, J=8.8 Hz, ArH), 4.05 (3H, s, OMe), 2.67 (3H, s, COMe); δ_C (100 MHz, $CDCl_3$) 198.2 (C), 169.6 (C), 161.3 (C), 145.9 (C), 128.6 (C), 127.9 (CH), 126.6 (CH), 53.3 (Me), 25.2 (Me); *m/z* (EI) 195 (M^+, 100%), 180 (16), 167 (13), 153 (24), 152 (50), 137 (56), 136 (17), 135 (28), 122 (23), 121 (68), 120 (22), 107 (31), 94 (17), 93 (19), 64 (15), 59 (19), 53 (15).

4.7.26. Methyl 3-(*tert*-butyldimethylsiloxy)-2-methylpyridine-6-carboxylate 18. Following general procedure 7 from **17a** (0.242 g, 1.0 mmol) and methyl propiolate (**7**, 0.168 g, 2.0 mmol), the *title compound* (0.082 g, 29%) was obtained in 6 h as a pale oil; (Found: M^+, 282.1526. $C_{14}H_{23}NO_3Si$ requires 282.1525); ν_{max} (film)/cm^{-1} 2956, 2934, 2887, 2861, 2254, 2235, 1719, 1574, 1463, 1408, 1392, 1364, 1325, 1258, 1199, 1120, 1007; δ_H (300 MHz, $CDCl_3$) 7.88 (1H, d, J=8.3 Hz, ArH), 7.06 (1H, d, J=8.3 Hz, ArH), 3.92 (3H, s, OMe), 3.51 (3H, s, Me), 0.98 (9H, s, CMe_3), 0.22 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 168.3 (C), 156.0 (C), 154.4 (C), 142.1 (C), 127.2 (CH), 127.0 (CH), 55.5 (Me), 28.2 (Me), 22.8 (Me), 20.8 (CMe_3), −1.3 ($SiMe_2$); *m/z* (CI) 282 (M^+, 100%).

4.7.27. 6-Acetyl-3-(*tert*-butyldimethylsiloxy)-2-methylpyridine 19. Following general procedure 7 from **17a** (0.242 g, 1.0 mmol) and 3-butyn-2-one (**8**, 0.136 g, 2.0 mmol), the *title compound* (0.073 g, 28%) was obtained in 6 h as a pale oil; (Found: M^+, 339.1507. $C_{14}H_{23}NO_3Si$ requires 339.1502); ν_{max} ($CHCl_3$)/cm^{-1} 2933, 2959, 2887, 2861, 2252, 1684, 1643, 1596, 1569, 1508, 1460, 1392, 1359, 1309, 1256, 1211, 1181, 1117, 1006; δ_H (300 MHz, $CDCl_3$) 7.83 (1H, d, J=8.3 Hz, ArH), 7.07 (1H, d, J=8.3 Hz, ArH), 2.66 (3H, s, Me), 2.49 (3H, s, Me), 1.02 (9H, s, CMe_3), 0.25 (6H, s, $SiMe_2$); δ_C (75 MHz, $CDCl_3$) 199.9 (C), 153.8 (C), 150.7 (C), 146.6 (C), 124.7 (CH), 121.5 (CH), 26.0 (Me), 20.5 (Me), 18.6 (CMe_3), −3.5 ($SiMe_2$); *m/z* (CI) 266 (MH^+, 100%), 152 (5).

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Novel mercaptoacetylative expeditious annulation of 5-mercaptopyrimidine ring on azoles using 1,3-oxathiolan-5-one

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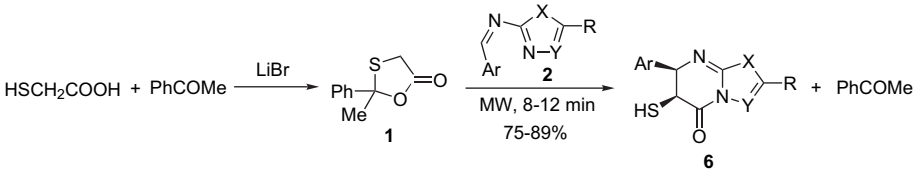
Abstract—Schiff bases of azoles containing the amino group as part of a partial amidine structure undergo mercaptoacetylative expeditious annulation with 2-methyl-2-phenyl-1,3-oxathiolan-5-one to yield highly substituted 6,7-dihydro-6-mercapto-5*H*-thiazolo/1,3,4-oxa(thia)-diazolo[3,2-*a*]pyrimidin-5-ones stereoselectively. The annulation is effected via an isolable intermediate under solvent-free microwave irradiation conditions in a one-pot procedure.

1. Introduction

Pyrimidines and azoles have a long history of applications in pharmaceutical and agrochemical industries.[1–6] Thus, heterocyclic systems resulting from the annulation of a pyrimidine ring on biologically versatile azoles appear to be attractive scaffolds to be utilised for exploiting chemical diversity. Highly substituted heterocycles are interesting as potential biodegradable pharmaceuticals and agrochemicals.[7–9] It is well known that the presence of a thiol function in many enzymes, called '–SH enzymes', is essential for their enzyme activity. Likewise, incorporation of a thiol



2-6	R	X	Y	Ar
a	Ph	O	N	Ph
b	Ph	O	N	4-ClC_6H_4
c	Ph	O	N	4-$MeOC_6H_4$
d	4-ClC_6H_4	O	N	Ph
e	4-ClC_6H_4	O	N	4-ClC_6H_4
f	4-ClC_6H_4	O	N	4-$MeOC_6H_4$
g	Ph	S	N	Ph
h	Ph	S	N	4-ClC_6H_4
i	Ph	S	N	4-$MeOC_6H_4$
j	H	S	C-Ph	Ph
k	H	S	C-Ph	4-ClC_6H_4
l	H	S	C-Ph	4-$MeOC_6H_4$

Scheme 1.

Keywords: Solvent-free; Microwaves; Schiff bases; Stereoselective synthesis; Azolo-pyrimidines.
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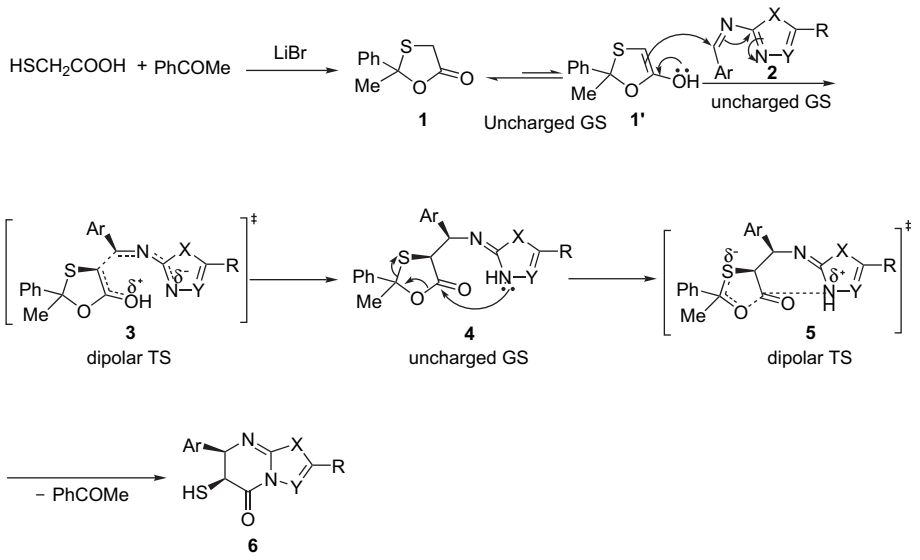

Scheme 2.

function in heterocycles, nucleosides, or nucleotides has led to a number of analogues possessing interesting biological and therapeutic properties.[10–17] Azolo-pyrimidines **6** incorporating a thiol function are hitherto unreported, and neither classical synthetic approaches to heterocycles nor classical functionalisation reactions of heterocycles can be readily used for their synthesis.

We have previously reported diastereoselective synthetic protocols for annulating pyrimidine ring on azoles incorporating an amino function at C-6 employing glycine derivatives.[18–22] As part of an ongoing programme of research, we had to develop a rapid and efficient synthetic approach to azolo-pyrimidine heterocyclic systems **6** incorporating a thiol function at C-6. For this purpose, we utilised the recently reported mercaptoacetyl transfer agent, 2-methyl-2-phenyl-1,3-oxathiolan-5-one **1**,[23] which leads to the desired mercaptoacetylative heteroannulation and is the key element in the present successful synthetic strategy for the target compounds **6** (Scheme 1). It is noteworthy that acetophenone, which is used to activate mercaptoacetic acid to act as a novel and efficient mercaptoacetyl transfer agent **1**, is automatically removed during the reactions yielding compounds **6**.

In order to achieve our goal expeditiously, we relied upon significant advantages of solvent-free microwave (MW) irradiation such as enhanced reaction rates, higher yields of pure products, easier work-up, rapid optimisation of reactions in parallel and several eco-friendly advantages in the context of green chemistry.[24–28] The application of MW irradiation to provide enhanced reaction rates and improved product yields in chemical synthesis has been extended to modern drug discovery processes,[29,30] and it is proving quite successful in the formation of carbon–heteroatom and carbon–carbon bonds.[31,32] Interestingly, the conjugate addition of **1** to azole Schiff bases **2** followed by ring transformation of the isolable adducts **4** to azolo-pyrimidinones **6** (Scheme 2) are among the few examples showing increased stereoselectivity under MW irradiation compared to conventional heating.

2. Results and discussion

After some preliminary experimentation, it was found that the envisaged annulation was successful with an intimate mixture of 2-methyl-2-phenyl-1,3-oxathiolan-5-one **1** and an azole Schiff base **2** under MW irradiation of 100 W in a CEM Discover microwave system for the time specified in Table 1. Isolation and purification by recrystallisation from ethanol afforded the azolo-pyrimidin-5-ones **6** in 75–89% yield (Table 1) with >95% diastereoselectivity.

For comparison purposes, the final temperature of the reaction mixture was recorded immediately after the MW irradiation and found to be <80 °C. The reactions were also carried out using a thermostated oil-bath at the same temperature (80 °C) as for the MW-activated method but for a longer (optimised) period of time (Table 1) to ascertain whether the MW method improves the yield or simply increases conversion rates. It was found that significantly lower yields

Table 1. Solvent-free microwave-activated synthesis of products **4** and **6**

Product	Time		Yield (%)[c,d]	
	MW[a] (min)	Thermal[b] (h)	MW	Thermal
4a	4	8	48	40
4h	4	8	51	45
4k	4	8	43	40
6a	12	14	76	44
6b	10	12	80	48
6c	12	13	78	45
6d	12	14	77	46
6e	8	12	89	52
6f	8	13	85	49
6g	12	14	75	43
6h	10	13	78	46
6i	12	14	77	45
6j	12	14	75	44
6k	10	13	77	45
6l	12	14	79	47

[a] Microwave irradiation time (power=100 W).
[b] Time for oil-bath heating at 80 °C.
[c] Yield of isolated and purified products.
[d] All compounds gave C, H and N analyses within ±0.35% and satisfactory spectral (IR, ^{1}H NMR, ^{13}C NMR and EIMS) data.

(40–52%) were obtained using oil-bath heating rather than the MW-activated method (Table 1).

This observation may be rationalised on the basis of the formation of a dipolar transition state (TS) from an uncharged ground state (GS) in these reactions (Scheme 2), and the greater stabilisation of more polar TS by dipole–dipole interactions with the electric field of microwaves as compared to the less polar GS, which may reduce the activation energy ($\Delta G^{\neq}$) resulting in the rate enhancement.[24]

The formation of **6** is rationalised by the conjugate addition of oxathiolan-5-one **1** to azole Schiff bases **2** to furnish adducts **4**, which undergo intramolecular nucleophilic attack of the nitrogen atom of the azole ring (*N*-3) at the carbonyl carbon (*C*-5) of the oxathiolan-5-one nucleus to yield **6** with the elimination of acetophenone (Scheme 2). This conclusion is based on the observation that the representative intermediate compounds **4a**, **4h** and **4k** could be isolated in 43–51% yield, these could be converted into the corresponding annulated products **6a**, **6h** and **6k** in quantitative yield, and that acetophenone was formed during the reaction (Scheme 2).

The formation of adducts **4** and their annulation to **6** were highly diastereoselective in favour of cis isomers. The diastereomeric ratios of the crude products were checked by ^{1}H NMR, prior to purification to ensure accurate and true diastereomeric ratios are reported. The diastereomeric ratio for compounds **6** in the case of MW activation was found to be >95:<5 and that from the oil-bath heating was >56:<44 as determined by ^{1}H NMR spectroscopy. The high diastereoselectivity (>95%) in favour of cis isomers under MW irradiation may be explained by considering that MW irradiation favours the reactions occurring via more polar TS[24] and that the TS leading to the formation of cis isomers is more polar than that leading to the trans isomer because, in general, cis isomers are more polar than the trans.[33]

3. Conclusion

In summary, we have developed a novel mercaptoacetic acid-based one-pot synthetic protocol for an expeditious and highly diastereoselective synthesis of potentially pharmaceutically and agrochemically useful 6-mercaptoazolo-pyrimidin-5-ones starting from readily available simple substrates employing solvent-free microwave irradiation conditions.

4. Experimental

4.1. General

Melting points were determined by open glass capillary method and are uncorrected. IR spectra in KBr were recorded on a Perkin–Elmer 993 IR spectrophotometer, ^{1}H NMR spectra were recorded on a Bruker WM-40 C (400 MHz) FT spectrometer in DMSO-d_6 using TMS as internal reference. ^{13}C NMR spectra were recorded on the same instrument at 100 MHz using the same solvent and internal reference. Mass spectra were recorded on a JEOL D-300 mass spectrometer. Elemental analyses were carried out in a Coleman automatic carbon, hydrogen and nitrogen analyser. A CEM Discover Focused Microwave Synthesis System operating at 2450 MHz was used at an output of 100 W for all the experiments. All chemicals used were reagent grade and were used as received without further purification. Silica gel-G was for TLC.

4.2. 2-Methyl-2-phenyl-1,3-oxathiolan-5-one 1

It was prepared by the condensation of acetophenone and mercaptoacetic acid in the presence of lithium bromide according to the recently reported method.[23]

4.3. 2,7-Diaryl-6,7-dihydro-6-mercapto-5*H*-thiazolo/1,3,4-oxa(thia)diazolo[3,2-*a*]pyrimidin-5-ones 6. General procedure

Thoroughly mixed 2-methyl-2-phenyl-1,3-oxathiolan-5-one **1** (2.0 mmol) and an azole Schiff base **2** (2.0 mmol) were taken in a 10 mL vial and subjected to MW irradiation at 100 W in a CEM Discover microwave system for 2 min. The reaction mixture was then thoroughly mixed outside the MW oven for 2 min and again irradiated for another 2 min. This intermittent irradiation-mixing cycle was repeated for the total irradiation time (Table 1). After completion of the reaction as indicated by TLC (hexane/AcOEt, 8:2, v/v), water (10 mL) was added to the reaction mixture and stirred well. The yellowish precipitate thus obtained was washed with water to give the crude product which was recrystallised from ethanol to afford a diastereomeric mixture (>95:<5; in the crude products the ratio was >94:<6 as determined by ^{1}H NMR spectroscopy). The product on second recrystallisation from ethanol furnished an analytically pure sample of a single diastereomer **6** (Table 1). On the basis of comparison of J values to literature ones,[18,34–38] the cis stereochemistry was assigned to **6**, as the coupling constant ($J_{6,7}$=4 Hz) for **6** was lower than that for very minor (<5%) diastereomer (trans), $J_{6,7}$=10 Hz.

4.3.1. Compound 6a. Yellowish needles (2.45 g, 76%), mp 215–217 °C. IR (KBr) ν_{max} 3046, 2550, 1680, 1602, 1586, 1446, 1310 cm^{-1}. ^{1}H NMR (DMSO-d_6/TMS) δ: 1.59 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.64 (d, 1H, J=4 Hz, H-7), 6.77 (dd, 1H, J=4, 8 Hz, H-6), 7.12–8.00 (m, 10H$_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.4 (7-C), 64.7 (6-C), 127.4, 128.2, 128.9, 129.6, 130.2, 130.9, 131.6, 132.6 (2×Ph), 159.6 (SC=N), 161.1 (2-C), 172.3 (C=O). Mass (m/z): 323 (M^+). Anal. Calcd for $C_{17}H_{13}N_3O_2S$: C, 63.14; H, 4.05; N, 12.99%. Found: C, 63.45; H, 4.29; N, 12.79%.

4.3.2. Compound 6b. Yellowish needles (2.86 g, 80%), mp 225–227 °C. IR (KBr) ν_{max} 3055, 2561, 1683, 1605, 1581, 1451, 1315 cm^{-1}. ^{1}H NMR (DMSO-d_6/TMS) δ: 1.61 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.64 (d, 1H, J=4 Hz, H-7), 6.79 (dd, 1H, J=4, 8 Hz, H-6), 7.16–8.01 (m, 9H$_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.4 (7-C), 64.7 (6-C), 127.2, 128.0, 128.6, 129.3, 130.0, 130.8, 131.7, 132.5 (Ph, 4-ClC_6H_4), 159.7 (SC=N), 161.2 (2-C), 172.0 (C=O). Mass (m/z): 357 (M^+). Anal. Calcd for $C_{17}H_{12}ClN_3O_2S$: C, 57.06; H, 3.38; N, 11.74%. Found: C, 57.41; H, 3.58; N, 11.94%.

4.3.3. Compound 6c. Yellowish needles (2.75 g, 78%), mp 220–221 °C. IR (KBr) ν_{max} 3061, 2582, 1685, 1604, 1576,

1460, 1320 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.60 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 3.75 (s, 3H, OMe), 6.65 (d, 1H, J=4 Hz, H-7), 6.78 (dd, 1H, J=4, 8 Hz, H-6), 7.13–7.99 (m, $9H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 54.6 (OMe), 61.5 (7-C), 64.8 (6-C), 127.3, 128.1, 128.9, 129.5, 130.3, 131.0, 131.6, 132.5 (Ph, 4-$MeOC_6H_4$), 159.8 (SC=N), 161.3 (2-C), 172.3 (C=O). Mass (*m*/*z*): 353 (M^+). Anal. Calcd for $C_{18}H_{15}N_3O_3S$: C, 61.18; H, 4.28; N, 11.89%. Found: C, 61.48; H, 4.03; N, 11.65%.

4.3.4. Compound 6d. Yellowish needles (2.75 g, 77%), mp 230–231 °C. IR (KBr) ν_{max} 3062, 2589, 1681, 1603, 1585, 1455, 1321 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.59 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.65 (d, 1H, J=4 Hz, H-7), 6.76 (dd, 1H, J=4, 8 Hz, H-6), 7.11–7.99 (m, $9H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.5 (7-C), 64.5 (6-C), 127.4, 128.1, 128.9, 129.5, 130.8, 131.6, 132.3, 133.0 (Ph, 4-ClC_6H_4), 159.8 (SC=N), 161.1 (2-C), 172.1 (C=O). Mass (*m*/*z*): 357 (M^+). Anal. Calcd for $C_{17}H_{12}ClN_3O_2S$: C, 57.06; H, 3.38; N, 11.74%. Found: C, 57.41; H, 3.58; N, 11.95%.

4.3.5. Compound 6e. Yellowish needles (3.50 g, 89%), mp 223–225 °C. IR (KBr) ν_{max} 3059, 2591, 1680, 1601, 1581, 1460, 1323 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.62 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.68 (d, 1H, J=4 Hz, H-7), 6.79 (dd, 1H, J=4, 8 Hz, H-6), 7.16–8.00 (m, $8H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.6 (7-C), 64.6 (6-C), 127.5, 128.2, 129.0, 129.7, 130.7, 131.5, 132.2, 133.0 (2×4-ClC_6H_4), 159.7 (SC=N), 161.2 (2-C), 172.3 (C=O). Mass (*m*/*z*): 393 (M^+). Anal. Calcd for $C_{17}H_{11}Cl_2N_3O_2S$: C, 52.05; H, 2.83; N, 10.71%. Found: C, 52.40; H, 2.60; N, 10.96%.

4.3.6. Compound 6f. Yellowish needles (3.29 g, 85%), mp 215–216 °C. IR (KBr) ν_{max} 3058, 2565, 1680, 1602, 1582, 1458, 1312 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.61 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 3.74 (s, 3H, OMe), 6.66 (d, 1H, J=4 Hz, H-7), 6.78 (dd, 1H, J=4, 8 Hz, H-6), 7.12–8.01 (m, $8H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 54.4 (OMe), 61.6 (7-C), 64.6 (6-C), 127.4, 128.1, 128.9, 129.8, 130.5, 131.2, 131.9, 132.7 (4-ClC_6H_4, 4-$MeOC_6H_4$), 159.7 (SC=N), 161.2 (2-C), 172.2 (C=O). Mass (*m*/*z*): 387 (M^+). Anal. Calcd for $C_{18}H_{14}ClN_3O_3S$: C, 55.74; H, 3.64; N, 10.83%. Found: C, 55.50; H, 3.89; N, 10.60%.

4.3.7. Compound 6g. Yellowish needles (2.54 g, 75%), mp 184–186 °C. IR (KBr) ν_{max} 3049, 2583, 1684, 1599, 1574, 1459, 1319 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.60 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.62 (d, 1H, J=4 Hz, H-7), 6.74 (dd, 1H, J=4, 8 Hz, H-6), 7.10–7.79 (m, $10H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.6 (7-C), 64.8 (6-C), 127.2, 127.9, 128.6, 129.4, 130.2, 131.0, 131.8, 132.7 (2×Ph), 150.0 (2-C), 159.9 (SC=N), 172.4 (C=O). Mass (*m*/*z*): 339 (M^+). Anal. Calcd for $C_{17}H_{13}N_3OS_2$: C, 60.15; H, 3.86; N, 12.38%. Found: C, 60.50; H, 3.64; N, 12.15%.

4.3.8. Compound 6h. Yellowish needles (2.91 g, 78%), mp 168–169 °C. IR (KBr) ν_{max} 3055, 2581, 1681, 1602, 1586, 1440, 1311 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.62 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.64 (d, 1H, J=4 Hz, H-7), 6.77 (dd, 1H, J=4, 8 Hz, H-6), 7.10–7.79 (m, $9H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.4 (7-C), 64.9 (6-C), 127.3, 128.0, 128.6, 129.6, 130.2, 131.0, 131.7, 132.6 (Ph, 4-ClC_6H_4), 150.3 (2-C), 160.1 (SC=N), 172.4 (C=O). Mass (*m*/*z*): 373 (M^+). Anal. Calcd for $C_{17}H_{12}ClN_3OS_2$: C, 54.61; H, 3.24; N, 11.24%. Found: C, 54.18; H, 3.49; N, 11.48%.

4.3.9. Compound 6i. Yellowish needles (2.84 g, 77%), mp 177–178 °C. IR (KBr) ν_{max} 3063, 2590, 1682, 1600, 1581, 1455, 1321 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.62 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 3.75 (s, 3H, OMe), 6.63 (d, 1H, J=4 Hz, H-7), 6.76 (dd, 1H, J=4, 8 Hz, H-6), 7.11–7.81 (m, $9H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 54.3 (OMe), 61.5 (7-C), 64.7 (6-C), 127.2, 127.9, 128.6, 129.5, 130.6, 131.2, 132.0, 132.8 (Ph, 4-$MeOC_6H_4$), 150.1 (2-C), 160.0 (SC=N), 172.3 (C=O). Mass (*m*/*z*): 369 (M^+). Anal. Calcd for $C_{18}H_{15}N_3O_2S_2$: C, 58.52; H, 4.09; N, 11.37%. Found: C, 58.87; H, 4.34; N, 11.17%.

4.3.10. Compound 6j. Yellowish needles (2.45 g, 75%), mp 164–165 °C. IR (KBr) ν_{max} 3051, 2572, 1680, 1603, 1583, 1454, 1322 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.58 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.58 (d, 1H, J=4 Hz, H-7), 6.72 (dd, 1H, J=4, 8 Hz, H-6), 7.62–7.81 (m, $11H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.4 (7-C), 64.6 (6-C), 118.5 (C-2), 127.0, 127.6, 128.4, 129.2, 129.8, 130.6, 131.2, 132.0 (2×Ph), 150.1 (3-C), 159.7 (SC=N), 172.0 (C=O). Mass (*m*/*z*): 326 (M^+). Anal. Calcd for $C_{17}H_{14}N_2OS_2$: C, 62.55; H, 4.32; N, 8.58%. Found: C, 62.20; H, 4.52; N, 8.35%.

4.3.11. Compound 6k. Yellowish needles (2.77 g, 77%), mp 118–120 °C. IR (KBr) ν_{max} 3059, 2566, 1682, 1601, 1580, 1448, 1317 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.62 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 6.62 (d, 1H, J=4 Hz, H-7), 6.76 (dd, 1H, J=4, 8 Hz, H-6), 7.12–7.83 (m, $10H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 61.6 (7-C), 64.8 (6-C), 118.6 (C-2), 127.2, 128.0, 128.7, 129.4, 130.2, 131.0, 131.7, 132.4 (Ph, 4-ClC_6H_4), 150.3 (3-C), 159.8 (SC=N), 172.2 (C=O). Mass (*m*/*z*): 360 (M^+). Anal. Calcd for $C_{17}H_{13}ClN_2OS_2$: C, 56.58; H, 3.63; N, 7.76%. Found: C, 56.88; H, 3.87; N, 7.54%.

4.3.12. Compound 6l. Yellowish needles (2.81 g, 79%), mp 180–181 °C. IR (KBr) ν_{max} 3066, 2592, 1685, 1605, 1574, 1460, 1323 cm^{-1}. ^1H NMR (DMSO-d_6/TMS) δ: 1.61 (d, 1H, J=8 Hz, SH, exchanges with D_2O), 3.74 (s, 3H, OMe), 6.60 (d, 1H, J=4 Hz, H-7), 6.74 (dd, 1H, J=4, 8 Hz, H-6), 7.10–7.95 (m, $10H_{arom}$). ^{13}C NMR (DMSO-d_6/TMS) δ: 54.2 (OMe), 61.7 (7-C), 64.9 (6-C), 118.8 (2-C), 127.3, 128.0, 128.6, 129.5, 130.2, 131.0, 131.8, 132.6 (Ph, 4-$MeOC_6H_4$), 150.3 (3-C), 159.9 (SC=N), 172.0 (C=O). Mass (*m*/*z*): 356 (M^+). Anal. Calcd for $C_{18}H_{16}N_2O_2S_2$: C, 60.65; H, 4.52; N, 7.86%. Found: C, 60.30; H, 4.75; N, 7.66%.

4.4. Isolation of 4a, 4h and 4k and their conversion into the corresponding annulated products 6a, 6h and 6k

The procedure followed was the same as described above for the synthesis of **6** except that the time of MW irradiation in this case was 4 min instead of 8–12 min for **6**. The adducts **4** were recrystallised from ethanol to give a diastereomeric mixture (>96:<4; in the crude isolates the ratio was >94:<6 as determined by ^1H NMR spectroscopy) which

was again recrystallised from ethanol to obtain an analytical sample of **4a**, **4h** and **4k**. The adducts **4a**, **4h** and **4k** were assigned the *erythro* (*syn*) stereochemistry, as their ^{1}H NMR spectra exhibited lower values of coupling constant $J_{SCH,NCH}$=4 Hz, than that of the very minor (<4%) diastereomer (*threo* or *anti*), $J_{SCH,NCH}$=9 Hz.[18,34–38] Finely powdered intermediate compounds **4a**, **4h** and **4k** were intermittently MW irradiated for 6 min in the same way as described for the synthesis of **6** to give the corresponding annulated products **6a**, **6h** and **6k** quantitatively.

4.4.1. Compound 4a. Yellowish needles (2.13 g, 48%), mp 225–226 °C. IR (KBr) ν_{max} 3309, 2972, 1778, 1605, 1574, 1460, 1318 cm^{-1}. ^{1}H NMR (DMSO-d_6/TMS) δ: 2.33 (s, 3H, Me), 6.68 (d, 1H, J=4 Hz, NCH), 6.79 (d, 1H, J=4 Hz, SCH), 7.65–7.86 (m, 15H$_{arom}$), 8.56 (br s, 1H, NH, exchanges with D_2O). ^{13}C NMR (DMSO-d_6/TMS) δ: 20.2 (Me), 64.5 (Ar–*C*), 69.4 (O=C–*C*), 127.0, 127.8, 128.5, 129.2, 129.9, 130.6, 131.2, 132.0, 132.7, 133.3, 134.0, 134.8 (3×Ph), 159.5 (SC=N), 161.2 (R–*C*), 172.1 (C=O). Mass (*m*/*z*): 443 (M^+). Anal. Calcd for $C_{25}H_{21}N_3O_3S$: C, 67.70; H, 4.77; N, 9.47%. Found: C, 67.36; H, 4.52; N, 9.72%.

4.4.2. Compound 4h. Yellowish needles (2.52 g, 51%), mp 218–220 °C. IR (KBr) ν_{max} 3307, 2970, 1775, 1603, 1586, 1458, 1315 cm^{-1}. ^{1}H NMR (DMSO-d_6/TMS) δ: 2.35 (s, 3H, Me), 6.69 (d, 1H, J=4 Hz, NCH), 6.78 (d, 1H, J=4 Hz, SCH), 7.66–7.85 (m, 14H$_{arom}$), 8.57 (br s, 1H, NH, exchanges with D_2O). ^{13}C NMR (DMSO-d_6/TMS) δ: 20.3 (Me), 64.6 (Ar–*C*), 69.5 (O=C–*C*), 127.2, 127.8, 128.6, 129.2, 129.9, 130.6, 131.2, 131.8, 132.4, 133.0, 133.7, 134.3 (2×Ph, 4-ClC_6H_4), 150.1 (R–*C*), 159.6 (SC=N), 172.2 (C=O). Mass (*m*/*z*): 495 (M^+). Anal. Calcd for $C_{25}H_{20}ClN_3O_2S_2$: C, 60.78; H, 4.08; N, 8.51%. Found: C, 60.43; H, 4.33; N, 8.75%.

4.4.3. Compound 4k. Yellowish needles (2.07 g, 43%), mp 115–116 °C. IR (KBr) ν_{max} 3303, 2971, 1776, 1601, 1585, 1440, 1310 cm^{-1}. ^{1}H NMR (DMSO-d_6/TMS) δ: 2.34 (s, 3H, Me), 6.67 (d, 1H, J=4 Hz, NCH), 6.77 (d, 1H, J=4 Hz, SCH), 7.64–7.84 (m, 15H$_{arom}$), 8.47 (br s, 1H, NH, exchanges with D_2O). ^{13}C NMR (DMSO-d_6/TMS) δ: 20.2 (Me), 64.5 (Ar–*C*), 69.5 (O=C–*C*), 118.2 (SCH), 127.1, 127.8, 128.4, 129.0, 129.7, 130.4, 131.0, 131.7, 132.3, 133.0, 133.7, 134.5 (2×Ph, 4-ClC_6H_4), 150.0 (Ph–*C*), 159.5 (SC=N), 172.3 (C=O). Mass (*m*/*z*): 482 (M^+). Anal. Calcd for $C_{25}H_{21}ClN_2O_2S_2$: C, 62.42; H, 4.40; N, 5.82%. Found: C, 62.77; H, 4.17; N, 5.60%.

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An autoxidation study of C2 substituted pyrimidine amino reductones

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Abstract—Three pyrimidine derivatives (**8a–c**), differing from isouramil and divicine at C2, have been synthesized and their autoxidation rates measured spectrophotometrically. The autoxidation rates of all five pyrimidines (**8a–c**, isouramil and divicine) were correlated with σ_p^+ values (rho=−1.28, r^2=0.949).

1. Introduction

The amino reductone structure,[1] i.e., enolamine, occurs in a number of natural products. For instance, several species of ancient organisms (fungi, cyanobacteria, and lichens) produce UV absorbing metabolites such as MAA's (mycosporine-like amino acids, Fig. 1) that are characterized by a cyclohexenone **1** or cyclohexenimine **2** chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol and having absorption maxima ranging from 310 to 360 nm.[2–3]

MAA's can be considered as potential sunscreens as their conjugated amino enolic chromophore has both broad absorption in the UV region and antioxidant properties desirable in a sun blocker.[4] Certain pyrimidine derivatives, i.e., isouramil **8d** (6-amino-2,5-dihydroxypyrimidin-4-one) and divicine **8e** (2,6-diamino-5-hydroxypyrimidin-4-one) incorporate the amino reductone group (Scheme 2). They are found in beans as glycosides and are thought to be the causative agents in favism.[5] A synergistic cytotoxicity has been demonstrated between carboplatin and divicine on murine erythroleukemic cells.[6] Divicine also enhances in vitro and in vivo lipopolysaccharide-induced release of tumor necrosis factor (TNF).[7]

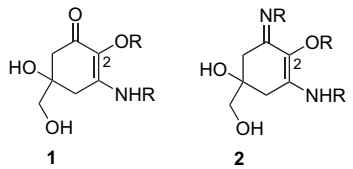

Figure 1. Structure of mycosporine-like amino acids.

A putative *cis*-enolamine **3** is thought to be the active intermediate in the formation of D-glucosamine-6-phosphate from D-fructose-6-phosphate using L-glutamine as the ammonia source, by glucosamine-6-phosphate synthase (GlmS). Because *N*-acetylglucosamine is an essential building block of both bacterial cell walls and fungal cell wall chitin, the enzyme is a potential target for antibacterial and antifungal agents.[8] Aminohexose reductones such as **4** obtained in the Maillard reaction are chiefly responsible for the development of aromas and colors during the thermal processing of foods (Fig. 2).[9]

Thus, an investigation of the redox properties of enolamines would shed light on their stability and behavior. In this paper

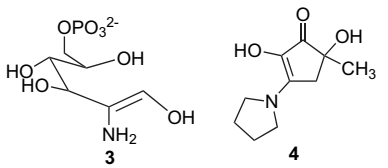

Figure 2. Naturally occurring *cis*-enoloamines.

Keywords: Amino reductones; Isouramil; Divicine; C2 pyrimidines; Autoxidation; Hammett correlation.
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we report the preparation of three pyrimidine derivatives (**8a–c**), differing from isouramil and divicine at C2, and the spectrophotometric measurement of their autoxidation rates.

2. Results and discussion

2.1. Synthesis

Pyrimidines **8a–c** were synthesized by condensing thiourea or acetamidine with 2-(tetrahydropyran-2-yloxy)-malonic acid diethyl ester (prepared in situ from tetrahydropyran-2-yloxyacetic acid ethyl ester, sodium hydride, and ethyl formate) to give **5a** and **5c**. Hydrolysis of **5a** and **5c** gave **6a** and **6c**, respectively, while treatment of **5c** with Raney Ni gave **6b**. Coupling of **6a** and **6b** with benzene diazonium chloride gave **7a** and **7b**, respectively. Methylation of **6c** followed by the same diazo coupling gave **7c**. Reduction of **7a–c** by dithionite gave **8a–c** (Scheme 1).

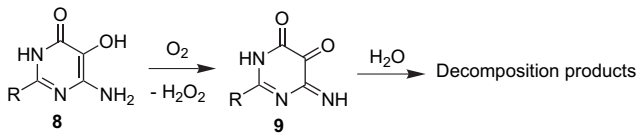
In addition to the spectroscopic and analytical data (see Section 4), the existence of an enolamine system (reductone) in compounds **8** was determined by various qualitative color tests including, ferric chloride solution,[10] 2,6-dichlorophenolindophenol solution,[11] and phosphomolybdic solution.[12]

2.2. Kinetic study of autoxidation

Compounds **8a–c**, differing from isouramil and divicine only at C2, were subjected to autoxidation under neutral conditions giving presumably H_2O_2 (Scheme 2).[5a,13–14]

The autoxidation rate was measured in air saturated buffer phosphate solutions 0.05 M at pH 7 and 25 °C. The solutions contained 1 mM EDTA to minimize catalysis of the oxidation by trace metallic cations.[5a,15] The rate of autoxidation was measured spectrophotometrically by following the decrease of the UV absorbance of the pyrimidines at their respective λ_{max} (Table 1).

All three compounds showed much slower oxidation rates compared with isouramil and divicine. This is probably the reason why there was no transient appearance of an absorption maximum around 240–255 nm, which is assumed to be due to the intermediacy of oxidized pyrimidine **9**, and was found in the autoxidation of isouramil, divicine, and related systems.[5a,14,16] The decrease in the absorbance of **8a–c**, followed a reasonably pseudo first order reaction in the pyrimidine concentration (the dissolved oxygen concentration was at least 13 times higher). However, Winterbourn et al. found that the reaction mechanism is complex and involves radical intermediates and chain reactions and that the dependency on the pyrimidine concentration is far from simple.[14,17] Therefore, we preferred to use the measured initial reaction rates for checking the quantitative dependency of the oxidation rate on the electron releasing power of the substituent in the C2 position. Our results for **8a–c** are summarized in



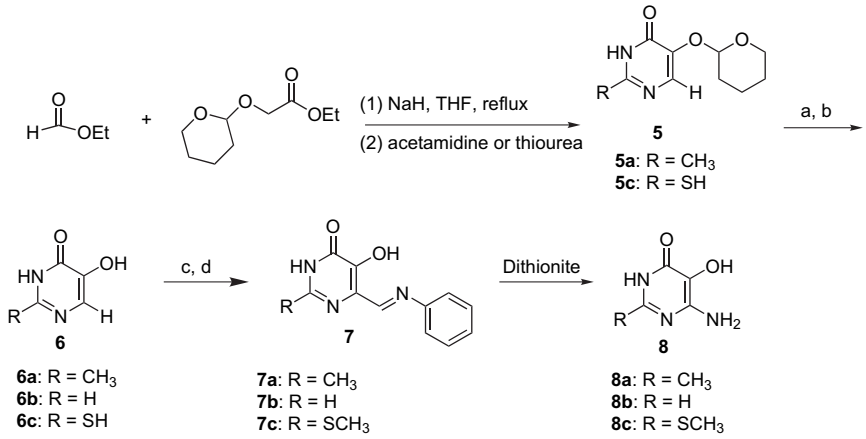
8a: R = CH_3
8b: R = H
8c: R = SCH_3
8d: R = OH (isouramil)
8e: R = NH_2 (divicine)

Scheme 2. Pyrimidine derivatives autoxidation.

Table 1. Spectral properties and initial autoxidation rates for compounds **8a–e**

Substituent R	λ_{max} nm (ε)	Initial rate μM/s	σ_p [a]	σ_p^+ [a]
H	274 (13,000)[b]	0.00087[c]	0	0
CH_3	275 (16,200)[b]	0.0015[c]	−0.17	−0.31
SCH_3	286 (10,800)[b]	0.0097[c]	0	−0.6
OH	280 (14,100)[d]	0.067[e]	−0.37	−1.6[f]
NH_2	285 (9800)[d]	0.061[e]	−0.66	−1.3

[a] Ref. 18.
[b] This work.
[c] This work; air saturated 0.05 M phosphate buffer pH=7.0, 1 mM EDTA, 25 °C, [pyrimidine]=2.4×10^{-5} M.
[d] Ref. 5a.
[e] Calculated from data in Ref. 14, see text.
[f] σ_p^+ for OH from Ref. 19.



5a: R = CH_3
5c: R = SH

6a: R = CH_3
6b: R = H
6c: R = SH

7a: R = CH_3
7b: R = H
7c: R = SCH_3

8a: R = CH_3
8b: R = H
8c: R = SCH_3

[a]H_3O^+ for **6a**, **6c**. [b]Raney Ni for **6b**. [c] PhN_2Cl for **7a** and **7b**. [d]$(MeO)_2SO_2$ then PhN_2Cl for **7c**.

Scheme 1. Synthesis of compounds **8**.

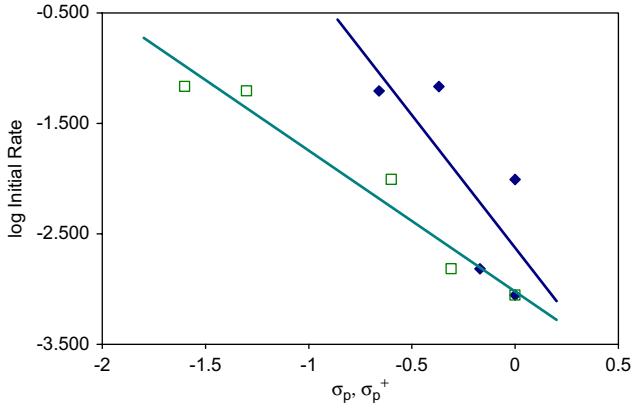
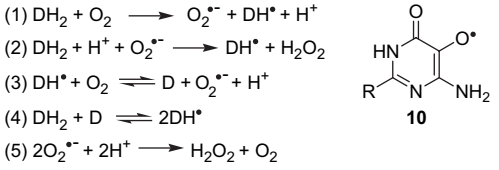


Figure 3. Hammett correlations against σ_p (◆) and σ_p^+ (□) for the autoxidation of compounds **8a–e** in air saturated solutions at pH 7.0 and 25 °C. Rho value for σ_p is −2.40, r^2=0.5868; rho value for σ_p^+ is −1.28, r^2=0.949.

Table 1. Table 1 also contains the initial rates for the reactions of isouramil and divicine (**8d** and **8e**) calculated from Winterbourn et al. data[14] (aerated 0.05 M phosphate buffer, pH 7, 23 °C, 50 mM DTPA and similar to our pyrimidine concentrations) using ΔH of 60.2 kJ/mol given by Chevion et al.[5a] A plot of the logarithmic values of the initial rates against Hammett σ_p constants did not give a reasonable correlation. However, the correlation was greatly improved when we used σ_p^+[18,19] values, rho value is −1.28 (r^2= 0.949) (Fig. 3).[20]

Winterbourn et al.[14,17] suggested the following complex chain mechanism to account for the autoxidation rates of isouramil and divicine. DH_2 stands for the reduced pyrimidine, $DH^\bullet$ for the pyrimidine radical (probably structure **10**) and D for the oxidized pyrimidine, most probably having structure **9** (Scheme 2). It is reasonable to expect the same mechanism for our compounds **8a**, **8b**, and **8c** (Scheme 3).



Scheme 3.

The observed initial reaction rates must be the result of a complex combination of the rates of the individual steps detailed in Scheme 3. Our limited data do not justify enlarging upon them, but still, the observed initial rate surely reflects the rate of the first initiation step (1) as well as the rates of the rate determining propagation steps (2) and (4). It is obvious that stabilizing the generated radical $DH^\bullet$ will enhance the rate of the above three steps. The formation of $DH^\bullet$ from DH_2 lowers the electron density on the oxygen atom and that explains the enhanced autoxidation rate with the electron releasing power of the substituent at position 2. The good correlation with σ_p^+ clearly indicates the role of resonance and partial distribution of charge in stabilizing the generated pyrimidine radical. A similar correlation was found between σ_p^+ and the one-electron reduction potential of 4-substituted phenoxyl radicals measured through their equilibrium with known redox pairs.[21,22] It is interesting to note that Xu et al.[23] checked the correlation of the first oxidation potential of substituted *N*-hydroxyacetanilides with Hammett σ^- values and found only a fair correlation (r^2=0.962). However, we found that their results correlate much better with σ^+ values (r^2=0.9943).

3. Conclusion

Several amino reductones occur naturally and are responsible for life-threatening hemolytic episodes in favism. On the other hand, amino reductones may also be useful sunscreens. Thus an understanding of their properties is desirable. We have found marked dependency of the autoxidation rate of five pyrimidine derivatives **8a–e** on the electron releasing power of the substituent at C2 and this can point a way to control the oxidation rate of future useful compounds.

4. Experimental

4.1. General procedures

All chemicals were of reagent grade and were used without further purification. ^{1}H NMR (300 MHz) spectra were recorded in $CDCl_3$. (*J* values are given in hertz). IR spectra were recorded on a Perkin–Elmer 2000 Fourier transformed infrared instrument. MS analyses were performed on Finnegan instrument (Model QDVO). All UV spectra were recorded on Unicam SP-1700. Nitrogen was bubbled for 10 min through 0.05 M phosphate buffer at pH 7 containing 1 mM EDTA. To 1.9 mL of the above buffer in a UV cell, 100 μL of freshly prepared pyrimidine solution (**8a–c**) were injected to give a final concentration of 5×10^{-5} M. The UV spectrum at the range 230–340 nm was recorded immediately. Injecting the same pyrimidine solution to air saturated buffer solution and recording the spectrum at increasing time intervals, showed a constant decrease in the UV absorption at the range 230–340 nm with no build up of a new absorption band at 240–255 nm as was found for isouramil and divicine.[5a] Kinetic measurements were performed by injecting 50 μL of the above pyrimidine solution into 2 cm^3 of the air saturated phosphate buffer thermo equilibrated at 25±1 °C, final pyrimidine concentration 2.4×10^{-5} M. The decrease of the absorbance at λ_{max} was recorded for 1–2 half-lives.

4.1.1. 2-Methyl-5-(tetrahydro-2*H*-pyran-2-yloxy)pyrimidin-4(3*H*)-one (5a).[24] To a suspension of sodium hydride (4.60 g in 55–60% paraffin oil), dry ether (50 cm^3) and dry ethyl formate (7.84 g) were added. Then (tetrahydropyran-2-yloxy)-acetic acid ethyl ester (20 g) was added dropwise under continuous stirring. After the mixture was refluxed for 2 h, acetamidine (4.3 g) was added. After the removal of ether from the reaction mixture, the remained ethanolic solution was refluxed for 4 h. Then the mixture was cooled and the volatile solvents were removed by rotory-evaporator. The residue was redissolved in water and filtered. The filtrate was acidified by acetic acid in an ice bath and the white precipitate was filtered, washed with water, and dried under reduced pressure at 100 °C. Yield (9.0 g, 58%), mp 149–151 °C; [Found: C 55.25; H 47.54; N 13.19. $C_{10}H_{16}N_2O_3$

requires C 55.59; H 7.60; N 13.20%]; δ_H (300 MHz, DMSO-d_6) 1.23–2.03 (6H, m), 2.27 (3H, s), 3.23–4.04 (2H, m), 5.47 (1H, br s), 7.60 (1H, s), 10.73 (1H, br s); ν_{max}/cm^{-1} 1670, 1610, 1385, 1310, 1205, 1190, 1120, 980, 910, 820, 775, and 740; MS(EI): *m*/*z* (%) 210 (100, M$^+$, $C_{10}H_{14}N_2O_3$), 126 (M$^+$, –C_5H_8O), 125 (M$^+$, –C_5H_9).

4.1.2. 5-Hydroxy-2-methylpyrimidin-4(3*H*)-one 6a. A few crystals of *p*-toluenesulfonic acid were added to a solution of **1a** (6.57 g) in hot methanol. After cooling the mixture in an ice bath, white crystals were formed. The crystals were filtered and dried under vacuum at 100 °C. Yield (2.5 g, 63%), mp>300 °C; δ_H (300 MHz, DMSO-d_6) 2.2 (3H, s), 7.27 (1H, s), 9.27 (1H, br s); ν_{max}/cm^{-1} 3300, 1670, 1620, 1420, 1380, 1245, 1110, 1020, 875, and 780; MS(EI): *m*/*z* (%) 126 (100, $C_5H_6N_2O_2$, M$^+$), 108 (M$^+$, –H_2O), 100 (M$^+$, −26).

4.1.3. 5-Hydroxy-2-methyl-6-phenylazo-3*H*-pyrimidin-4-one 7a. Diazotation of aniline (1.17 g) was done in hydrochloric acid (3.9 cm^3) and water (8 cm^3) by addition of sodium nitrite (0.87 g) in water (6 cm^3) at 0–5 °C. Then sodium acetate (3.1 g) was added slowly under continuous stirring, followed by the addition of a solution of **6a** (1.59 g) in 10% sodium hydroxide (10.4 cm^3). After stirring for 30 min, the reaction mixture was left for overnight at 4 °C. Then the reaction was warmed to 40 °C for 1 h and filtered. The red crystals formed were washed and dried under vacuum at 100 °C. Yield (1.61 g, 56%), mp 243–245 °C; [Found: C 57.60; H 4.22. $C_{11}H_{10}N_4O_2$ requires C 57.89; H 4.35%]; δ_H (300 MHz, DMSO-d_6) 1.95 (3H, s), 6.44–7.73 (5H, m), 11.19 (1H, br s), 11.64 (1H, br s); ν_{max}/cm^{-1} 3220, 3180, 1710, 1670, 1600, 1520, 1470, 1430, 1360, 1280, 1250, 1050, 800, 775, 715, 700, and 660; MS(EI): *m*/*z* (%) 230 (25, $C_{11}H_{10}N_4O_2$, M$^+$), 105 (100, $C_6H_5N_2^+$).

4.1.4. 6-Amino-5-hydroxy-2-methylpyrimidin-4(3*H*)-one 8a.[25] A solution of **7a** (1.61 g) in water (15 cm^3) was heated to 60–70 °C, an excess of sodium dithionite was added to the solution in batches until a bright yellow color was obtained and the solution was cooled in an ice bath. The white crystals formed were washed with water and dried under vacuum at 100 °C. Yield (0.44 g, 44%), mp>300 °C; [Found: C 42.53; H 4.98; N 29.46. $C_5H_7N_3O_2$ requires C 42.55; H 4.96; N 29.79%]; δ_H (300 MHz, DMSO-d_6) 2.13 (3H, s), 5.36 (2H, br s), 7.85 (1H, br s), 11.66 (1H, br s); ν_{max}/cm^{-1} 3420, 3320, 3160, 1600, 1440, 1380, 1280, 1210, 1020, 990, 900, 790, and 770; MS(EI): *m*/*z* (%) 141 (100, $C_5H_7N_3O_2$, M$^+$).

4.1.5. 2-Mercapto-5-(tetrahydro-pyran-2-yloxy)-3*H*-pyrimidin-4-one 5c. Identical to the procedure for the synthesis of **5a**, except for the addition of thiourea (15.29 g) instead of acetamidine. White crystals were obtained. Yield (25.0 g, 54.5%), mp>300 °C; [Found: C 47.15; H 5.38; S 14.49. $C_9H_{12}N_2O_3S$ requires C 47.37; H 5.26; S 14.00]; δ_H (300 MHz, DMSO-d_6) 0.8–1.97 (6H, m), 3.23–3.80 (2H, m), 5.27 (1H, br s), 7.16 (1H, d, J_{HNCH}=6.0 Hz), 10.8 (1H, br s), 11.3 (1H, br s); ν_{max}/cm^{-1} 3150, 3080, 1630, 1570, 1250, 1200, 1180, 1150, 1110, 1020, 980, 940, 900, 870, 810, and 670; MS(EI): *m*/*z* (%) 147 (100, M$^+$−81).

4.1.6. 5-Hydroxy-2-mercapto-3*H*-pyrimidin-4-one 6c. A suspension of **5c** (6.0 g) in 1 M H_2SO_4 (30 cm^3) was stirred for 2 h. Then the product was filtered and washed with water, methanol, and ether, and dried under vacuum at 100 °C. Yield (3.2 g, 84.4%), mp>300 °C; [Found: C 33.53; H 2.60; N 19.30; S 22.96. $C_4H_4N_2O_2S$ requires C 33.33; H 2.78; N 19.44; S 22.22%]; δ_H (300 MHz, DMSO-d_6) 6.97 (1H, d, J_{HNCH}=6.0 Hz), 9.60 (1H, br s), 10.27 (1H, br s); ν_{max}/cm^{-1} 3240, 3100, 1660, 1580, 1400, 1290, 1230, 1170, 1140, 890, 820, 760, 750, and 690; MS(EI): *m*/*z* (%) 144 (100, $C_4H_4N_2O_2S$, M$^+$).

4.1.7. 5-Hydroxy-2-methylsulfanyl-6-phenylazo-3*H*-pyrimidin-4-one 7c. Compound **6c** (2.9 g) was dissolved in a solution of sodium hydroxide (1.8 g) in water (12 cm^3) and heated to 40 °C. Dimethylsulfate (3.0 g) was added dropwise while vigorously stirring and then the mixture was cooled in an ice bath and filtered. The filtrate was acidified with concentrated hydrochloric acid and was left overnight at 4 °C. The formed crystals were filtered, successively washed with water, methanol, and ether, and dried under vacuum at 100 °C. The 2-methylthio-4,5-dihydroxypyrimidine formed (1.4 g) was subjected to the same diazotization procedure as **3a**. Yield (1.5 g, 74.0%), mp 213–215 °C; δ_H (300 MHz, DMSO-d_6) 2.73 (3H, s); 6.97–7.83 (5H, m); 10.73 (1H, br s); ν_{max}/cm^{-1} 3480, 3200, 3100, 1710, 1660, 1590, 1510, 1450, 1250, 1150, 1030, 990, 770, 750, 690, and 640; MS(EI): *m*/*z* (%) 262 (10, $C_{11}H_{10}N_4O_2S$, M$^+$), 105 (100, $C_6H_5N_2^+$), 91 ($C_6H_5N^+$), 77 ($C_6H_5^+$).

4.1.8. 6-Amino-5-hydroxy-2-(methylthio)pyrimidin-4-(3*H*)-one 8c. Identical to the procedure for the synthesis of **8a**, except for the use of **7c** (1.0 g) instead of **7a**. White crystals were obtained. Yield (0.43 g, 66%), mp 243–245 °C; [Found: C 34.27; H 4.08; N 24.21; S 19.00. $C_5H_7N_3O_2S$ requires C 34.38; H 4.05; N 24.28; S 18.50%); δ_H (300 MHz, DMSO-d_6) 2.45 (3H, s); 5.87 (2H, br s), 7.90 (1H, br s); ν_{max}/cm^{-1} 3250, 3380, 1640, 1600, 1570, 1410, 1330, 1240, 970, 830, and 760; MS(EI): *m*/*z* (%) 173 (100, $C_5H_7N_3O_2S$, M$^+$).

4.1.9. 5-Hydroxypyrimidin-4(3*H*)-one 6b. To a solution of water (76 cm^3) and concentrated aqueous ammonia (7.6 cm^3), **5c** (11.0 g) was added followed by the addition of Raney nickel (40.0 g). The mixture was refluxed for 4 h then it was cooled and filtered. All the volatile solvents were removed by rotor-evaporator and the residue was redissolved in methanol. After addition of ether, pink crystals precipitated out of the solution, and were dried under vacuum at 100 °C. Yield (2.5 g, 46%), mp 265–267 °C; δ_H (300 MHz, DMSO-d_6) 7.40 (1H, s), 7.67 (1H, s); ν_{max}/cm^{-1} 1640, 1600, 1360, 1300, 1270, 1100, 930, 880, 790, 780, and 615; MS(EI): *m*/*z* (%) 112 (100, $C_4H_4N_2O_2$, M$^+$).

4.1.10. 5-Hydroxy-6-phenylazo-3*H*-pyrimidine-4-one 7b. Identical to the procedure for the synthesis of **7a** except for the addition of **6b** (1.12 g) instead of **6a**. White crystals were obtained. Yield (1.9 g, 88%), mp 244–245 °C; δ_H (300 MHz, DMSO-d_6) 6.83–7.76 (6H, m), 11.88 (1H, br s); ν_{max}/cm^{-1} 3490, 3290, 1700, 1650, 1615, 1600, 1590, 1500, 1450, 1300, 1240, 1170, 1120, 1015, 900, 875, 750, 730, 680, 660, 640, 660, and 640; MS(EI): *m*/*z* (%) 216 (15, $C_{10}H_8N_4O_2$, M$^+$), 105 (100, $C_6H_5N_2^+$), 91 ($C_6H_5N^+$), 77 ($C_6H_5^+$).

4.1.11. 6-Amino-5-hydroxypyrimidin-4(3*H*)-one 8b. Identical to the procedure for the synthesis of **8a** except for the

addition of **7b** (1.0 g) instead of **7a**. Yield (0.45 g, 76%), mp>300 °C; [Found: C 38.02; H 3.75; N 33.35. $C_4H_5N_3O_2$ requires C 37.80; H 3.94; N 33.07%]; δ_H (300 MHz, DMSO-d_6) 5.83 (2H, br s), 7.56 (1H, s), 11.79 (1H, br s); ν_{max}/cm^{-1} 3470, 3140, 1670, 1640, 1620, 1440, 1370, 1250, 1170, 1010, 890, 810, 770, and 650; MS(EI): *m/z* (%) 127 (100, $C_4H_5N_3O_2$, M^+).

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20. One referee suggested that compound **8d** is almost certainly in the 2-oxo form. However, to the best of our knowledge the structure of the most stable/common tautomer of isouramil, compound **8d**, is not yet established and it appears in the literature in various ways. We think that the similarity in the autoxidation rate of isouramil and divicine (compound **8e** which is generally believed to exist mainly as the 2-NH_2 tautomer) and especially the good fit of the reaction rate of isouramil to the Hammett correlation line that was drawn for the other four pyrimidines using sigma plus, indicate that indeed, the main tautomer of compound **8d** does have 2-OH. The similarity in the UV spectra of the five systems (Table 1) gives supporting evidence to the above claim. Furthermore, the 2-SH derivative which exists as the thiourea tautomer, showed in contrast two UV peaks, at 300 nm (17,000) and 238 nm (16,400) (unpublished results).
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An in-depth analysis of the effect of substituents on imines in cycloaddition reactions with nitrosoalkenes

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Abstract—An in-depth experimental and theoretical analysis of the reactions of simple acyclic imines with nitrosoalkenes is reported. The effect of the substituents on nitrogen as well as carbon atom of imines on the cycloaddition pathways followed is systematically explored. The reactions of various functionalized imines with nitrosoalkenes leading to the formation of imidazoles and imidazole-*N*-oxides have also been explored. The plausible mechanisms leading to various heterocycles have been proposed.

1. Introduction

The C-nitroso group of arylnitroso, α-chloronitroso, cyano–nitroso, C-nitroso sugar derivatives, and acylnitroso compounds is known to effectively participate as a 2π component in hetero Diels–Alder reactions.[1] Of these, the acylnitroso species has been exploited much more extensively than the other dienophiles.[2] On the other hand, α-nitrosostyrenes have been known to participate as 4π components in Diels–Alder reactions with various polarized and unpolarized alkenes,[3a,b,c] allenes,[3d] all carbon dienes,[3e] enamines,[3f,g,h] and enol ethers.[3i] In contrast, there are not many such reports of the cycloadditions of nitrosoalkenes with carbon–nitrogen double bond.[4] Mackay et al.[5] reported an unusual [3+2] cycloaddition of α-nitrosoalkenes with carbon–nitrogen double bonds of oxazines and failed to observe such a reaction of nitrosoalkenes with various other cyclic and acyclic compounds bearing a carbon–nitrogen double bond.

Subsequent disclosures from our laboratory have shown a generalized and unusual [3+2] cycloaddition mode with various polarized 1,3-diazabuta-1,3-dienes[6] resulting in an easy access to synthetically and biologically significant imidazoline *N*-oxides. Few generalizations have been enunciated to account for the nature and preponderance of cycloadducts formed in such reactions. Mackay et al.[5] proposed that the electronic nature of the α-substituents on nitrosoalkenes determine their preferred conformations, which in turn become instrumental in controlling the distribution of products in these reactions. However, in recent reports by Tahdi et al.,[7] the effect of substituents present on α-carbon atom of nitrosoalkenes on the cycloaddition pathways followed is not generalized.[7] In view of these contradictions and in order to have a deeper insight regarding the key factors influencing the [3+2] versus [4+2] cycloaddition reactions, we examined the reactions of various imines with α-substituted nitrosoalkenes.

The treatment of benzylidene-phenyl-amine with α-phenyl nitrosoalkenes (entry a; Scheme 1), generated in situ from the corresponding α-bromooxime and sodium bicarbonate, in methylene chloride resulted in the exclusive formation of **3a**, a [3+2] cycloadduct. In order to scrutinize the effect of the electronic nature of α-substituents on nitrosoalkenes, the reactions of benzylidene-phenyl-amine were repeated with other nitrosoalkenes viz. *p*-tolyl and *p*-nitrophenyl substituted nitrosoalkenes (entry b and c; Scheme 1). These reactions also led to the exclusive formation of nitrones **3b** and **3c**. This observation got generalized by the sole formation of nitrones **3** in the reactions of other *N*-aryl imines with nitrosoalkenes (Scheme 1). The presence of oxazines **4** even in traces was excluded by analysis of the crude product with the help of high-resolution ^{1}H NMR spectroscopy. These experiments indicated that the nature of substituents on the nitrosoalkenes do not play any significant role in influencing the competitive [3+2] versus [4+2] cycloaddition pathways as proposed earlier.[5] It was felt that the substituents on imines might be crucial in influencing the pathways followed and the distribution of the products formed in their reactions with nitrosoalkenes. In order to substantiate this proposition, we considered it worthwhile to examine the reactions of *N*-alkyl imines with nitrosoalkenes. Interestingly, the reactions of benzylidene-cyclohexyl-amine with all the

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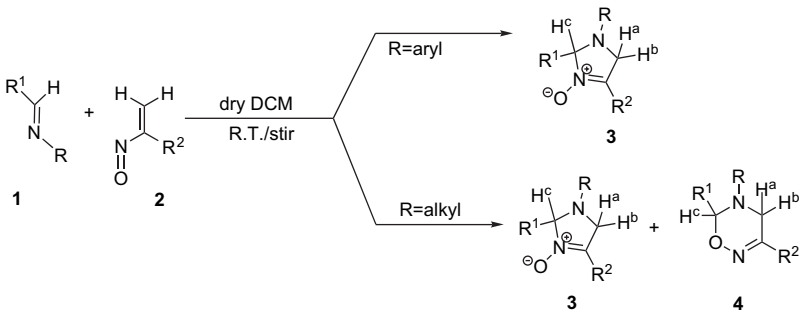


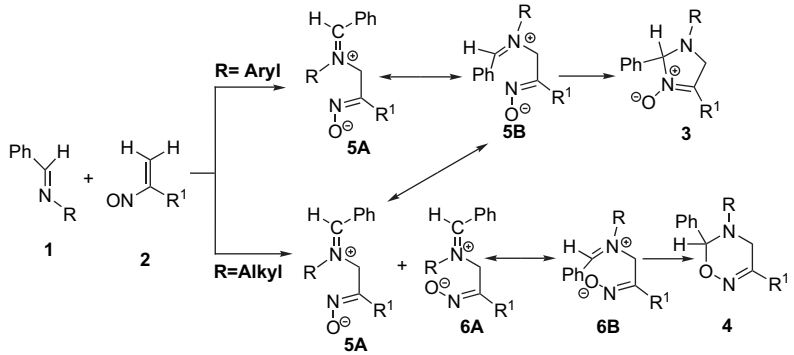
a. R=C_6H_5,R^1=C_6H_5,R^2=C_6H_4-CH_3(*p*)
b. R=C_6H_5,R^1=C_6H_5,R^2=C_6H_5
c. R=C_6H_5,R^1=C_6H_5,R^2=C_6H_4-NO_2(*p*)
d. R=cyclohexyl,R^1=C_6H_5,R^2=C_6H_5
e. R=cyclohexyl,R^1=C_6H_5,R^2=C_6H_4-CH_3(*p*)
f. R=cyclohexyl,R^1=C_6H_5,R^2=C_6H_4-NO_2(*p*)
g. R=C_6H_4-CH_3(*p*),R^1=C_6H_5,R^2=C_6H_5
h. R=C_6H_4-CH_3(*p*),R^1=C_6H_5,R^2=C_6H_4-CH_3(*p*)
i. R=C_6H_4-CH_3(*p*),R^1=C_6H_5,R^2=C_6H_4-NO_2(*p*)
j. R=C_6H_4-OCH_3(*p*),R^1=C_6H_4-NMe_2(*p*),R^2=C_6H_5
k. R=C_6H_4-OCH_3(*p*),R^1=C_6H_4-NMe_2(*p*),R^2=C_6H_4-CH_3(*p*)
l. R=C_6H_4-OCH_3(*p*),R^1=C_6H_4-NMe_2(*p*),R^2=C_6H_4-NO_2(*p*)
m. R=benzyl,R^1=C_6H_4-NMe_2(*p*),R^2=C_6H_5
n. R=benzyl,R^1=C_6H_4-NMe_2(*p*),R^2=C_6H_4-CH_3(*p*)
o. R=benzyl,R^1=C_6H_4-NMe_2(*p*),R^2=C_6H_4-NO_2(*p*)
p. R=n-butyl,R^1=C_6H_5,R^2=C_6H_5
q. R=n-butyl,R^1=C_6H_5,R^2=C_6H_4-CH_3(*p*)
r. R=n-butyl,R^1=C_6H_5,R^2=C_6H_4-NO_2(*p*)
s. R=iso-propyl,R^1=C_6H_5,R^2=C_6H_5
t. R=iso-propyl,R^1=C_6H_5,R^2=C_6H_4-CH_3(*p*)
u. R=iso-propyl,R^1=C_6H_5,R^2=C_6H_4-NO_2(*p*)
v. R=furyl,R^1=C_6H_5,R^2=C_6H_5
w. R=furyl,R^1=C_6H_5,R^2=C_6H_4-CH_3(*p*)
x. R=furyl,R^1=C_6H_5,R^2=C_6H_4-NO_2(*p*)

Scheme 1.

forementioned nitrosoalkenes (entry d, e, f) resulted in the isolation of a mixture of nitrones **3** and oxazines **4** in good yields; oxazines obtained in higher proportions (40:60). The reproducible formation of similar mixtures of nitrones and oxazines in the reactions of other *N*-alkyl imines with nitrosoalkenes once again supported the deliberation regarding the decisive role of iminic substituents in the product distribution. Accordingly, the present manuscript is a generalized and consummate account of our earlier communication[8] on the effect of substituents on various imines on the mode of cycloadditions followed in their reactions with nitrosoalkenes.

The structures **3** and **4** have been assigned to [3+2] and [4+2] cycloadducts on the basis of analytical data and spectral evidences. The products **3m** and **4m**, for example, were characterized as [4-(1-benzyl-3-oxy-4-phenyl-2,5-dihydro-1*H*-imidazol-2-yl)-phenyl]-dimethyl-amine and [4-(5-benzyl-3-phenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazin-6-yl)-phenyl]-dimethyl-amine. The mass spectrum of product **3m**, for example, analyzed for $C_{24}H_{25}N_3O$, exhibited an intense M-16 peak (*m*/*z*=355) diagnostic of nitrone,[3a] while the mass spectrum of **4m** exhibited a molecular ion peak at *m*/*z*=371 for $C_{24}H_{25}N_3O$. In the ^{1}H NMR of **3m**, the methylene protons exhibited two doublets of doublets at δ 3.92, 4.35 (J=14.1 and 4.2 Hz, H^a) and 4.35 (J=14.2 and 3.6 Hz, H^b) corresponding to a CH_2 of a nitrone, downfield from the position δ 3.95 in case of oxazine **4m**. A doublet of doublet (J=4.2 and 3.6 Hz, H^c) corresponding to the CH of nitrone **3f** appeared at δ 5.49, while that for oxazine **4m** appeared as a singlet at δ 6.00. The ^{13}C NMR spectra were also in agreement with the nitrone and oxazine structures assigned above to **3m** and **4m**.

The nitrones **3** and oxazines **4** are formed in competition and are not in equilibrium, these were separately shown to be stable to the reaction conditions, though rearrangements of oxazines to nitrones[9] and nitrones to oxazines[10] are well documented in the literature. The plausible mechanism for the formation of nitrones and oxazines is illustrated in Scheme 2. The addition of nitrosoalkene to a C=N bond is less likely to proceed via a concerted single step as reported earlier.[3,11] The reaction presumably proceeds through



Scheme 2.

zwitterionic intermediates, **5A** and **6A**, which are formed from the *cisoid* and *transoid* conformations of the nitrosoalkenes. These intermediates are interconvertible to other intermediates **5B** and **6B**, respectively.

However, the interconversion between sets of **5A** and **6A** and **5B** and **6B** does not seem to be credible as suggested earlier,[10,3,4] since this requires rotation around a C=N–O bond, which becomes stronger after nucleophilic attack, i.e., changes from a partial double bond in the nitrosoalkene to a typical double bond in the zwitterionic intermediate. Finally, the intermediates **5B** and **6B** cyclize to form the nitrones **3** and oxazines **4**, respectively. It seems that formation of the intermediate **6A** is discouraged in the case of aryl-substituted imines because of the steric repulsions between the oxygen lone pairs and the π electron cloud on the aryl substituent. In the case of alkyl-substituted imines; the formation of both intermediates **5A** and **6A** is possible because of the reduced steric repulsion between the alkyl group and the nitrogen or oxygen of the nitrosoalkenes. The above mechanistic rationale is in agreement with the formation of both [4+2] and [3+2] cycloadducts in the case of alkyl imines and exclusively [3+2] adduct in the case of aryl imines.

The experimental observations and the mechanism proposed above have been supported by the theoretical calculations at semi-empirical AM1 and ab initio[12] HF/6-31G* levels of computational analysis implemented in Gaussian series of programs.[13] The schematic representation of the reaction mechanism studied theoretically is shown in Figure 1.

The schematic representation of the conformations of optimized structures of the intermediates is shown in Figure 2. The optimized parameters like bond lengths, bond angles, and dihedral angles of the intermediates are characterized in Figure 3. The activation energies of the various reactants, products, and the transition states were estimated from HF/6-31G* point energy calculations on the structures previously optimized (Table 1). The relative energies (kcal/mol) of various intermediates and products bearing *N*-alkyl and *N*-aryl substituents using HF theoretical methods at room temperature using 6-31+G* basis sets are given in Table 2. All attempts to locate a transition state for a concerted process, as proposed by Gilchrist[3] for the formation of six-membered ring, between H_2C=NH and H_2C=CH–N=O at AM1 and HF/6-31+G* level calculations have revealed that the only pathway operative in this reaction involves the initial nucleophilic attack of iminic nitrogen on the terminal carbon of nitrosoethylene. Complete optimizations carried out at HF/6-31+G* theory level on the reaction path involving

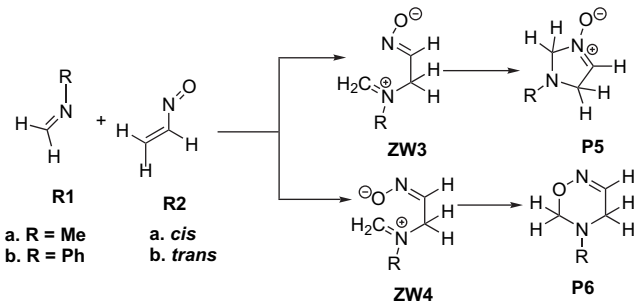


Figure 1.

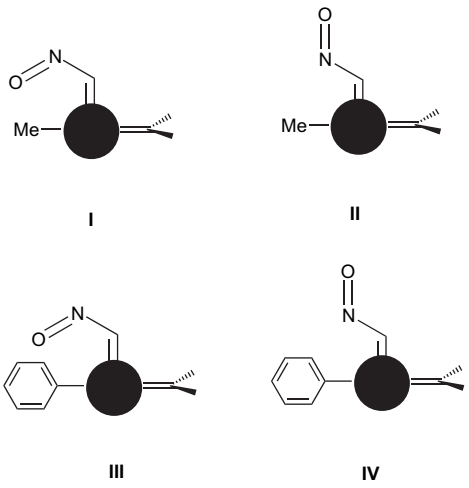
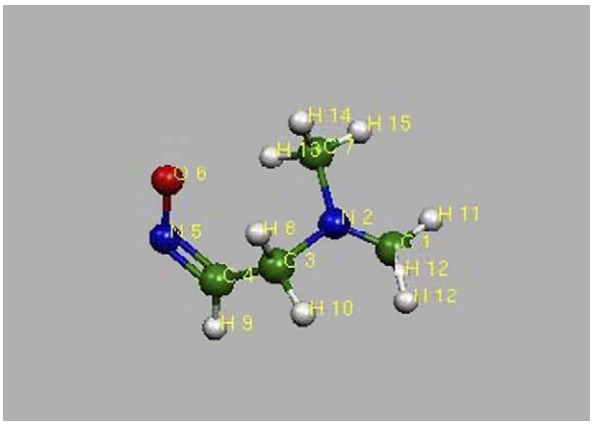


Figure 2. Schematic representation of the conformations of the intermediates on the reaction path between nitrosoalkenes and imines.

H_2C=NMe and *cis*- and *trans*-conformers of H_2C=CH–N=O have exhibited that the initial nucleophilic attack occurs in such a way so as to form intermediates **I** and **II**, respectively. The intermediates **I** and **II** have been found to be 17.6 and 27.6 kcal/mol higher in energy than the corresponding starting materials. The corresponding final products are stable by −37.6 and −20.2 kcal/mol as compared to the starting materials (Fig. 3) indicating that the formation of six-membered ring, in the subsequent ring closure step, is more favorable from kinetic as well as thermodynamic aspects.

Further, HF/6-31+G* calculations for the reaction path involving H_2C=N–Ph and H_2C=CH–N=O indicated that the formation of a zwitterionic intermediate is feasible for the *s-trans* arrangement of nitrosoethylene. However, such an intermediate could not be located for the *s-cis* arrangement. This may be due to the repulsive interactions operating between the approaching phenyl (π-electrons) and oxygen (non-bonding electrons) during the initial nucleophilic attack. Due to these repulsive forces, the formation of the corresponding zwitterionic intermediate is avoided. However, in the case of *s-trans* of nitrosoethylene, the formation of intermediate **IV** is conceivable after a twist in the C–C–N–C angle probably because of the decreased repulsive interactions. The relative energies of the intermediate **IV** and the nitrone are 28.6 and −21.1 kcal/mol with respect to the corresponding starting materials. Thus, the presence of a phenyl group on nitrogen does not allow the formation of zwitterionic intermediate **6**, thereby justifying the absence of the six-membered oxazine in the title reactions of imines bearing *N*-aryl substituents. This rationale is further substantiated by the formation of nitrones and oxazines in the reactions of benzylidene-benzyl-amine (entry m, n, o) and benzylidene-furyl-amine (entry v, x, y) with nitrosoalkenes **2**, where the above referred electronic repulsions and steric interactions are supposedly decreased, the phenyl ring being one carbon atom away from the nitrogen of imine due to the insertion of a methylene group (Fig. 4).

Solvent effects have not been taken into account in our study. However, solvents of different polarities might also change the chemoselectivity of the reaction. However, the solvent used experimentally, dichloromethane, a non-polar solvent,



I

C1-N2=1.253924 ; C3-N2-C1=121.6523538

N2-C3=1.518186 ; C4-C3-N2=112.238191

C4-C3=1.4871451; N5-C4-C3=120.9890215

N5-C4=1.2968952; O6-N5-C4=117.6119068

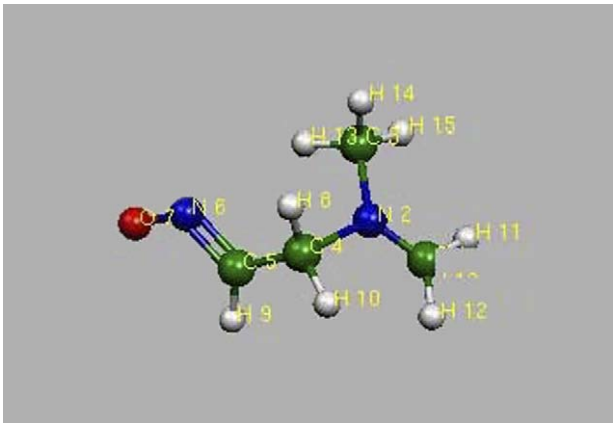
O6-N5=1.2646881; C7-N2-C3=116.6974679

C7-N2=1.4734058; C4-C3-N2-C1=-111.0999956

C7-N2-C3=116.697468; N5-C4-C3-N2=-88.4786299

N2-C3-C4=112.238191;O6-N5-C4-C3=6.0749739

O6-N5-C4-C3=6.0749739;C7-N2-N3-C4=66.1768982



II

N2-C1=1.252758; C5-C4-N2=111.492389

C3-N2=1.4656284; N6-C5-C4=116.6413079

C4-N2=1.558485; O7-N6-C5=119.2266812

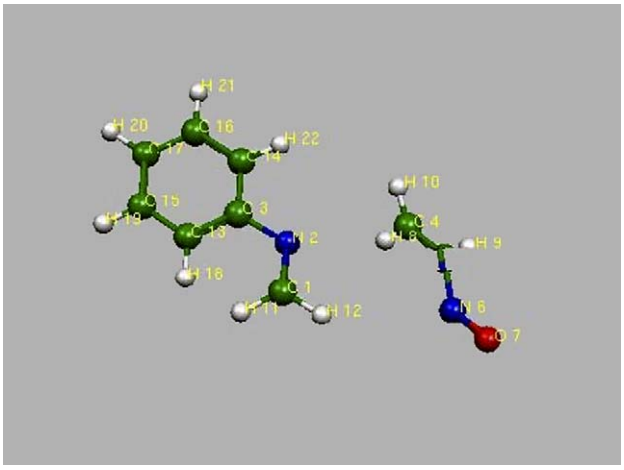
C5-C4=1.4636224; C4-N2-C3-C1=-175.1989112

N6-C5=1.3089472; C5-C4-N2-C3=69.9372295

O7-N6=1.2393158; N6-C5-C4-N2=-93.2134719

C3-N2-C1=122.39158; O7-N6-C5-C4=177.5004877

C4-N2-C3=115.4419577



IV

N2-C1=1.2564137; C15-C13=1.3874312

C3-N2=1.4378906; C16-C14=1.3863046

C4-N2=1.6058972; C17-C15=1.3867999

C5-C4=1.4519722; C3-N2-C1=121.538060

N6-C5=1.3147996; C4-N2-C3=117.348833

O7-N6=1.2357110; C5-C4-N2=113.606416

C13-C3=1.3849957; N6-C5-C4=116.527161

C14-C3=1.3856619; O7-N6-C5=119.1016681

C4-N2-C3-C1=-176.3054821;C13-C3-N2-C1=83.3302754

C5-C4-N2-C3=-173.3125322;C16-C14-C3-N2=1.3863045

N6-C5-C4-N2=-86.9930372; C17-C15-C13-C3=-0.4286934

O7-N6-C5-C4=177.5699184

Figure 3. Three dimensional representation of the geometries of the intermediates **I**, **II**, and **IV** (**III** does not exist) optimized at HF/6-31+G* theory level.

Table 1. Yields of nitrones **3** and oxazines **4** in Scheme 1

Entry no.	**3** (Yield %)	**4** (Yield %)	Entry no.	**3** (Yield %)	**4** (Yield %)
a	79	—	m	37	55
b	78	—	n	30	50
c	73	—	o	36	54
d	35	53	p	34	56
e	32	48	q	30	50
f	34	51	r	31	53
g	75	—	s	35	47
h	80	—	t	37	48
i	78	—	u	31	52
j	85	—	v	34	49
k	81	—	w	31	54
l	88	—	x	34	50

would not have any significant influence on the activation energies (Table 3).

The results presented above are in agreement with the earlier report regarding the exclusive formation of nitrones, i.e., [3+2] adducts in the reactions of nitrosoalkenes with *N*-aryl-formamidines.[6a]

In continuation of these studies, it was felt worthwhile to examine the effect of substituents on carbon of imines in such reactions. In this series, the reactions of *N*-aryl-benzamidines with nitrosoalkenes yielding imidazoles have already been studied in our laboratory. In-depth study of these reactions has been explored by investigating these reactions with

Table 2. Absolute energies (ZPE corrected values that has been scaled by a factor of 0.9153 for HF level) of various reactants, intermediates and products at rt

Strs	HF/6-31+G*	ZPVE	Scaling factor (0.9153)	Total energy
R1a	−133.0655661	0.073537	0.067308416	−132.9982577
R1b	−323.5871725	0.130540	0.119483262	−323.4676892
R2a	−206.6777504	0.052863	0.048385504	−206.629402
R2b	−206.6857993	0.053289	0.048775422	−206.6364239
ZW3a	−339.7067992	0.132047	0.12086219	−339.5859366
ZW3b	−530.2269237	0.187762	0.171858559	−530.0550651
ZW4a	—	—	—	—
ZW4b	−339.7152183	0.133285	0.121995761	−339.5932225
P5a	−339.7829033	0.136773	0.125188327	−339.657715
P5b	−530.3061588	0.193126	0.176768228	−530.1293906
P6a	−339.8031696	0.137465	0.125821715	−339.6773479
P6b	−530.3208283	0.193775	0.177362258	−530.143466

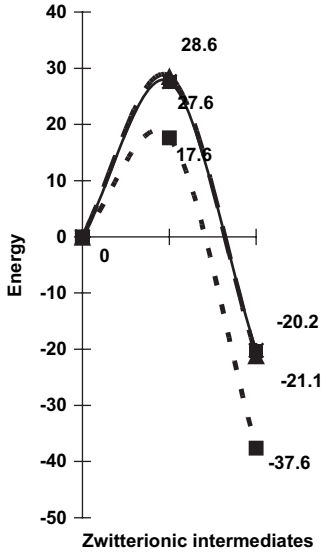
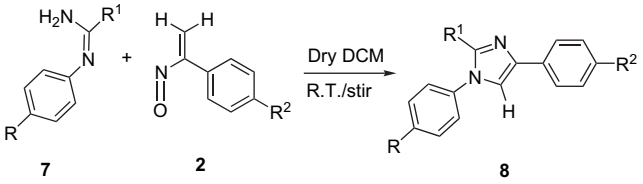


Figure 4. Potential energy surface showing the relative energy of zwitterionic intermediates on the reaction path between nitrosoalkene and imine. The path involving intermediate **I** leads to the formation of oxazine, the path involving intermediates **II** and **IV** lead to the formation of nitrones. Path involving **III**, which should have led to an oxazine, could not be traced due of the instability of intermediate **III** on PE surface.



a. R = H, R^1 = SCH_3, R^2 = CH_3
b. R = CH_3, R^1 = SCH_3, R^2 = CH_3
c. R = CH_3, R^1 = SCH_3, R^2 = NO_2
d. R = CH_3, R^1 = Piperidine, R^2 = NO_2
e. R = H, R^1 = morpholine, R^2 = NO_2
f. R = CH_3, R^1 = pyrrollidine, R^2 = CH_3

Scheme 3.

Table 4. Yields of imidazoles **8** in Scheme 3

Entry no.	Yield %
8a	78
8b	80
8c	81
8d	80
8e	81
8f	85

imines bearing polar donating amines and thiomethyl at carbon of imines, i.e., *N*-aryl-secondary amine-carboxamidines commonly known as guanidines and 2-methyl-1-aryl-isothioureas **7**.

Interestingly, these reactions also resulted in the exclusive isolation of imidazoles **8** in very good yields (Scheme 3, Table 4), characterized on the basis of spectral and analytical evidences (Table 4).

The one pot synthesis of imidazoles reported here assumes significance because:

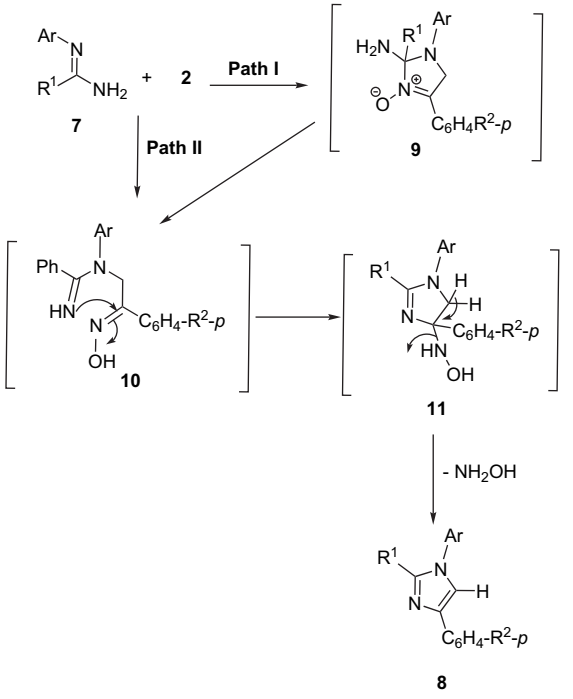
1. These observations are at variance with the earlier reports by Mackay et al. that nitrosoalkenes did not react with the carbon–nitrogen double bonds of *N*,*N*-dimethyl-*N*′-phenyl-benzamidine and 1-phenyl-ethanone *O*-methyl-oxime.
2. The earlier reported reactions of *N*-aryl-benzamidines with nitrosoalkenes leading to the formation of imidazoles involve very cumbersome experimental procedures and the use of iron carbonyls.[10]

It is possible to discern various possible mechanistic pathways to the formation of imidazoles in this case. The

Table 3. Relative energies (kcal/mol) of various intermediates and products bearing alkyl and aryl substituents using HF theoretical methods at rt using 6-31+G* basis sets

Reaction	Relative energies of zwitterionic intermediates		Relative energies of products formed	
	Before adding ZPVE	After adding ZPVE	Before adding ZPVE	After adding ZPVE
Me–N=CH_2+*cis* H_2C=CH–N=O	17.6 (**I**)	21.6 (**I**)	−37.6 (**I**)	−31.2 (**I**)
Me–N=CH_2+*trans* H_2C=CH–N=O	27.6 (**II**)	30.78 (**II**)	−20.2 (**II**)	−15.86 (**II**)
Ph–N=CH_2+*trans* H_2C=CH–N=O	28.6 (**IV**)	30.59 (**IV**)	−21.1 (**IV**)	−14.45 (**IV**)

plausible mechanism, consistent with the one prescribed earlier for the reaction of benzamidines with nitrosoalkenes,[6a,b] is depicted in Scheme 4.

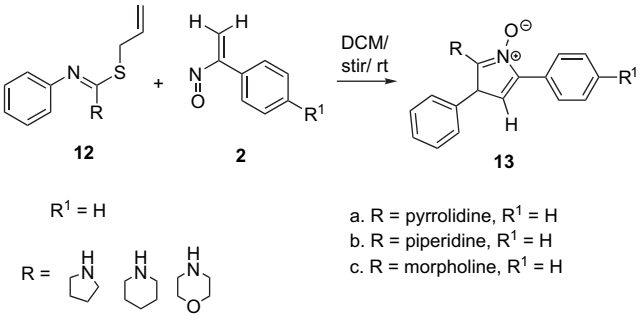


Scheme 4.

The reaction may lead to the initial formation of 2-amino-*N*-oxide intermediate **9**, which probably being unstable is transformed to another intermediate **10**. The intermediate **10** then cyclizes, as shown, to yield another intermediate **11**, which ultimately undergoes elimination of NH_2OH to yield the imidazoles **8**. The formation of **8** in this case could also be explained via initial displacement of halide from α-chlorooxime leading to the intermediate **10**, which as described above then yields **8** via **11**.

In order to authenticate the synthetic versatility of these reactions, it was felt that the presence of a suitably disposed dipolarophile in the molecular structure might result in intramolecular 1,3-dipolar cycloadditions of the generated nitrone. Keeping this in view, we have examined the reactions of *S*-allylated imines **12** with nitrosoalkenes. Interestingly, the reactions of *N*-phenyl-secondaryamino-1-carboximidothioic acid allyl ester **12a–d** with nitrosoalkenes **2** following the procedure described earlier resulted in the formation of imidazole-*N*-oxides **13** in good yields (Table 5), instead of the expected dipolar adducts (Scheme 5). The formation of the imidazole-*N*-oxides **13** in these reactions is at odds with our earlier assumption that the allylated nitrones **15** formed above will be stable enough so as to follow intramolecular 1,3-dipolar cycloadditions utilizing the *S*-allyl moiety thus leading to the formation of tricyclic heterocycle **16** (Table 5).

The products formed **13** were characterized as imidazoles on the basis of spectral and analytical data. The ^{1}H NMR spectrum of the products showed the absence of allylic function as well as the methylene protons and the presence of a characteristic olefenic proton. The compound **13a**, for example, characterized as 1,4-diphenyl-2-pyrrolidin-1-yl-1*H*-imidazole and analyzed for $C_{19}H_{19}N_3O$ exhibited two multiplets at δ 1.79 and 3.30 corresponding to the pyrrolidine protons. A characteristic singlet corresponding to olefenic proton appeared at δ 6.88. The ^{13}C NMR spectral data is in perfect consistent with the suggested structure. The mass spectra of the compound showed a molecular ion peak (M^+) at *m/z*: 305.

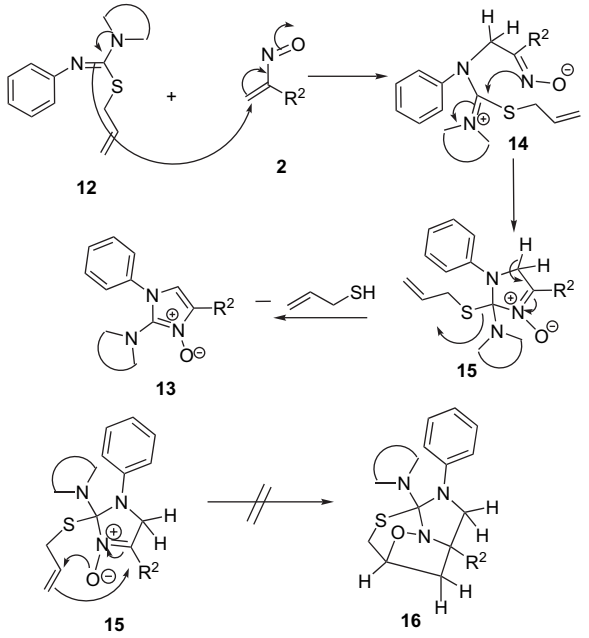


Scheme 5.

Table 5. Yields of imidazole-*N*-oxides **16** in Scheme 5

Entry no.	Yield %
16a	77
16b	80
16c	79

The most plausible mechanism for the formation of imidazole-*N*-oxides **13** has been depicted in Scheme 6. It is assumed that the nitrogen atom of the *S*-allylated imine **12** attacks the electrophilic carbon of nitrosoalkene **2**, as expected, to form an intermediate **14**, which then cyclizes to form another intermediate **15**. This intermediate **15** after the elimination of a molecule of allyl thiol leads to the formation of **13**.



Scheme 6.

In view of the above observations, it is generalized that the aryl imines cycloaddition to nitrosoalkenes in a [3+2] manner, while alkyl imines cycloaddition to nitrosoalkenes in competitive [3+2] and [4+2] manner. It may be inferred that the substituents on nitrogen as well as carbon atoms of simple and functionalized imines play a pivotal role in determining the mechanistic pathways and products formed in their reactions with nitrosoalkenes.

These cycloaddition reactions offer a remarkable practical ingress to a diversity of functional and synthetically flexible nitrogen containing molecular assemblies having biological and pharmacological significance.[14]

2. Experimental

2.1. General

Melting points were determined by open capillary method using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform with Brucker AC-E 200 (200 MHz) and AC-E 300 (300 MHz) spectrometers using TMS as internal standard. Chemical shift values are expressed as δ (parts per million) downfield from TMS and J values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, q: quartet and br: broad peak. ^{13}C NMR spectra were also recorded on a Brucker AC-200E (50.4 MHz) or Brucker AC-300E (75.0 MHz) spectrometers in a deuterochloroform using TMS as internal standard. Mass spectra were recorded on Shimadzu GCMS-QP-2000 mass spectrometer. Elemental analyses were performed on Heraus CHN-O-Rapid Elemental Analyzer.

2.2. Starting materials

All the acyclic imines (Schiff's bases)[15a] 1,2-methyl-1-aryl-isothioureas **7a**–**c**,[15b] *N*-aryl-secondary amine-carboxamidines **7d**–**f**,[15b] *S*-allylated imines **12**, and bromooximes of acetophenone, *p*-nitroacetophenone and *p*-methylacetophenone **2**[15c] were prepared by reported procedures.

2.3. Reactions of acyclic imines (Schiff's bases) with α-nitrosoalkenes. General procedure for nitrones 3 and oxazines 4

A solution of Schiff's base **1** (4 mmol) and α-bromooxime **2** (4.2 mmol) in dry CH_2Cl_2 was stirred at rt in the presence of anhydrous sodium bicarbonate (6 mmol) for 24–25 h. The deposited salt and the excess of sodium bicarbonate were filtered off and washed with small portions (2×10 ml) of CH_2Cl_2. The combined filtrates were washed with water, extracted with dichloromethane, dried over Na_2SO_4, and concentrated under reduced pressure. The nitrones **3** and oxazines **4** were isolated and purified with the help of column chromatography on 60–120 mesh silica gel. The nitrones and oxazines were recrystallized from benzene–hexane (2:1) and EtOAc–hexane (1:5), respectively.

2.3.1. 1,2-Diphenyl-4-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3a). White crystalline solid; yield 79%; mp 165–166 °C; [Found: C, 80.49; H, 6.21; N, 11.62. $C_{22}H_{20}N_2O$ requires C, 80.46; H, 6.14; N, 11.69%]; ν_{max}/cm^{-1} (KBr): 1218 (N–O), 1545, 1595 (C═N); δ_H (200 MHz): δ 2.28 (s, 3H, –CH_3), 4.93 (dd, J=14.1 and 2.4 Hz, 1H, –CH_2), 5.17 (dd, J=14.1 and 5.2 Hz, 1H, –CH_2), 6.20 (dd, J=2.4 and 5.2 Hz, 1H, methine), 6.60 (d, J=8.2 Hz, 2H, ArH), 7.13 (d, J=8.2 Hz, 2H, ArH), 7.49–7.65 (m, 6H, ArH), 8.25–8.43 (m, 4H, ArH); ^{13}C NMR: 21.1 (CH_3), 57.4 (CH_2), 94.3 (methine), 118.9, 122.1, 124.2, 125.7, 127.9, 133.5, 134.1, 134.9, 136.3, 139.1, 141.8, 148.2, and 153.1; *m*/*z*: 328 (M^+) and 312 (M^+-16).

2.3.2. 1,2,4-Triphenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3b). White crystalline solid; yield 78%; mp 140–141 °C; [Found: C, 80.18; H, 5.80; N, 8.98. $C_{21}H_{18}N_2O$ requires C, 80.23; H, 5.77; N, 8.91%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1547 (C═N), 1589 (C═N); δ_H (200 MHz): δ 4.88 (dd, J=14.2 and 2.4 Hz, 1H, –CH_2), 5.16 (dd, J=14.2 and 5.2 Hz, 1H, –CH_2), 6.18 (dd, J=2.4 and 5.3 Hz, 1H, methine), 6.56–6.84 (m, 3H, ArH), 7.18–7.47 (m, 6H, ArH), 7.60–7.62 (m, 3H, ArH), 8.32–8.35 (m, 3H, ArH); ^{13}C NMR: 58.1 (CH_2), 89.3 (CH), 120.2, 121.1, 123.2, 124.7, 126.8, 128.5, 134.2, 135.2, 136.3, 138.3, 140.2, 147.2, and 152.1; *m*/*z*: 314 (M^+) and 298 (M^+-16).

2.3.3. 4-(4-Nitro-phenyl)-1,2-diphenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3c). White crystalline solid; yield 73%; mp 181–182 °C; [Found: C, 70.22; H, 4.84; N, 11.62. $C_{21}H_{17}N_3O_3$ requires C, 70.18; H, 4.77; N, 11.69%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1545, 1592 (C═N); δ_H (200 MHz): δ 5.18 (dd, J=14.4 and 2.9 Hz, 1H, –CH_2), 5.46 (dd, J=14.4 and 5.4 Hz, 1H, –CH_2), 6.49 (dd, J=2.9 and 5.4 Hz, 1H, methine), 7.05–7.52 (m, 5H, ArH), 7.65–7.88 (m, 5H, ArH), 8.54 (d, J=9.0 Hz, 2H, ArH), 8.74 (d, J=9.0 Hz, 2H, ArH); ^{13}C NMR: 56.3 (CH_2), 94.4 (methine), 118.2, 120.9, 123.3, 125.1, 125.9, 133.2, 133.8, 134.4, 135.1, 137.6, 140.5, 145.2, and 152.2; *m*/*z*: 359 (M^+) and 343 (M^+-16).

2.3.4. 1-Cyclohexyl-2,4-diphenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3d). White crystalline solid; yield 35%; mp 165–166 °C; [Found: C, 78.78; H, 7.50; N, 8.71. $C_{21}H_{24}N_2O$ requires C, 78.71; H, 7.55; N, 8.74%]; ν_{max}/cm^{-1} (KBr): 1218 (N–O), 1525, 1598 (C═N); δ_H (200 MHz): δ 1.26–2.03 (m, 10H, cyclohexyl), 2.71–2.73 (m, 1H, –CH, cyclohexyl), 4.32 (dd, J=14.7 and 3.6 Hz, 1H, –CH_2), 4.66 (dd, J=14.4 and 4.2 Hz, 1H, –CH_2), 5.78 (dd, J=3.6 and 4.2 Hz, 1H, methine), 7.36–7.53 (m, 6H, ArH), 7.69–7.74 (m, 2H, ArH), 8.33–8.37 (m, 2H, ArH); ^{13}C NMR: 25.5, 25.7, 30.7, 31.2, 31.9, 37.8, 52.9 (CH_2), 90.7 (methine), 122.5, 126.3, 129.1, 129.9, 132.7, 136.8, 139.0, 142.9, and 163.8; *m*/*z*: 320 (M^+) and 304 (M^+-16).

2.3.5. 5-Cyclohexyl-3,6-diphenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4d). White crystalline solid; yield 53%; mp 141–142 °C; [Found: C, 78.76; H, 7.48; N, 8.76. $C_{21}H_{24}N_2O$ requires C, 78.71; H, 7.55; N, 8.74%]; ν_{max}/cm^{-1} (KBr): 1620 (C═N); δ_H (200 MHz): δ 1.21–2.03 (m, 10H, cyclohexyl), 2.82–2.85 (m, 1H, –CH, cyclohexyl), 3.59 (s, 2H, –CH_2), 6.00 (s, 1H, methine), 7.21–7.52 (m, 5H, ArH), 7.73–8.10 (m, 5H, ArH); ^{13}C NMR: 25.6, 25.7, 25.8, 31.1, 31.9, 38.2, 61.7 (CH_2), 89.2 (methine), 123.7, 124.9,

126.2, 127.8, 128.4, 137.7, 140.2, 147.7, and 153.5; *m/z*: 334 (M^+).

2.3.6. 1-Cyclohexyl-2-phenyl-4-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3e). White crystalline solid; yield 32%; mp 175–176 °C; [C, 79.05; H, 7.90; N, 8.29. $C_{22}H_{26}N_2O$ requires C, 79.00; H, 7.84; N, 8.38%]; ν_{max}/cm^{-1} (KBr): 1220 (N–O), 1545, 1591 (C═N); δ_H (200 MHz): δ 1.15–1.89 (m, 10H, cyclohexyl), 2.43 (s, 3H, CH_3), 2.83–2.86 (m, 1H, –CH, cyclohexyl), 3.95 (dd, J=14.1 and 3.2 Hz, 1H, –CH_2), 4.61 (dd, J=14.1 and 4.5 Hz, 1H, –CH_2), 5.70 (dd, J=3.2 and 4.5 Hz, 1H, methine), 6.59 (d, J=8.1 Hz, 2H, ArH), 7.03 (d, J=8.1 Hz, 2H, ArH), 7.12–7.52 (m, 5H, ArH); ^{13}C NMR: 20.9, 25.1, 25.4, 25.8, 30.7, 31.1, 38.7, 52.9 (CH_2), 89.1 (CH), 120.6, 124.2, 126.1, 127.3, 128.1, 132.2, 134.3, 139.4, and 161.7; *m/z*: 334 (M^+) and 318 (M^+-16).

2.3.7. 5-Cyclohexyl-6-phenyl-3-*p*-tolyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4e). White crystalline solid; yield 48%; mp 153–154 °C; [Found C, 78.97; H, 7.91; N, 8.45. $C_{22}H_{26}N_2O$ requires C, 79.00; H, 7.84; N, 8.38%]; ν_{max}/cm^{-1} (KBr): 1621 (C═N); δ_H (200 MHz): δ 1.21–2.07 (m, 10H, cyclohexyl), 2.39 (s, 3H, CH_3), 2.84–2.87 (m, 1H, –CH, cyclohexyl), 3.61 (s, 2H, –CH_2), 5.99 (s, 1H, methine), 6.58 (d, J=8.2 Hz, 2H, ArH), 7.01 (d, J=8.0 Hz, 2H, ArH), 7.10–7.46 (m, 5H, ArH); ^{13}C NMR: 21.0, 25.4, 25.7, 25.8, 30.9, 31.2, 38.3, 61.4 (CH_2), 89.3 (CH), 121.1, 123.9, 126.0, 127.8, 128.3, 133.0, 134.5, 138.3, and 153.6; *m/z*: 334 (M^+).

2.3.8. 1-Cyclohexyl-4-(4-nitro-phenyl)-2-phenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3f). White crystalline solid; yield 34%; mp 169–170 °C; [Found C, 69.08; H, 6.31; N, 11.52. $C_{21}H_{23}N_3O_3$ requires C, 69.02; H, 6.34; N, 11.50%]; ν_{max}/cm^{-1} (KBr): 1221 (N–O), 1547, 1595 (C═N); δ_H (200 MHz): δ 1.18–1.95 (m, 10H, cyclohexyl), 2.65–2.66 (m, 1H, –CH, cyclohexyl), 3.98 (dd, J=14.7 and 3.6 Hz, 1H, –CH_2), 4.62 (dd, J=14.7 and 4.2 Hz, 1H, –CH_2), 5.72 (dd, J=3.6 and 4.2 Hz, 1H, methine), 7.43–7.61 (m, 5H, ArH), 8.27 (d, J=8.6 Hz, 2H, ArH), 8.42 (d, J=8.6 Hz, 2H, ArH); ^{13}C NMR: 25.3, 25.5, 25.8, 30.9, 31.4, 38.8, 53.1 (CH_2), 89.9 (methine), 123.7, 127.2, 128.5, 129.8, 133.1, 136.7, 138.0, 148.1, and 161.9; *m/z*: 365 (M^+) and 349 (M^+-16).

2.3.9. 5-Cyclohexyl-3-(4-nitro-phenyl)-6-phenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4f). White crystalline solid; yield 51%; mp 153–154 °C; [Found: C, 69.10; H, 6.30; N, 11.55. $C_{21}H_{23}N_3O_3$ requires C, 69.02; H, 6.34; N, 11.50%]; ν_{max}/cm^{-1} (KBr): 1620 (C═N); δ_H (200 MHz): δ 1.21–2.05 (m, 10H, cyclohexyl), 2.85–2.87 (m, 1H, –CH, cyclohexyl), 3.62 (s, 2H, –CH_2), 6.00 (s, 1H, methine), 7.30–7.57 (m, 5H, ArH), 7.73 (d, J=8.5 Hz, 2H, ArH), 8.19 (d, J=8.5 Hz, 2H, ArH); ^{13}C NMR: 25.5, 25.6, 25.7, 31.1, 31.4, 38.9 (–CH, cyclohexyl), 61.6 (–CH_2), 89.4 (methine), 123.6, 125.2, 126.5, 128.2, 128.5, 138.0, 140.1, 148.2, and 153.5; *m/z*: 365 (M^+).

2.3.10. 2,4-Diphenyl-1-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3g). White crystalline solid; yield 75%; mp 175–176 °C; [Found: C, 80.50; H, 6.16; N, 8.48. $C_{22}H_{20}N_2O$ requires C, 80.46; H, 6.14; N, 8.53%]; ν_{max}/cm^{-1} (KBr): 1221 (N–O), 1545, 1600 (C═N); δ_H (200 MHz): δ 2.25 (s, 3H, –CH_3), 4.88 (dd, J=14.1 and 3.2 Hz, 1H, –CH_2), 5.14 (dd, J=14.1 and 5.0 Hz, 1H, –CH_2), 6.20 (dd, J=3.2 and 5.0 Hz, 1H, methine), 6.52 (d, J=8.4 Hz, 2H, ArH), 7.04 (d, J=8.4 Hz, 2H, ArH), 7.42–7.48 (m, 6H, ArH), 7.62–7.63 (m, 2H, ArH), 8.34–8.35 (m, 2H, ArH); ^{13}C NMR: 24.9, 55.1 (CH_2), 89.2 (methine), 118.2, 121.2, 122.9, 123.6, 126.2, 133.1, 133.9, 134.1, 135.2, 137.5, 141.1, 147.2, and 152.1; *m/z*: 328 (M^+) and 312 (M^+-16).

2.3.11. 2-Phenyl-1,4-di-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3h). White crystalline solid; yield 80%; mp 135–136 °C; [Found: C, 80.72; H, 6.40; N, 8.22. $C_{23}H_{22}N_2O$ requires C, 80.67; H, 6.48; N, 8.18%]; ν_{max}/cm^{-1} (KBr): 1220 (N–O), 1545, 1601 (C═N); δ_H (200 MHz): δ 2.21 (s, 3H, –CH_3), 2.25 (s, 3H, CH_3), 4.91 (dd, J=14.1 and 3.2 Hz, 1H, –CH_2), 5.17 (dd, J=14.1 and 5.2 Hz, 1H, –CH_2), 6.22 (dd, J=3.2 and 5.2 Hz, 1H, methine), 6.48 (d, J=8.6 Hz, 2H, ArH), 7.02 (d, J=8.6 Hz, 2H, ArH), 7.35–7.51 (m, 9H, ArH); ^{13}C NMR: 24.5, 25.1, 55.2 (CH_2), 90.1 (methine), 118.7, 120.5, 123.2, 123.8, 124.5, 125.9, 134.1, 134.9, 136.4, 139.1, 140.1, 146.5, and 153.1; *m/z*: 342 (M^+) and 326 (M^+-16).

2.3.12. 4-(4-Nitro-phenyl)-2-phenyl-1-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3i). White crystalline solid; yield 78%; mp 160–161 °C; [Found: C, 70.82; H, 5.16; N, 11.29. $C_{22}H_{19}N_3O_3$ requires C, 70.76; H, 5.13; N, 11.25%]; ν_{max}/cm^{-1} (KBr): 1221 (N–O), 1547, 1597 (C═N); δ_H (200 MHz): δ 2.20 (s, 3H, –CH_3), 5.02 (dd, J=14.1 and 2.8 Hz, 1H, –CH_2), 5.27 (dd, J=14.1 and 5.4 Hz, 1H, –CH_2), 6.30 (dd, J=2.8 and 5.4 Hz, 1H, methine), 6.61 (d, J=8.4 Hz, 2H, ArH), 7.13 (d, J=8.4 Hz, 2H, ArH), 7.50–7.70 (m, 5H, ArH), 8.39 (d, J=8.9 Hz, 2H, ArH), 8.59 (d, J=8.9 Hz, 2H, ArH); ^{13}C NMR: 20.1 (CH_3), 52.5 (CH_2), 90.1 (methine), 112.9, 117.3, 123.6, 127.2, 127.8, 127.9, 128.6, 129.7, 129.9, 132.5, 141.3, 147.7, and 152.2; *m/z*: 373 (M^+) and 357 (M^+-16).

2.3.13. {4-[1-(4-Methoxy-phenyl)-3-oxy-4-phenyl-2,5-dihydro-1*H*-imidazol-2-yl]-phenyl}-dimethyl-amine (3j). Yellow crystalline solid; yield 85%; mp 139–140 °C; [Found: C, 74.45; H, 6.45; N, 10.82. $C_{24}H_{25}N_3O_2$ requires C, 74.39; H, 6.50; N, 10.84%]; ν_{max}/cm^{-1} (KBr): 1223 (N–O), 1546, 1589 (C═N); δ_H (200 MHz): δ 2.91 [(s, 6H, –$N(CH_3)_2$], 3.72 (s, 3H, –OCH_3), 4.76 (dd, J=14.1 and 3.2 Hz, 1H, –CH_2), 5.05 (dd, J=14.1 and 5.5 Hz, 1H, –CH_2), 6.00 (dd, J=3.2 and 5.5 Hz, 1H, methine), 6.53 (d, J=8.8 Hz, 2H, ArH), 6.69 (d, J=8.7 Hz, 2H, ArH), 6.79 (d, J=8.8 Hz, 2H, ArH), 7.20–8.24 (m, 7H, ArH); ^{13}C NMR: 40.4 (NMe_2), 52.5 (–CH_2–), 55.6 (OCH_3), 90.6 (methine), 112.1, 113.8, 115.0, 122.2, 124.1, 128.1, 128.9, 133.2, 135.8, 138.5, 148.9, 152.1, and 153.5; *m/z*: 387 (M^+) and 371 (M^+-16).

2.3.14. {4-[1-(4-Methoxy-phenyl)-3-oxy-4-*p*-tolyl-2,5-dihydro-1*H*-imidazol-2-yl]-phenyl}-dimethyl-amine (3k). Yellow crystalline solid; yield 81%; mp 177–178 °C; [Found: C, 74.84; H, 6.75; N, 10.45. $C_{25}H_{27}N_3O_2$ requires C, 74.79; H, 6.78; N, 10.47%]; ν_{max}/cm^{-1} (KBr): 1225 (N–O), 1547, 1595 (C═N); δ_H (200 MHz): δ 2.35 (s, 3H, CH_3), 2.91 [(s, 6H, –$N(CH_3)_2$], 3.72 (s, 3H, –OCH_3), 4.71 (dd, J=14.2 and 3.2 Hz, 1H, –CH_2), 5.10 (dd, J=14.2 and 5.4 Hz, 1H, –CH_2), 6.03 (dd, J=3.2 and 5.4 Hz, 1H, methine), 6.62 (d, J=8.8 Hz, 2H, ArH), 6.71 (d, J=8.8 Hz,

2H, ArH), 6.91 (d, J=8.8 Hz, 2H, ArH), 6.99 (d, J=8.8 Hz, 2H, ArH), 7.27 (d, J=8.8 Hz, 2H, ArH), 7.43 (d, J=8.8 Hz, 2H, ArH); ^{13}C NMR: 21.6 (CH_3), 40.3 (NMe_2), 52.8 (–CH_2–), 55.5 (OCH_3), 90.6 (methine), 113.9, 114.3, 115.2, 122.3, 126.9, 127.5, 128.1, 129.1, 134.6, 135.7, 140.2, 146.1, and 153.1; *m/z*: 401 (M^+) and 385 (M^+-16).

2.3.15. {4-[1-(4-Methoxy-phenyl)-4-(4-nitro-phenyl)-3-oxy-2,5-dihydro-1*H*-imidazol-2-yl]-phenyl}-dimethyl-amine (3l). Red crystalline solid; yield 88%; mp 166–167 °C; [Found: C, 66.63; H, 5.52; N, 12.99. $C_{24}H_{24}N_4O_4$ requires C, 66.69; H, 5.59; N, 12.96%]; ν_{max}/cm^{-1} (KBr): 1220 (N–O), 1542, 1591 (C═N); δ_H (200 MHz): δ 2.96 [(s, 6H, –$N(CH_3)_2$], 3.73 (s, 3H, –OCH_3), 4.79 (dd, J=14.1 and 3.1 Hz, 1H, –CH_2), 5.15 (dd, J=14.1 and 5.4 Hz, 1H, –CH_2), 6.08 (dd, J=3.1 and 5.4 Hz, 1H, methine), 6.59 (d, J=8.8 Hz, 2H, ArH), 6.70 (d, J=8.7 Hz, 2H, ArH), 6.81 (d, J=8.8 Hz, 2H, ArH), 7.44 (d, J=8.7 Hz, 2H, ArH), 8.29 (d, J=8.8 Hz, 2H, ArH), 8.49 (d, J=8.8 Hz, 2H, ArH); ^{13}C NMR: 40.3 (NMe_2), 52.7 (–CH_2–), 55.5 (OCH_3), 90.7 (methine), 112.1, 113.7, 114.9, 122.2, 123.9, 127.2, 128.8, 132.9, 135.2, 138.3, 147.8, 151.7, and 152.8; *m/z*: 432 (M^+) and 416 (M^+-16).

2.3.16. [4-(1-Benzyl-3-oxy-4-phenyl-2,5-dihydro-1*H*-imidazol-2-yl)-phenyl]-dimethyl-amine (3m). Creamish crystalline solid; yield 37%; mp 178–179 °C; [Found: C, 77.57; H, 6.82; N, 11.33. $C_{24}H_{25}N_3O$ requires C, 77.60; H, 6.78; N, 11.37%]; ν_{max}/cm^{-1} (KBr): 1221 (N–O), 1547, 1600 (C═N); δ_H (200 MHz): δ 2.90 [(s, 6H, –$N(CH_3)_2$], 3.21 (d, J=13.5 Hz, 1H, benzylic), 3.92 (dd, J=14.1 and 4.2 Hz, 1H, CH_2), 4.05 (d, J=13.5 Hz, 1H, benzylic), 4.35 (dd, J=14.2 and 3.6 Hz, 1H, CH_2), 5.49 (dd, J=4.2 and 3.6 Hz, 1H, methine), 6.81 (d, J=8.5 Hz, 2H, ArH), 7.31–7.57 (m, 5H, ArH), 7.62 (d, J=8.5 Hz, 2H, ArH), 7.95–8.10 (m, 5H, ArH); ^{13}C NMR: 41.1 (NMe_2), 52.7 (CH_2, benzylic), 56.1 (CH_2), 94.3 (methine), 112.1, 121.2, 123.6, 126.7, 128.1, 128.7, 129.9, 132.5, 134.1, 136.9, 148.1, 152.1, and 153.2; *m/z*: 371 (M^+) and 355 (M^+-16).

2.3.17. [4-(5-Benzyl-3-phenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazin-6-yl)-phenyl]-dimethyl-amine (4m). White crystalline solid; yield 55%; mp 172–173 °C; [Found: C, 77.68; H, 6.82; N, 11.33. $C_{24}H_{25}N_3O$ requires C, 77.60; H, 6.78; N, 11.37%]; ν_{max}/cm^{-1} (KBr): 1621 (C═N); δ_H (200 MHz): δ 2.92 [(s, 6H, –$N(CH_3)_2$], 3.25 (d, J=13.6 Hz, 1H, benzylic CH_2), 3.95 (s, 2H, CH_2, oxazine), 4.09 (d, J=13.6 Hz, 1H, benzylic –CH_2), 6.00 (s, 1H, methine), 6.78 (d, J=8.5 Hz, 2H, ArH), 7.15–7.32 (m, 5H, ArH), 7.48 (d, J=8.5 Hz, 2H, ArH), 7.65–7.98 (m, 5H, ArH); ^{13}C NMR: 40.2 (NMe_2), 52.1 (CH_2, oxazine), 53.6 (CH_2, benzylic), 92.9 (methine), 112.2, 122.7, 123.9, 127.5, 128.3, 129.0, 130.9, 135.5, 137.1, 138.2, 147.1, 152.5, and 153.5; *m/z*: 371 (M^+).

2.3.18. [4-(1-Benzyl-3-oxy-4-*p*-tolyl-2,5-dihydro-1*H*-imidazol-2-yl)-phenyl]-dimethyl-amine (3n). Yellow crystalline solid; yield 30%; mp 161–162 °C; [Found: C, 77.94; H, 7.02; N, 10.87. $C_{25}H_{27}N_3O$ requires C, 77.89; H, 7.06; N, 10.90%]; ν_{max}/cm^{-1} (KBr): 1225 (N–O), 1548, 1601 (C═N); δ_H (200 MHz): δ 2.32 (s, 3H, CH_3), 2.91 [(s, 6H, –$N(CH_3)_2$], 3.56 (d, J=13.0 Hz, 1H, benzylic), 3.82 (dd, J=14.2 and 4.2 Hz, 1H, CH_2), 4.06 (d, J=13.0 Hz, 1H, benzylic), 4.32 (dd, J=14.2 and 3.6 Hz, 1H, CH_2), 5.49 (dd, J=4.1 and 3.5 Hz, 1H, methine), 6.78 (d, J=8.4 Hz, 2H, ArH), 6.88 (d, J=8.6 Hz, 2H, ArH), 6.99 (d, J=8.6 Hz, 2H, ArH), 7.12–7.89 (m, 7H, ArH); ^{13}C NMR: 20.9 (CH_3), 41.1 (NMe_2), 52.9 (CH_2, benzylic), 56.1 (CH_2), 94.1 (methine), 112.5, 120.9, 122.5, 125.5, 128.1, 128.8, 131.2, 134.1, 134.7, 138.2, 146.1, 151.9, and 152.9; *m/z*: 385 (M^+) and 369 (M^+-16).

2.3.19. [4-(5-Benzyl-3-*p*-tolyl-5,6-dihydro-4*H*-[1,2,5]oxadiazin-6-yl)-phenyl]-dimethyl-amine (4n). White crystalline solid; yield 50%; mp 142–143 °C; [Found: C, 77.92; H, 7.02; N, 10.93. $C_{25}H_{27}N_3O$ requires C, 77.89; H, 7.06; N, 10.90%]; ν_{max}/cm^{-1} (KBr): 1624 (C═N); δ_H (200 MHz): δ 2.32 (s, 3H, CH_3), 2.90 [(s, 6H, –$N(CH_3)_2$], 3.23 (d, J=13.8 Hz, 1H, benzylic), 3.95 (s, 2H, CH_2, oxazine), 4.15 (d, J=13.8 Hz, 1H, benzylic), 5.98 (s, 1H, methine), 6.75 (d, J=8.0 Hz, 2H, ArH), 6.95 (d, J=8.0 Hz, 2H, ArH), 7.18 (d, J=8.5 Hz, 2H, ArH), 7.21–7.40 (m, 5H, ArH), 7.48 (d, J=8.5 Hz, 2H, ArH); ^{13}C NMR: 20.9 (CH_3), 40.3 (NMe_2), 52.5 (CH_2, oxazine), 54.3 (CH_2, benzylic), 93.1 (methine), 114.1, 119.1, 123.5, 126.2, 129.1, 129.9, 132.2, 134.1, 135.2, 137.7, 146.1, 152.1, and 153.5; *m/z*: 385 (M^+).

2.3.20. {4-[1-Benzyl-4-(4-nitro-phenyl)-3-oxy-2,5-dihydro-1*H*-imidazol-2-yl]-phenyl}-dimethyl-amine (3o). White crystalline solid; yield 36%; mp 147–148 °C; [Found: C, 69.26; H, 5.85; N, 13.39. $C_{24}H_{24}N_4O_3$ requires C, 69.21; H, 5.81; N, 13.45%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1547, 1595 (C═N); δ_H (200 MHz): δ 2.90 [(s, 6H, –$N(CH_3)_2$], 3.62 (d, J=13.0 Hz, 1H, benzylic), 3.87 (dd, J=14.0 and 4.6 Hz, 1H, CH_2), 4.08 (d, J=13.0 Hz, 1H, benzylic), 4.36 (dd, J=14.0 and 3.2 Hz, 1H, CH_2), 5.46 (dd, J=4.6 and 3.2 Hz, 1H, methine), 6.80 (d, J=8.4 Hz, 2H, ArH), 7.25–7.36 (m, 5H, ArH), 7.54 (d, J=8.4 Hz, 2H, ArH), 8.21 (d, J=9.0 Hz, 2H, ArH), 8.35 (d, J=9.0 Hz, 2H, ArH); ^{13}C NMR: 40.3 (NMe_2), 53.9 (–CH_2–, benzylic), 56.3 (–CH_2); 94.6 (methine), 112.1, 121.2, 123.6, 126.9, 127.7, 128.6, 130.0, 133.3, 133.6, 136.5, 147.6, 151.8, and 152.9; *m/z*: 416 (M^+) and 400 (M^+-16).

2.3.21. {4-[5-Benzyl-3-(4-nitro-phenyl)-5,6-dihydro-4*H*-[1,2,5]oxadiazin-6-yl]-phenyl}-dimethyl-amine (4o). Yellow crystalline solid; yield 54%; mp 115–117 °C; [Found: C, 69.27; H, 5.86; N, 13.39. $C_{24}H_{24}N_4O_3$ requires C, 69.21; H, 5.81; N, 13.45%]; ν_{max}/cm^{-1} (KBr): 1625 (C═N); δ_H (200 MHz): δ 2.95 [(s, 6H, –$N(CH_3)_2$], 3.47 (d, J=14.0 Hz, 1H, benzylic), 3.49 (d, J=13.9 Hz, 1H, benzylic), 3.62 (d, J=5.0 Hz, 1H, –CH_2), 3.87 (d, 1H, J=5.1 Hz), 5.76 (s, 1H, methine), 6.73 (d, J=8.8 Hz, 2H, ArH), 7.27–7.41 (m, 5H, ArH), 7.47 (d, J=8.7 Hz, 2H, ArH), 7.70 (d, J=8.9 Hz, 2H, ArH), 8.17 (d, J=8.9 Hz, 2H, ArH); ^{13}C NMR: 40.3 (NMe_2), 52.1 (CH_2, benzylic), 56.1 (–CH_2), 91.3 (methine), 112.4, 122.5, 124.1, 127.3, 128.1, 129.1, 131.5, 134.2, 134.8, 137.1, 148.5, 152.5, and 153.2; *m/z*: 416 (M^+).

2.3.22. 1-Butyl-2,4-diphenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3p). White crystalline solid; yield 34%; mp 146–147 °C; [Found: C, 77.60; H, 7.45; N, 9.48. $C_{19}H_{22}N_2O$ requires C, 77.52; H, 7.53; N, 9.52%]; ν_{max}/cm^{-1} (KBr): 1221 (N–O), 1545, 1594 (C═N); δ_H (200 MHz): δ 0.92 (t, J=7.4 Hz, 3H, CH_3), 1.37–1.65 (m, 4H, 2×CH_2), 2.72–2.81 (m, 2H, CH_2), 4.32 (dd, J=14.7 and 3.6 Hz, 1H,

CH_2), 4.71 (dd, J=14.7 and 4.2 Hz, 1H, CH_2), 5.85 (dd, J=3.5 and 4.1 Hz, 1H, methine), 6.99–7.32 (m, 6H, ArH), 7.42–7.59 (m, 4H, ArH); ^{13}C NMR: 14.3, 21.7, 23.9, 45.2, 53.1, 91.2, 122.9, 123.5, 125.9, 127.9, 129.7, 137.9, 141.7, 150.2, and 153.2; *m/z*: 294 (M^+) and 278 (M^+-16).

2.3.23. 5-Butyl-3,6-diphenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4p). White crystalline solid; yield 56%; mp 129–130 °C; [Found: C, 77.55; H, 7.48; N, 9.47. $C_{19}H_{22}N_2O$ requires C, 77.52; H, 7.53; N, 9.52%]; ν_{max}/cm^{-1} (KBr): 1621 (C=N); δ_H (200 MHz): δ 0.95 (t, 3H, CH_3), 1.39–1.63 (m, 4H, 2×CH_2), 2.71–2.84 (m, 2H, CH_2), 3.89 (AB quartet, J=7.7 Hz, 2H, CH_2), 5.99 (s, 1H, methine), 6.95–7.32 (m, 5H, ArH), 7.45–7.98 (m, 5H, ArH); ^{13}C NMR: 13.7, 20.2, 32.1, 46.6, 52.1, 91.5, 125.7, 121.9, 124.3, 126.9, 129.9, 140.2, 143.9, 151.1, and 153.1; *m/z*: 294 (M^+).

2.3.24. 1-Butyl-2-phenyl-4-*p*-tolyl-2,5-dihydro-1H-imidazole 3-oxide (3q). White crystalline solid; yield 30%; mp 155–156 °C; [Found: C, 77.82; H, 7.81; N, 9.13. $C_{20}H_{24}N_2O$ requires C, 77.89; H, 7.84; N, 9.08%]; ν_{max}/cm^{-1} (KBr): 1220 (N–O), 1547, 1596 (C=N); δ_H (200 MHz): δ 0.92 (t, J=7.5 Hz, 3H, CH_3), 1.38–1.62 (m, 4H, 2×CH_2), 2.32 (s, 3H, CH_3), 2.73–2.81 (m, 2H, CH_2), 4.40 (dd, J=14.7 and 3.6 Hz, 1H, CH_2), 4.79 (dd, J=14.7 and 4.2 Hz, 1H, CH_2), 5.79 (dd, J=3.6 and 4.2 Hz, 1H, methine), 6.60 (d, J=8.5 Hz, 2H, ArH), 7.02 (d, J=8.5 Hz, 2H, ArH), 7.35–7.61 (m, 5H, ArH); ^{13}C NMR: 14.1, 21.2, 23.5, 46.1, 55.3, 94.3, 121.1, 122.4, 126.3, 128.1, 129.2, 138.7, 142.2, 148.5, and 152.9; *m/z*: 308 (M^+) and 292 (M^+-16).

2.3.25. 5-Butyl-6-phenyl-3-*p*-tolyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4q). White crystalline solid; yield 50%; mp 140–141 °C; [Found: C, 77.89; H, 7.80; N, 9.14. $C_{20}H_{24}N_2O$ requires C, 77.89; H, 7.84; N, 9.08%]; ν_{max}/cm^{-1} (KBr): 1625 (C=N); δ_H (200 MHz): δ 0.94 (t, J=7.2 Hz, 3H, CH_3), 1.38–1.62 (m, 4H, 2×CH_2), 2.35 (s, 3H, CH_3), 2.73–2.81 (m, 2H, CH_2), 3.62 (AB quartet, J=7.6 Hz, 2H, CH_2, oxazine), 5.80 (s, 1H, methine), 6.58 (d, J=8.2 Hz, 2H, ArH), 6.98 (d, J=8.2 Hz, 2H, ArH), 7.07–7.42 (m, 5H, ArH); ^{13}C NMR: 13.7, 20.2, 21.2, 30.2, 45.1, 52.0, 91.1, 121.1, 122.8, 123.9, 126.2, 128.9, 139.1, 141.4, 149.1, and 153.1; *m/z*: 308.42 (M^+).

2.3.26. 1-Butyl-4-(4-nitro-phenyl)-2-phenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3r). White crystalline solid; yield 31%; mp 125–126 °C; [Found: C, 67.29; H, 6.20; N, 12.35. $C_{19}H_{21}N_3O_3$ requires C, 67.24; H, 6.24; N, 12.38%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1549, 1600 (C=N); δ_H (200 MHz): δ 0.92 (t, J=7.4 Hz, 3H, CH_3), 1.38–1.63 (m, 4H, 2×CH_2), 2.75–2.85 (m, 2H, CH_2), 4.35 (dd, J=14.7 and 3.6 Hz, 1H, CH_2), 4.75 (dd, J=14.7 and 4.2 Hz, 1H, CH_2), 5.75 (dd, J=3.2 and 4.5 Hz, 1H, methine), 7.41–7.68 (m, 5H, ArH), 7.91 (d, J=8.8 Hz, 2H, ArH), 8.32 (d, J=8.7 Hz, 2H, ArH); ^{13}C NMR: 13.5, 21.2, 28.9, 45.3, 51.4, 93.8, 122.7, 123.5, 127.1, 129.4, 129.9, 139.4, 143.1, 150.2, and 153.2; *m/z*: 339 (M^+) and 326 (M^+-16).

2.3.27. 5-Butyl-3-(4-nitro-phenyl)-6-phenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4r). White crystalline solid; yield 53%; mp 105–106 °C; [Found: C, 67.29; H, 6.20; N, 12.35. $C_{19}H_{21}N_3O_3$ requires C, 67.24; H, 6.24; N, 12.38%]; ν_{max}/cm^{-1} (KBr): 1620 (C=N); δ_H (200 MHz): δ 0.94 (t, J=7.2 Hz, 3H, CH_3), 1.39–1.63 (m, 4H, 2×CH_2), 2.74–2.82 (m, 2H, CH_2), 3.65 (AB quartet, J=7.7 Hz, 2H, CH_2, oxazine), 5.79 (s, 1H, methine), 7.28–7.59 (m, 5H, ArH), 7.78 (d, J=8.8 Hz, 2H, ArH), 8.23 (d, J=8.8 Hz, 2H, ArH); ^{13}C NMR: 13.8, 20.2, 29.9, 43.8, 51.5, 91.0, 123.7, 125.4, 126.6, 128.5, 128.6, 137.1, 140.3, 148.3, and 152.1; *m/z*: 339 (M^+).

2.3.28. 1-Isopropyl-2,4-diphenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3s). White crystalline solid; yield 35%; mp 120–121 °C; [Found: C, 77.19; H, 7.12; N, 10.05. $C_{18}H_{22}N_2O$ requires C, 77.11; H, 7.19; N, 9.99%]; ν_{max}/cm^{-1} (KBr): 1225 (N–O), 1545, 1599 (C=N); δ_H (200 MHz): δ 1.15–1.21 (m, 6H, 2×CH_3), 3.19–3.45 (m, 1H, –CH, isopropyl), 4.32 (dd, J=14.7 and 3.6 Hz, 1H, CH_2), 4.72 (dd, J=14.7 and 4.2 Hz, 1H, CH_2), 5.79 (dd, J=3.6 and 4.2 Hz, 1H, methine), 6.82–7.35 (m, 6H, ArH), 7.51–7.69 (m, 2H, ArH), 7.92–8.05 (m, 2H, ArH); ^{13}C NMR: 12.3, 22.4, 49.1, 89.7, 127.5, 129.1, 130.2, 130.8, 131.7, 133.5, 134.7, 142.1, and 153.9; *m/z*: 280 (M^+) and 264 (M^+-16).

2.3.29. 5-Isopropyl-3,6-diphenyl-5,6-dihydro-4*H*-[1,2,5]-oxadiazine (4s). White crystalline solid; yield 47%; mp 101–102 °C; [Found: C, 77.08; H, 7.12; N, 10.06. $C_{18}H_{22}N_2O$ requires C, 77.14; H, 7.19; N, 9.99%]; ν_{max}/cm^{-1} (KBr): 1623 (C=N); δ_H (200 MHz): δ 1.15–1.23 (m, 6H, 2×CH_3), 3.21–3.48 (m, 1H, –CH, isopropyl), 3.69 (s, 2H, –CH_2, oxazine), 5.99 (s, 1H, methine), 6.75–7.10 (m, 5H, ArH), 7.25–7.95 (m, 5H, ArH); ^{13}C NMR: 11.8, 21.9, 48.8, 89.8, 129.1, 129.7, 130.2, 132.1, 132.9, 134.1, 134.7, 140.7, and 152.1; *m/z*: 280 (M^+).

2.3.30. 1-Isopropyl-2-phenyl-4-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3t). White crystalline solid; yield 37%; mp 133–134 °C; [Found: C, 77.58; H, 7.50; N, 9.48. $C_{19}H_{22}N_2O$ requires C, 77.52; H, 7.53; N, 9.52%]; ν_{max}/cm^{-1} (KBr): 1221 (N–O), 1547, 1595 (C=N); δ_H (200 MHz): δ 1.13–1.21 (m, 6H, 2×CH_3), 2.32 (s, 3H, CH_3), 3.15–3.42 (m, 1H, –CH, isopropyl), 4.35 (dd, J=14.2 and 4.2 Hz, 1H, CH_2), 4.62 (dd, J=14.2 and 4.2 Hz, 1H, CH_2), 5.75 (dd, J=3.7 and 4.1 Hz, 1H, methine), 6.65 (d, J=8.5 Hz, 2H, ArH), 6.99 (d, J=8.5 Hz, 2H, ArH), 7.10–7.49 (m, 5H, ArH); ^{13}C NMR: 11.6, 20.6 (CH_3), 21.4 (CH), 44.1 (CH_2), 90.1 (methine), 127.8, 128.1, 129.5, 130.1, 130.9, 131.2, 134.1, 140.1, and 153.1; *m/z*: 294 (M^+) and 278 (M^+-16).

2.3.31. 5-Isopropyl-6-phenyl-3-*p*-tolyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4t). White crystalline solid; yield 48%; mp 105–106 °C; [Found: C, 77.59; H, 7.48; N, 9.58. $C_{19}H_{22}N_2O$ requires C, 77.52; H, 7.53; N, 9.52%]; ν_{max}/cm^{-1} (KBr): 1620 (C=N); δ_H (200 MHz): δ 1.15–1.22 (m, 6H, 2×CH_3), 2.32 (s, 3H, CH_3), 3.25–3.50 (m, 1H, –CH, isopropyl), 3.95 (s, 2H, oxazine CH_2), 5.98 (s, 1H, methine), 6.94 (d, J=8.6 Hz, 2H, ArH), 7.02 (d, J=8.6 Hz, 2H, ArH), 7.12–7.45 (m, 5H, ArH); ^{13}C NMR: 11.4, 20.5 (CH_3), 22.0 (CH), 45.4 (CH_2), 93.1 (methine), 128.1, 129.2, 129.9, 131.1, 132.8, 133.2, 134.1, 137.4, and 152.5; *m/z*: 294 (M^+) and 278 (M^+-16).

2.3.32. 1-Isopropyl-4-(4-nitro-phenyl)-2-phenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3u). White crystalline solid; yield 31%; mp 111–112 °C; [Found: C, 66.51; H, 5.85; N, 12.94. $C_{18}H_{19}N_3O_3$ requires C, 66.45; H, 5.89; N, 12.91%];

ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1545, 1596 (C═N); δ_H (200 MHz): δ 1.15–1.22 (m, 6H, 2×CH_3), 3.21–3.48 (m, 1H, –CH, isopropyl), 4.22 (dd, J=14.7 and 3.6 Hz, 1H, CH_2), 4.68 (dd, J=14.7 and 4.2 Hz, 1H, CH_2), 5.72 (dd, J=3.6 and 4.2 Hz, 1H, methine), 7.35–7.61 (m, 5H, ArH), 7.82 (d, J=8.7 Hz, 2H, ArH), 8.27 (d, J=8.7 Hz, 2H, ArH); ^{13}C NMR: 11.4, 21.3 (CH, isopropyl), 43.5 (CH_2), 90.1 (methine), 127.2, 127.9, 128.4, 129.1, 130.5, 130.7, 132.4, 133.9, and 154.2; m/z: 325 (M^+) and 309 (M^+-16).

2.3.33. 5-Isopropyl-3-(4-nitro-phenyl)-6-phenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4u). White crystalline solid; yield 52%; mp 98–99 °C; [Found: C, 66.50; H, 5.85; N, 12.98. $C_{18}H_{19}N_3O_3$ requires C, 66.45; H, 5.89; N, 12.91%]; ν_{max}/cm^{-1} (KBr): 1621 (C═N); δ_H (200 MHz): δ 1.17–1.25 (m, 6H, 2×CH_3), 3.25–3.51 (m, 1H, –CH, isopropyl), 3.61 (s, 2H, –CH_2, oxazine), 5.91 (s, 1H, methine), 7.29–7.55 (m, 5H, ArH), 7.73 (d, J=8.7 Hz, 2H, ArH), 8.19 (d, J=8.7 Hz, 2H, ArH); ^{13}C NMR: 11.2, 21.2, 42.4, 94.8, 127.5, 128.2, 128.7, 128.9, 130.1, 130.5, 132.1, 134.2, and 153.5; m/z: 325 (M^+); Anal. Calcd for: C, 66.45; H, 5.89; N, 12.91.

2.3.34. 1-Furan-2-ylmethyl-2,4-diphenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3v). White crystalline solid; yield 34%; mp 138–139 °C. [Found: C, 66.50; H, 5.85; N, 12.98. $C_{18}H_{19}N_3O_3$ requires C, 66.45; H, 5.89; N, 12.91%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1545, 1597 (C═N); δ_H (200 MHz): δ 3.81 (d, J=14.6 Hz, 1H, furyl), 3.92 (d, J=14.6 Hz, 1H, furyl), 4.2 (dd, J=14.2 and 4.5 Hz, 1H, –CH_2–), 4.56 (dd, J=14.2 and 3.3 Hz, 1H, –CH_2–), 5.62 (dd, J=4.5 and 3.3 Hz, 1H, methine), 6.23–6.32 (m, 2H, H_b and H_c), 6.42–7.53 (m, 11H, 10ArH and 1H, H_a); ^{13}C NMR: 54.2, 56.5, 94.5 (methine), 111.8, 119.3, 122.4, 126.8, 127.5, 129.2, 132.6, 134.3, 137.5, 139.2, 148.2, 152.2, and 154.1; m/z: 318 (M^+) and 302 (M^+-16); Anal. Calcd for $C_{20}H_{18}N_2O_2$: C, 75.45; H, 5.70; N, 8.80; Found: C, 75.50; H, 5.76; N, 8.82.

2.3.35. 5-Furan-2-ylmethyl-3,6-diphenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4v). White crystalline solid; yield 49%; mp 121–122 °C. [Found: C, 75.51; H, 5.78; N, 8.85. $C_{20}H_{18}N_2O_2$ requires C, 75.45; H, 5.70; N, 8.80%]; ν_{max}/cm^{-1} (KBr): 1625 (C═N); δ_H (200 MHz): δ 3.62 (s, 2H, CH_2), 3.82 (d, J=14.7 Hz, 1H, furyl), 3.91 (d, J=14.7 Hz, 1H, furyl), 5.78 (s, 1H, methine), 6.26–6.33 (m, 2H, H_b and H_c), 6.78–7.32 (m, 5H, 4ArH and 1H, H_a), 7.34–7.48 (m, 6H, ArH); ^{13}C NMR: 53.8, 56.5, 94.5, 112.0, 117.9, 120.8, 121.3, 125.7, 126.5, 127.3, 130.5, 132.6, 135.4, 146.9, 151.2, and 153.1; m/z: 318 (M^+).

2.3.36. 1-Furan-2-ylmethyl-2-phenyl-4-*p*-tolyl-2,5-dihydro-1*H*-imidazole 3-oxide (3w). White crystalline solid; yield 31%; mp 145–146 °C. [Found: C, 75.93; H, 6.12; N, 8.45. $C_{21}H_{20}N_2O_2$ requires C, 75.88; H, 6.06; N, 8.43%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1550, 1595 (C═N); δ_H (200 MHz): δ 2.39 (s, 3H, CH_3), 3.83 (d, J=14.7 Hz, 1H, furyl), 3.94 (d, J=14.7 Hz, 1H, furyl), 4.24 (dd, J=14.1 and 4.4 Hz, 1H, –CH_2–), 4.54 (dd, J=14.1 and 3.5 Hz, 1H, –CH_2–), 5.60 (dd, J=4.4 and 3.5 Hz, 1H, methine), 6.29–6.31 (m, 2H, H_b and H_c), 6.99–7.01 (d, J=8.4 Hz, 2H, ArH), 7.03–7.06 (d, J=8.4 Hz, 2H, ArH), 7.17–7.45 (m, 6H, 5ArH and 1H, H_a); ^{13}C NMR: 21.5 (CH_3), 54.0, 56.4, 94.6 (methine), 112.4, 120.9, 124.0, 127.0, 128.1, 128.8, 131.1, 133.4, 134.2, 137.3, 147.4, 152.1, and 152.9; m/z: 332 (M^+) and 316 (M^+-16).

2.3.37. 5-Furan-2-ylmethyl-6-phenyl-3-*p*-tolyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4w). White crystalline solid; yield 54%; mp 130–131 °C. [Found: C, 75.92; H, 6.10; N, 8.45. $C_{21}H_{20}N_2O_2$ requires C, 75.88; H, 6.06; N, 8.43%]; ν_{max}/cm^{-1} (KBr): 1621 (C═N); δ_H (200 MHz): δ 2.35 (s, 3H, CH_3), 3.63 (s, 2H, CH_2), 3.83 (d, J=14.7 Hz, 1H, furyl), 3.94 (d, J=14.7 Hz, 1H, furyl), 5.78 (s, 1H, methine), 6.27–6.33 (m, 2H, H_b and H_c), 6.99–7.02 (d, J=8.2 Hz, 2H, ArH), 7.13–7.80 (m, 8H, 7ArH and 1H, H_a); ^{13}C NMR: 20.9, 53.7, 56.4, 94.4, 111.8, 118.3, 120.8, 121.9, 122.8, 124.6, 127.0, 129.2, 130.1, 133.2, 147.5, 151.4, and 153.0; m/z: 332 (M^+).

2.3.38. 1-Furan-3-ylmethyl-4-(4-nitro-phenyl)-2-phenyl-2,5-dihydro-1*H*-imidazole 3-oxide (3x). Bright yellow crystalline solid; yield 30%; mp 155–156 °C. [Found: C, 66.16; H, 4.76; N, 11.59. $C_{20}H_{17}N_3O_4$ requires C, 66.11; H, 4.72; N, 11.56%]; ν_{max}/cm^{-1} (KBr): 1220 (N–O), 1551, 1589 (C═N); δ_H (200 MHz): δ 3.84 (d, J=14.8 Hz, 1H, furyl CH_2), 3.98 (d, J=14.8 Hz, 1H, furyl), 4.26 (dd, J=14.1 and 4.5 Hz, 1H, –CH_2–), 4.56 (dd, J=14.1 and 3.7 Hz, 1H, –CH_2–), 5.61 (dd, J=4.4 and 3.5 Hz, 1H, methine), 6.27–6.36 (m, 2H, H_b and H_c), 7.27–7.62 (m, 6H, 5ArH and H_a), 8.26 (d, J=8.9 Hz, 2H, ArH), 8.39 (d, J=8.9 Hz, 2H, ArH); ^{13}C NMR: 53.9 (–CH_2–), 56.3 (–CH_2), 94.6 (methine), 112.1, 121.2, 123.6, 126.9, 127.7, 128.6, 130.0, 133.3, 133.6, 136.5, 147.6, 151.8, and 152.9; m/z: 363 (M^+) and 347 (M^+-16).

2.3.39. 5-Furan-2-ylmethyl-3-(4-nitro-phenyl)-6-phenyl-5,6-dihydro-4*H*-[1,2,5]oxadiazine (4x). Yellow crystalline solid; yield 50%; mp 126–127 °C. [Found: C, 66.15; H, 4.76; N, 11.58. $C_{20}H_{17}N_3O_4$ requires C, 66.11; H, 4.72; N, 11.56%]; ν_{max}/cm^{-1} (KBr): 1625 (C═N); δ_H (200 MHz): δ 3.65 (s, 2H, CH_2), 3.82 (d, J=14.6 Hz, 1H, furyl), 3.93 (d, J=14.6 Hz, 1H, furyl), 5.77 (s, 1H, methine), 6.28–6.33 (m, 2H, H_b and H_c), 7.26–7.56 (m, 6H, 5ArH and 1H, H_a), 7.74 (d, J=8.8 Hz, 2H, ArH), 8.23 (d, J=8.8 Hz, 2H, ArH); ^{13}C NMR: 53.8 (–CH_2–), 56.3, 94.5 (methine), 111.9, 118.2, 121.3, 122.3, 123.7, 125.9, 127.8, 129.1, 130.5, 133.4, 147.5, 151.5, and 153.1; m/z: 363 (M^+).

2.4. Reactions of 2-methyl-1-aryl-isothioureas 7a–c and *N*-aryl-secondary amine-carboxamidines 7d–f with α-nitrosoalkenes

A solution of carboxamidines or isothioureas **7** (4 mmol) and α-bromooxime **2** (4.2 mmol) in dry CH_2Cl_2 (20 ml) was stirred at rt in the presence of sodium bicarbonate for 2–3 h. Work up identical with that employed for the nitrone **3** gave the crude products **8**, which were purified by column chromatography on silica gel (EtOAc–hexane :: 1:9) to yield the corresponding imidazoles **8** (75–85%).

2.4.1. 2-Methylsulfanyl-1-phenyl-4-*p*-tolyl-1*H*-imidazole (8a). Mp 145–146 °C; yield 78%; [Found: C, 72.85; H, 5.73; N, 10.01. $C_{17}H_{16}N_2S$ requires C, 72.82; H, 5.75; N, 9.99%]; ν_{max}/cm^{-1} (KBr): 1592 (C═N); δ_H (200 MHz): δ 2.35 (s, 3H, CH_3), 2.47 (s, 3H, SCH_3), 6.51 (d, J=8.2 Hz, 2H, ArH), 7.21 (d, J=8.2 Hz, 2H, ArH), 7.45

(s, 1H, H_a), 7.47–7.52 (m, 5H, ArH); δ_C (200 MHz): δ 18.9 (SCH_3); 20.9 (CH_3); 121.0, 123.1, 125.2, 126.9, 128.0, 129.4, 129.7, 133.5, 136.7, and 137.7; *m/z*: 280.39 (M^+), 280.10 (100%), 281.11 (19.1%), and 282.10 (4.6%).

2.4.2. 4-[4-(4-Nitro-phenyl)-1-phenyl-1*H*-imidazol-2-yl]-morpholine (8e). Mp 165–166 °C; yield 81%; [Found: C, 65.14; H, 5.20; N, 15.97. $C_{19}H_{18}N_4O_3$ requires C, 65.13; H, 5.18; N, 15.99%]; ν_{max}/cm^{-1} (KBr): 1595 (C=N); δ_H (200 MHz): δ 2.92 (m, 4H, $-CH_2-N-CH_2-$), 3.67 (m, 4H, $-CH_2-O-CH_2-$), 7.19–7.32 (m, 6H, 5 aromatic+1 olefenic), 8.24 (d, 2H, *J*=8.0 Hz, arom), 8.40 (d, 2H, *J*=8.0 Hz, arom); δ_C (200 MHz): δ 58.9 ($-CH_2-N-CH_2-$), 71.4 ($-CH_2-O-CH_2-$), 121.0, 123.2, 124.1, 125.0, 127.9, 128.0, 129.4, 136.7, 137.0, 142.6, and 148.4; *m/z*: 350.37 (M^+), 350.14 (100%), 351.14 (21.5%), and 352.14 (3.1%).

2.4.3. 2-Pyrrolidin-1-yl-1, 4-di-*p*-tolyl-1*H*-imidazole (8f). Mp 132–133 °C; yield 85%; [Found: C, 79.49; H, 7.31; N, 13.21. $C_{21}H_{23}N_3$ requires C, 79.46; H, 7.30; N, 13.24%]; ν_{max}/cm^{-1} (KBr): 1601 (C=N); δ_H (200 MHz): δ 1.59 (m, 4H, $-CH_2-CH_2-$), 2.32 (s, 3H, CH_3), 2.81 (m, 4H, $-CH_2-N-CH_2-$), 6.79 (d, *J*=8.0 Hz, 2H, ArH), 7.05 (m, 3H, 2 aromatic+1 olefenic), 7.12 (d, 2H, *J*=8.0 Hz, arom), 7.36 (d, 2H, *J*=8.0 Hz, arom); δ_C (200 MHz): δ 20.5 (CH_3), 20.9 (CH_3), 25.1 ($-CH_2-CH_2-$), 47.7 ($-CH_2-N-CH_2-$), 120.9, 123.0, 125.2, 126.9, 129.7, 130.1, 133.5, 133.7, 137.0, 137.2, and 137.7; *m/z*: 317.43 (M^+), 317.19 (100%), 318.19 (24.5%), and 319.20 (2.7%).

2.5. Reactions of *N*-phenyl-secondaryamino-1-carboximidothioic acid allyl ester 15a–d and 2-allyl-1,1-dimethyl-3-phenyl-isothiourea 15e–f with α-nitrosoalkenes

A solution of *S*-allylated imines **15** (4 mmol) and α-bromooxime **2** (4.2 mmol) in dry CH_2Cl_2 (20 ml) was stirred at rt in the presence of sodium bicarbonate for 2–3 h. Work up identical with that employed for the nitrones **3** and **8** gave the crude products, which were purified by column chromatography on silica gel (EtOAc–hexane:: 1:10) to yield the corresponding products **16** (70–80%).

2.5.1. 1,4-Diphenyl-2-pyrrolidin-1-yl-1*H*-imidazole 3-oxide (16a). Mp 142–143 °C; yield 77%; [Found: C, 74.76; H, 6.20; N, 13.75. $C_{19}H_{19}N_3O$ requires C, 74.73; H, 6.27; N, 13.76%]; ν_{max}/cm^{-1} (KBr): 1223 (N–O), 1583, 1596 (C=N); δ_H (200 MHz): δ 1.79 (m, 4H, $-CH_2-CH_2-$), 3.30 (m, 4H, $-CH_2-N-CH_2-$), 6.88 (m, 1H, olefenic), 7.08–7.32 (m, 10H, ArH); δ_C (200 MHz): δ 22.6, 45.7, 69.2, 119.6, 122.7, 125.2, 126.5, 127.1, 128.7, 130.2, 132.5, 139.1, and 154.2; *m/z*: 305 (M^+).

2.5.2. 1-(3-Oxy-1,4-diphenyl-1*H*-imidazol-2-yl)-piperidine (16b). Mp 153–154 °C; yield 80%; [Found: C, 75.24; H, 6.62; N, 13.17. $C_{20}H_{21}N_3O$ requires C, 75.21; H, 6.63; N, 13.16%]; ν_{max}/cm^{-1} (KBr): 1219 (N–O), 1599, 1652 (C=N); δ_H (200 MHz): δ 1.58 (m, 6H, $-CH_2-CH_2-CH_2-$), 2.83 (m, 2H, $-N-CH_2-$), 3.42 (m, 2H, $-N-CH_2-$), 6.49 (m, 1H, olefenic), 6.83–7.45 (m, 10H, ArH); δ_C (200 MHz): δ 25.2 ($-CH_2-CH_2-$), 26.2 (CH_2), 55.1 ($-CH_2-N-CH_2-$), 70.1, 119.8, 123.2, 125.9, 126.2, 127.5, 128.5, 130.0, 140.2, and 153.2; *m/z*: 319 (M^+).

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15. (a) *Preparation of Schiff's bases*: A mixture of primary amine (5 mmol) in CH_2Cl_2 (30 ml) was stirred in the presence of $MgSO_4$ (7.5 mmol) at rt for 3–4 h. The reaction mixture was filtered and the residue was washed with CH_2Cl_2 (10 ml). The combined was then washed with water (3×50 ml) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude product thus obtained was recrystallized with EtOAc–hexane :: 1:9); (b) McNeil Lab., G.B. 1573532, 1976; D.E. 2711757; *Chem. Abstr.* EN 88, 37603; (c) Korter, H.; Scholl, R. *Ber.* **1901**, *34*, 1901.

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Synthesis of some multi-β-substituted cationic porphyrins and studies on their interaction with DNA

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Abstract—A series of multi-β-substituted cationic porphyrins, 2,3,12,13-tetraphenyl-5-(*N*-trimethyl-4-ammoniumphenyl)-10,15,20-triphenylporphyrin **2**; 2,3,12,13-tetramethyl-5-(*N*-trimethyl-4-ammoniumphenyl)-10,15,20-triphenylporphyrin **3**; 2,3,7,8,12,13,17,18-octaphenyl-5-(*N*-trimethyl-4-ammoniumphenyl)-10,15,20-triphenylporphyrin **4**, and 2,3,7,8,12,13,17,18-octaphenyl-5,10-di(*N*-trimethyl-4-ammoniumphenyl)-15,20-diphenylporphyrin **5**, have been synthesized. Their photooxidative abilities and interaction with DNA were investigated by UV, fluorescence, CD, and gel electrophoresis. It is found that substituents at β-position of the porphyrins have significant effect on interactions and binding mode of the porphyrins with DNA. Increasing positive charges in the porphyrins strengthen their interactions with DNA.

1. Introduction

Interactions of a variety of drugs and other relatively small molecules with DNA have aroused great interest of many groups.[1–4] Among these agents, porphyrin family has caused particular attention.[5–8]

Fiel and collaborators[9] had studied the interaction of porphyrins with nucleic acid and found that a variety of porphyrins could bind to DNA.[10–12] As the backbone of nucleic acid has many negative charges, cationic porphyrin derivatives will be more easy to bind to DNA. One widely investigated cationic porphyrin is tetra-(4-*N*-methylpyridium)porphyrin [T4MPyP]. T4MPyP and its analogues have been known to bind to DNA.[13–17]

Natural porphyrin derivatives such as the photosynthetic centers, vitamin B_{12} and P-450[18–20] are β-substituted porphyrins. Their nonplanar conformation was affected by the β-substituents. We could imagine that interactions of nonplanar porphyrins with DNA will have different binding modes compared to that of planar T4MpyP. This assumption was made and supported by Neidle's group.[21] They found that Ni^{2+}-T4MPyP had a highly nonplanar conformation whereas T4MPyP was a typically planar, and the binding mode to DNA was totally changed from intercalation (for planar T4MPyP) to minor groove binding (for nonplanar NiT4MPyP). It suggests that porphyrin structure change can induce their DNA binding mode. Recently, several groups have intensively studied the effect of β-substituents on porphyrin properties.[22–25] It is found that substituents at β-position of porphyrins exerted much larger steric and electronic effects on the porphyrin ring than those at *meso*-aryl position.[26] Therefore, synthesis of β-substituted cationic porphyrins and study on their interaction abilities to DNA will be of great significance for biological and clinical applications. In our previous paper,[27] we synthesized two kinds of β-tetrasubstituted cationic porphyrins and preliminarily investigated their photocleavage abilities to plasmid DNA. Herein, we shall report in detail our synthesis of β-octa-substituted cationic porphyrins and their interactions with DNA.

2. Results

2.1. Synthesis of β-substituted cationic porphyrins

Four β-substituted cationic porphyrins **2**, **3**, **4**, and **5** have been synthesized and shown in Scheme 1.

β-Tetrasubstituted cationic porphyrins **2** and **3** were prepared from β-tetrabromo porphyrin as starting material by following the general method shown in Scheme 2. After bromination of compound **6** with NBS, tetrabromo porphyrin **7** was obtained.[28] Mono-4-nitro-phenyl porphyrin **8** was obtained by reacting fuming HNO_3 with porphyrin **7** in $CHCl_3$ at 5 °C.[29] After coupling boronic acids with compound **8** by

Keywords: β-Substituted cationic porphyrins; DNA; Interaction; Photocleavage.
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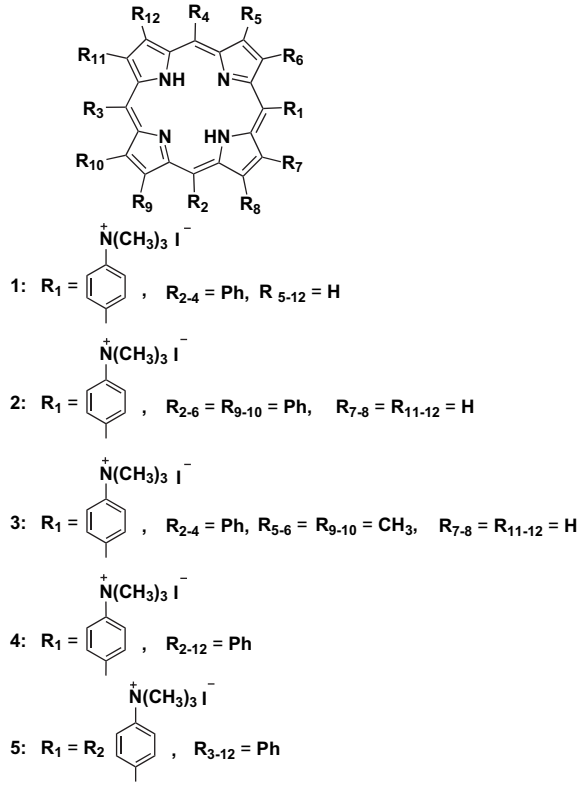
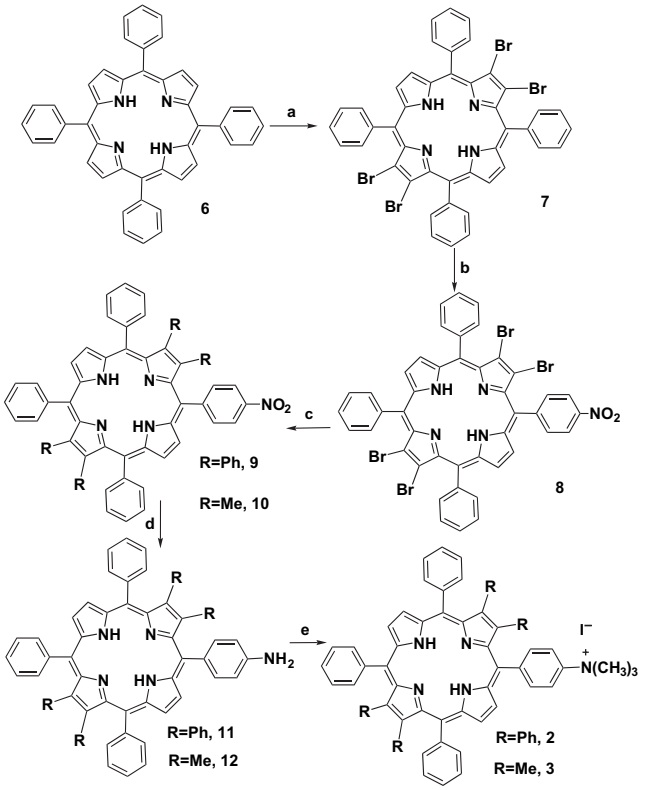


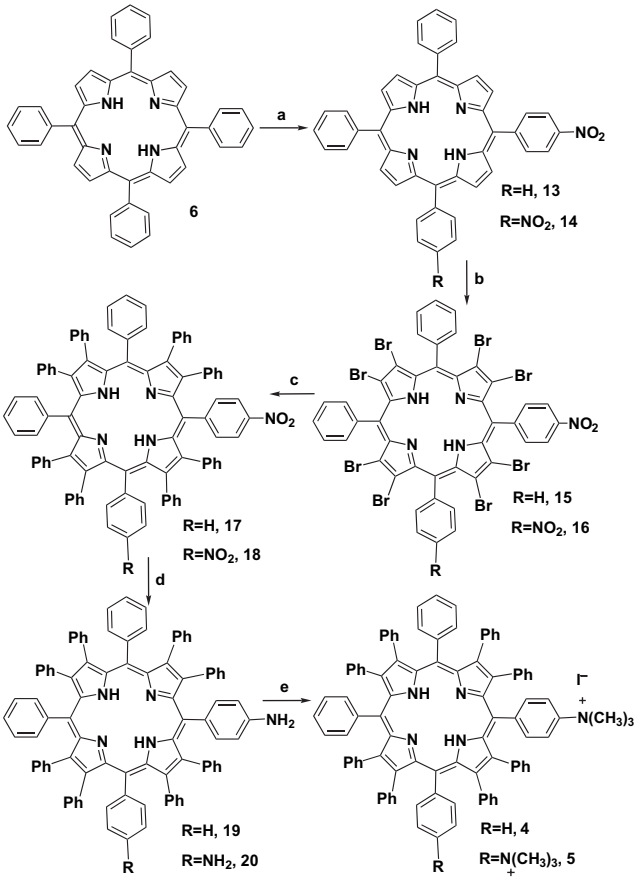
Scheme 1. Structures of the β-substituted cationic porphyrins.



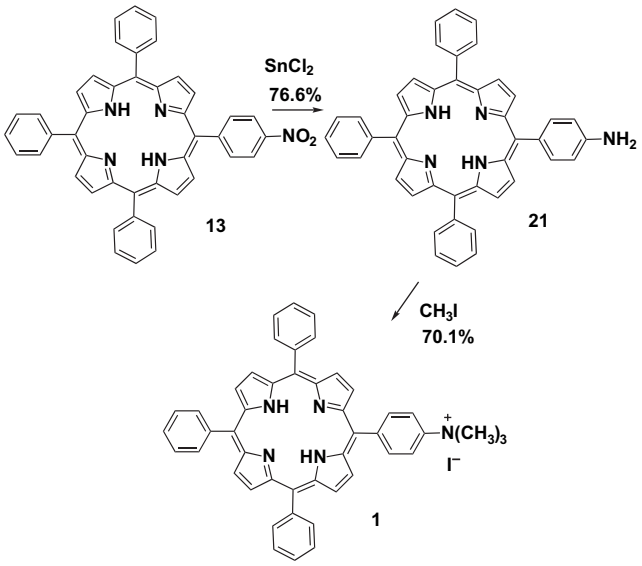
Scheme 2. Synthesis of quaternary ammonium porphyrins **2** and **3**: (a) NBS, $CHCl_3$, reflux 3 h, 75.5%; (b) fuming HNO_3, 5 °C, 3 h, 24.3%; (c) $MeB(OH)_2$, K_2CO_3, $Pd(Ph_3P)_4$, toluene, 95–105 °C, 3 days, 87.2%; $PhB(OH)_2$, Na_2CO_3, $Pd(Ph_3P)_4$, DMF, 12 h, 75.9%; (d) $SnCl_2\cdot 2H_2O$, concd HCl, 70 °C, 3 h (for Me, 87.7%), 12 h (for Ph, 82.6%); (e) CH_3I, DMF, 40 °C, 5 h, 61.1% (Me), 42.3% (Ph).

Suzuki coupling reaction,[30] porphyrins **9** and **10** were obtained in good yields.[31] $SnCl_2$ was chosen as the reducing agent to nitro porphyrin by Kruper[29] because it was moderate to the porphyrin ring but effective to nitro group. It took long time to convert the nitro group into amino group. After methylation of the amino group of compounds **11** and **12**, the desired β-tetrasubstituted cationic porphyrins **2** and **3** were obtained.

β-Octaphenyl substituted cationic porphyrins **4** and **5** were prepared from β-octabromo substituted porphyrins by following the general method shown in Scheme 3. Mono nitrated porphyrin **13** was obtained in high yield by mixing starting material TPP **6** with fuming HNO_3[29] with dinitrated porphyrin **14** obtained in low yield (<20%) by over nitration. Because of the strong oxidizing property of fuming HNO_3, many by-products would be formed as increase of the quantity of fuming HNO_3 and the reaction time. $NaNO_2$ in TFA is a moderate alternative nitrating agent. Dinitrated porphyrin was obtained easily and rapidly under this condition with good yield (>45%).[32] Octabromination of porphyrin **13** and **14** was realized by adding Br_2[28] because of its strong brominating ability. Nitro group increases the acidity of the proton on the central nitrogen, allowing the metalation at room temperature. After metalation by



Scheme 3. Synthesis of quaternary ammonium porphyrins **4** and **5**: (a) fuming HNO_3, 5 °C, 3 h (for 13, 50.5%); $NaNO_2$, TFA, rt, 90 s (for **14**, 45.7%); (b) (1) $Cu(OAc)_2$, $CHCl_3$, rt, 1 h; (2) Br_2, $CHCl_3$, rt, 1 day; (3) $HClO_4$, $CHCl_3$, rt, 12 h, 64.2% (**15**), 67.3% (**16**); (c) $PhB(OH)_2$, K_2CO_3, $Pd(Ph_3P)_4$, toluene, 95–105 °C, 7 days, 55.6% (**17**), 52.3% (**18**); (d) $SnCl_2\cdot 2H_2O$, concd HCl, 80 °C, 2 h, 60.8% (**19**); 58.4% (**20**); (e) CH_3I, DMF, 45 °C, 1 day, 85.5% (**4**), 78.9% (**5**).



Scheme 4. Synthesis of quaternary ammonium porphyrin **1**.

superfluous copper salt, Br_2 was added without removal of the remnant copper salt to complete the bromination of porphyrin **13** and **14**. This process prevents the demetalation and induce the completion of octabromination. After demetalation by $HClO_4$, β-octabromo porphyrins **15** and **16** were obtained. Seven days were taken to complete the coupling reaction. Because of the strong steric effect of the β-phenyl group and the poor water solubility, the nitro group on the *meso*-phenyl was difficult to be reduced in concd HCl. Therefore, addition of a small quantity of DMF as co-solvent in the reaction would solve this problem by increasing the solubility of porphyrin to obtain the desired product in good reduction yield. Finally, methylation of the amino groups in porphyrin **19** and **20** was performed by mixing with methyl iodide in DMF, respectively. Porphyrins **4** and **5** were obtained in the yields of 85.5 and 78.9%.

For control experiments, we synthesized cationic porphyrin **1** without β-substituents by mixing porphyrin **21**[29] with methyl iodide in DMF and the yield was 70.1% (Scheme 4).[33] New compounds have been fully characterized by ^{1}H NMR, HRMS, and UV.

2.2. Binding studies

UV–vis absorption spectroscopy, CD and fluorescence were used to determine the bindings of β-substituted cationic porphyrins **1–5** to DNA. In general, hypochromic shift and

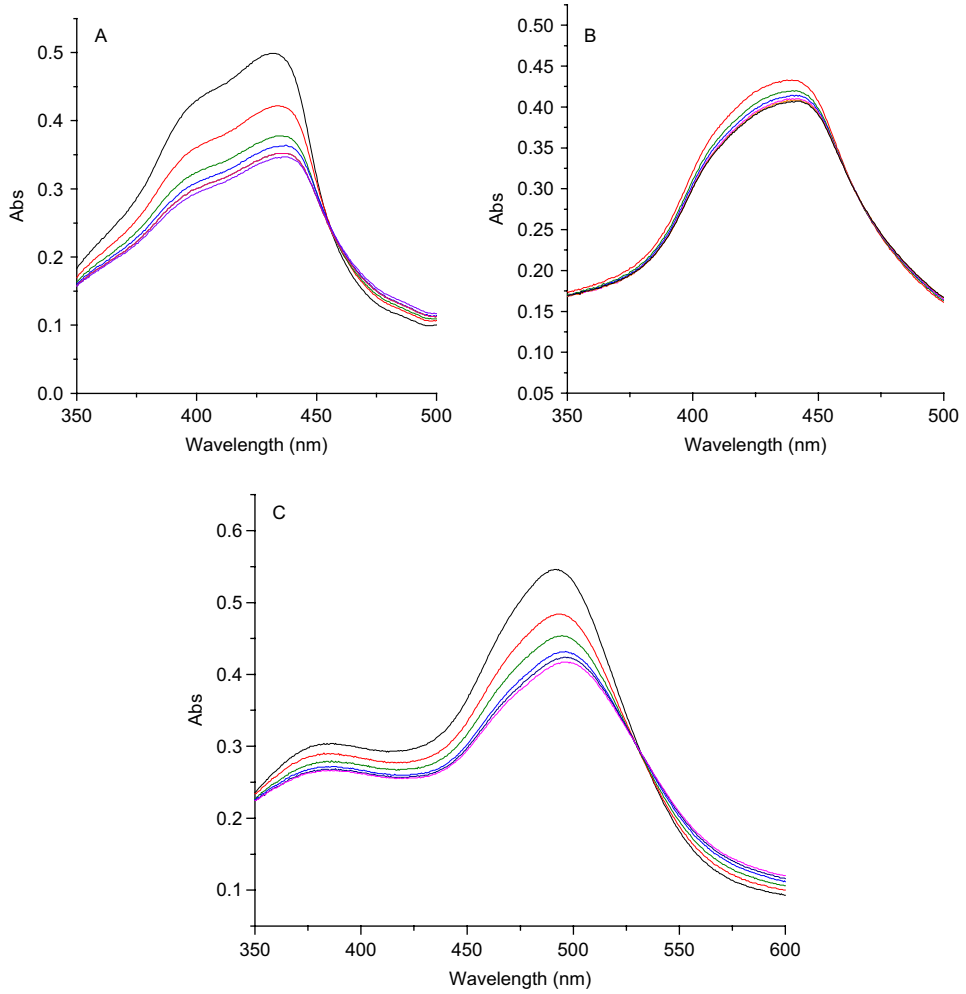


Figure 1. UV–vis absorbance spectra of 10 μM porphyrin **1** (A), **3** (B), and **5** (C) with increasing concentrations of calf thymus DNA base pair from 0, 1.16, 3.32, 4.48, 8.96, and 17.92 μM, respectively. (from upper to lower). The spectra were recorded in 0.05 M Tris–HCl, (pH 7.3), 2% DMF, containing 0.1 M NaCl.

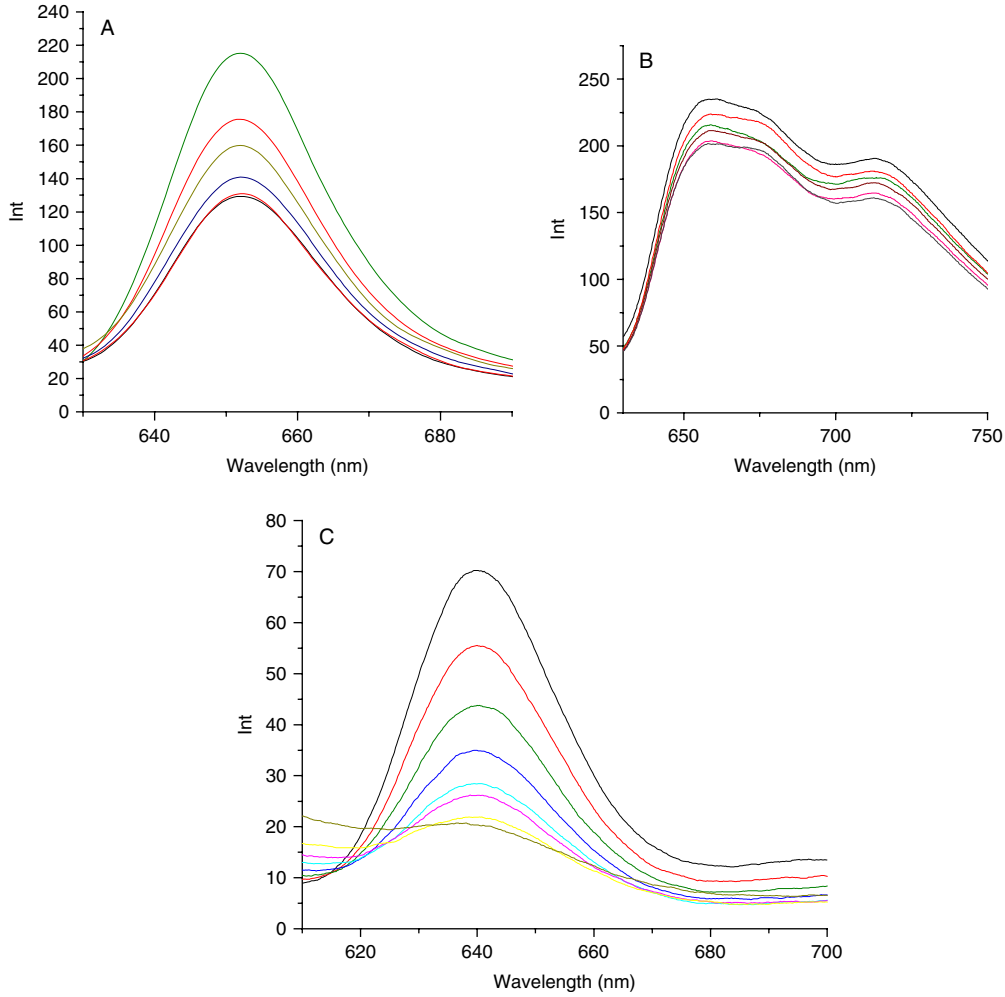


Figure 2. Fluorescence emission spectra of porphyrin **1** (A), 10 μM; **3** (B), 10 μM; **5** (C), 20 μM with increasing concentrations of calf thymus DNA. [Bp]=0, 1.16, 2.32, 3.48, 4.64, 6.96 μM for A and B. [Bp]=0, 0.58, 1.16, 1.74, 2.32, 2.90, 3.48, 4.06 μM. All curves decreased with the addition of DNA. The spectra were recorded in 3 mM Tris–HCl, 0.3 mM EDTA (pH=8.0), 2% DMF.

bathochromic shift in the Soret band, and the change of CD signal of porphyrins upon interaction with DNA indicate binding modes of porphyrins to DNA. The change of the fluorescence emission spectra of porphyrins upon mixing with DNA can point to the nature of the binding.[34]

2.3. UV–vis absorption spectroscopy

The effect of stoichiometric addition of calf thymus DNA on the UV–vis absorption spectra of porphyrins **1**, **3**, and **5** was shown in Figure 1. Increasing the concentration of CT DNA resulted in a hypochromic shift at the Soret band of porphyrins.

In Figure 1, isosbestic point can be observed in the titration curve for each porphyrin, which suggests the formation of a well-defined porphyrin-DNA complex.[35]

As for binding affinity, the most steric hindered porphyrin **5** with two positive charges exhibits the largest binding constant, suggesting that the number of positive charge is essential for the binding. The Columbic force between the cation of porphyrin and the phosphate of nucleotide could favor the interaction of porphyrin with DNA. It is interesting to find that β-phenyl substituted porphyrin **2** and **4** possess stronger binding ability than the less steric hindered β-methyl substituted porphyrin **3**. Probably, electronic effect, at this time, exerts its influence to compensate the disadvantage caused by steric effect on the binding.

2.4. Fluorescence studies[36]

The effect of stoichiometric addition of calf thymus DNA on the fluorescence spectrum of porphyrins **1**, **3**, and **5** is shown in Figure 2, these three porphyrins display distinct fluorescence emission spectrum.

β-Unsubstituted porphyrin **1** (Fig. 2A) displays an intermediate decrease by about 40.3% during the DNA titration, indicating intermediate binding ability compared to porphyrin **3** and **5**. β-Tetramethyl porphyrin **3** (Fig. 2B) exhibits a weak binding by the result of 15.1% maximum hypochromicity. Although the weakest among all the cationic porphyrins, the fluorescence of the dicationc β-octaphenyl porphyrin **5** (Fig. 2C) is quenched rapidly and dramatically (70.1%) by CT DNA, suggesting the strongest binding affinity, that is in good accordance with the result obtained by EB competitive method.

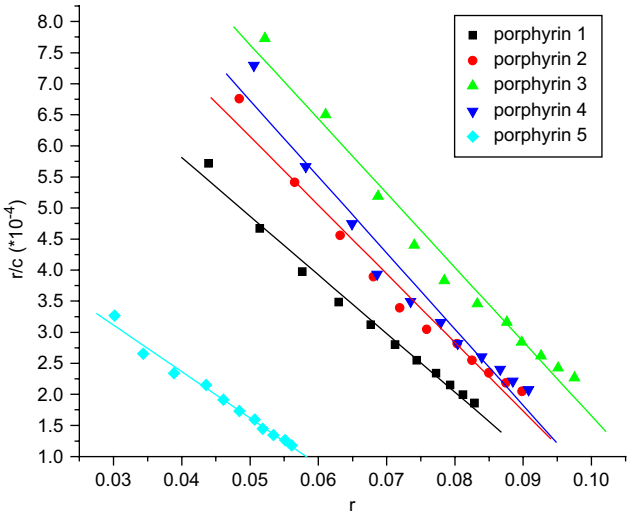


Figure 3. The Scatchard plot drawn by EB fluorescence competitive method. All [porphyrin]=2 μM, DNA [bp]=5.80 μM in 0.05 M Tris–HCl, (pH 7.3), 2% DMF, containing 0.1 M NaCl.

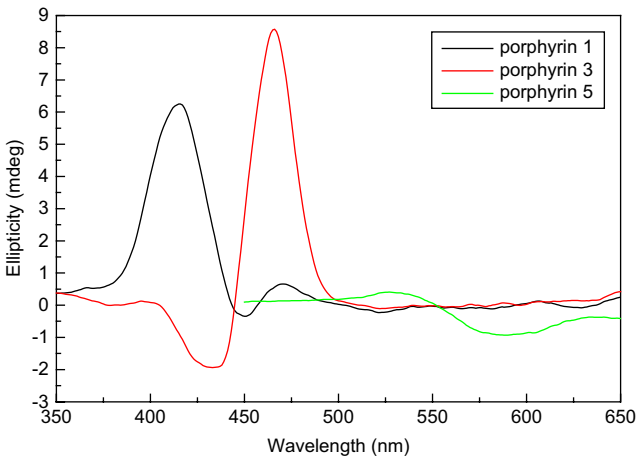


Figure 4. Induced CD spectra of 20 μM porphyrin **1**, **3**, and **5** in the presence of calf thymus DNA at R=0.5. The spectra were recorded in 3 mM Tris–HCl, 0.3 mM EDTA (pH 8.0), 4% DMF.

The binding affinity between porphyrin and DNA is expressed as shown by the apparent binding constants (K_{app}). It is calculated and determined by ethidium bromide (EB) fluorescence competitive method. In this method, EB is usually used as DNA probe and competed DNA binding sites with porphyrin. The results can be reflected by variation of fluorescence intensity. All measurements and calculation methods were performed by the reported methods.[37,38] The Scatchard plot is drawn by this method and had been shown in Figure 3.

Data of UV-titration and K_{app} are summarized in Table 1. As shown in Table 1, porphyrin **1** displays the largest hypochromicity of 32.0%, but its moderate bathochromic shift (6 nm) implies that this compound adopts a nonintercalation mode with CT DNA. The bathochromic shift values obtained for the interactions of porphyrin **2**, **3**, **4**, and **5** with calf thymus DNA are small and suggested that the binding mode of these four β-substituted porphyrins should not be intercalation.[39] Obviously steric hindrance of these porphyrins controls their binding mode with CT DNA.

Table 1. Values of hypochromicity, bathochromic, and binding constant

Por	% Hypochromicity with CT DNA	Bathochromic shift (nm)	K_{app}
1	32.0	6	2.00×10^5
2	10.7	<3	1.77×10^5
3	14.3	6	3.53×10^4
4	16.5	<3	1.01×10^5
5	24.0	5	7.54×10^5

2.5. Circular dichroism (CD) studies

To further clarify the binding mode, the induced CD spectra of the porphyrins were recorded at the presence of calf thymus DNA. Figure 4 shows the induced CD spectra of porphyrins **1**, **3**, and **5** bound to calf thymus DNA. In the case of duplex DNA, a positive induced CD band in the Soret region indicates its groove binding, and a negative induced CD band indicates intercalation.[6–8] Conservative CD signal is a signature of self-stacked porphyrins bound externally on the polymer surface.[6–8] Porphyrin **1** shows a strong positive peak and a weak positive peak (black); porphyrin **3** shows a strong positive peak and a weak negative peak (red), whereas porphyrin **5** shows conservative CD profiles (green). No ellipticity is in the CD of either porphyrins or DNA alone as control experiment. Porphyrins **1** and **3** should be located at the groove of calf thymus DNA and might interact with DNA in groove binding mode. Conservative CD signal of porphyrin **5** suggests its external binding mode on the DNA surface.

2.6. Structural optimization

The structural optimization of porphyrin **1**, **2**, **3**, **4**, and **5** was calculated by semiempirical AM1 method and shown in Figure 5. Porphyrin **1** without β-substituents has a nearly planar ring (Fig. 4A), whereas the steric effect of nonplanar porphyrins **2**, **3**, **4**, and **5** might block their intercalations into the base pair of DNA. Tetramethyl substituents at β-positions of porphyrin (Fig. 5C) might display moderate steric hindrance, and the porphyrin ring has a little distortion. Phenyl substituted porphyrins (Fig. 5B, D and E) show much more intense steric effect. Porphyrin **5** has eight phenyl groups at its β-position, so the steric hindrance effect is the largest and the porphyrin ring adopts a saddle conformation. Further computational research on the binding mode of these porphyrins with DNA is underway.

2.7. Photosensitized cleavage of DNA

A convenient way to test the ability of porphyrins to photodamage DNA is to measure the conversion of supercoiled DNA (form I) to form II.[40] The cleaving abilities of porphyrins **1**, **2**, **3**, **4**, and **5** to plasmid DNA (pBR322) upon illumination were investigated using agarose gel electrophoresis. All experiments were performed in buffer (pH=8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 2% DMF) and the samples were irradiated by high-pressure mercury lamp 50-W for 60 min at room temperature and the distance from the sample to the filament of the mercury lamp was 20 cm. Results of DNA cleavage are illustrated in Figure 6.

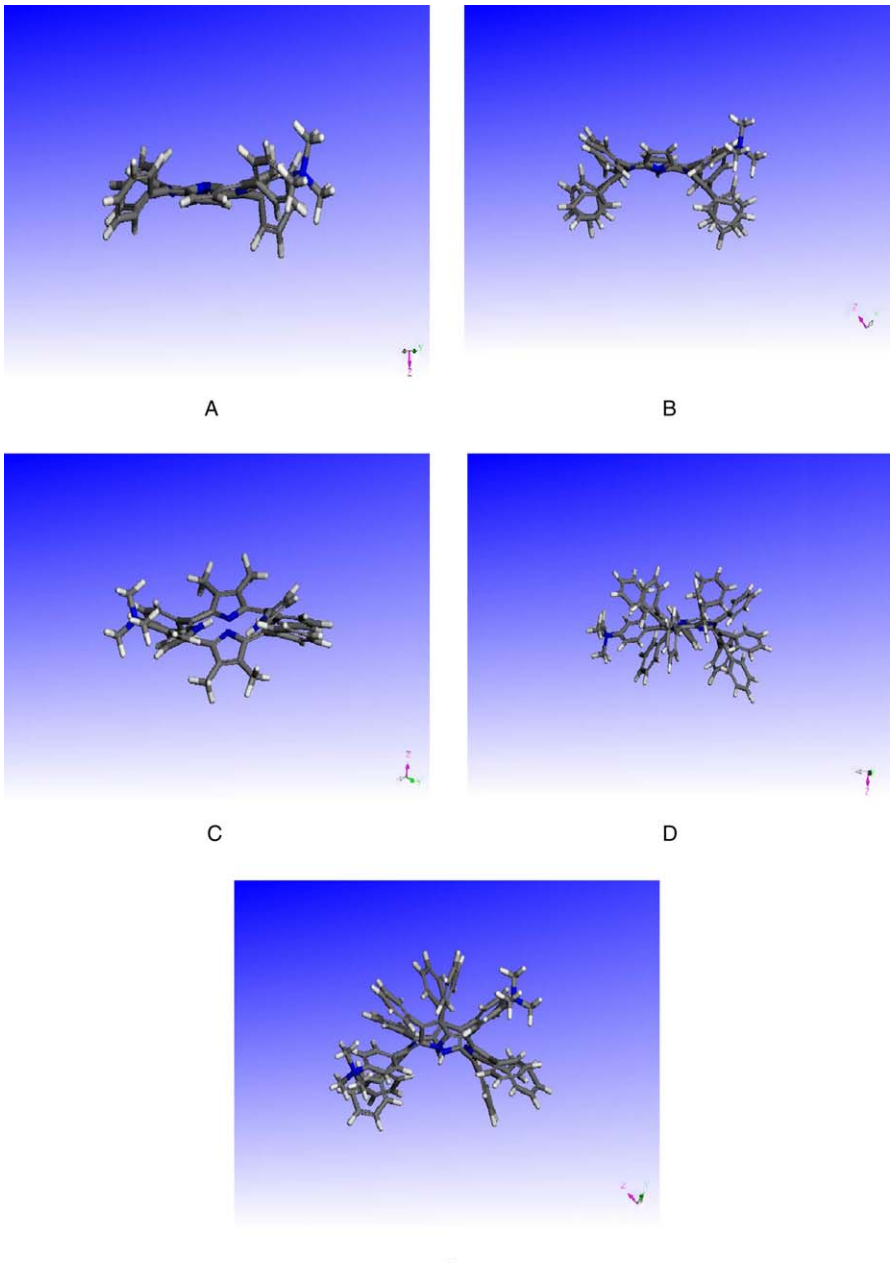


Figure 5. Structural optimization of porphyrin **1** (A), **2** (B), **3** (C), **4** (D), and **5** (E). The geometry of compounds was optimized by semiempirical AM1 method.

Control experiments indicated that no cleavage of DNA happened in the presence of DMF (Fig. 6A, lane 2). No cleavage of DNA was also observed if compound **3** was mixed with DNA without illumination (Fig. 6A, lane 3). In the presence of light, most cationic porphyrins, except β-nonsubstituted porphyrin **1**, were able to convert supercoiled DNA into form II at the concentration of 20 μM. Comparing with the mono-cationic β-substituted porphyrin **2**, **3**, and **4**, tetramethyl substituted porphyrin **3** had the strongest ability to cleave DNA. Tetraphenyl substituted porphyrin **2** had the weakest ability to cleave DNA among these three mono cationic porphyrins (Fig. 6A). Dicationic porphyrin **5** displayed stronger cleavage ability than all mono-cationic porphyrins. For porphyrin **5** (5 μM), the amount of DNA converted to form II in 60 min was almost 90%, but 50% for the strongest mono cationic porphyrin **3** at the same concentration (Fig. 6B). It suggests that charges might be one of important factor to interact with DNA.

3. Discussion

It is reported that the species responsible for causing DNA cleavage is believed to be singlet oxygen.[41–43] Our previous research[27] suggested that the rate of singlet oxygen production for β-nonsubstituted porphyrin and β-tetrasubstituted porphyrin was not significantly different. Measurement of singlet oxygen production by measuring the decomposition of 1,3-diphenylisobenzofuran (DPBF)[44–47] for these porphyrins **1**, **2**, **3**, **4**, and **5** has been carried out and the results are shown in Figure 7. The slope of the plots of bleached absorption versus illumination time is proportional to the rate of

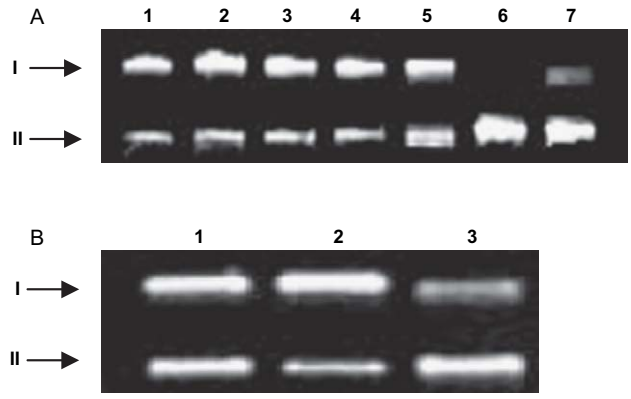


Figure 6. Agarose gel electrophoresis (1%) of supercoiled pBR322 DNA photosensitized with porphyrin **1**–**5**. 10 μL reaction mixtures contained 75 ng of plasmid DNA, 3 mM Tris–HCl, 0.3 mM EDTA (pH 8.0), 2% DMF. (A): lane 1: DNA+*hν* (60 min); lane 2: DNA+DMF (2%)+*hν* (60 min); lane 3: DNA+**3** (20 μM); lane 4: DNA+**1** (20 μM)+*hν* (60 min); lane 5: DNA+**2** (20 μM)+*hν* (60 min); lane 6: DNA+**3** (20 μM)+*hν* (60 min); lane 7: DNA+**4** (20 μM)+*hν* (60 min). (B): lane 1: DNA+**3** (5 μM)+*hν* (60 min); lane 2: DNA+**4** (5 μM)+*hν* (60 min); lane 3: DNA+**5** (5 μM)+*hν* (60 min).

production of singlet oxygen.[44–47] It is found that the rates of singlet oxygen production for these five porphyrins are not significantly different from each other (Table 2). Therefore, substitution at β-position of these porphyrins does not change the yields of singlet oxygen production. It implies that the photocleavge abilities of DNA by these differently β-substituted porphyrins are probably related to their conformations.

Many factors affect the porphyrin's interaction with DNA. In our case, the steric effect of the porphyrin skeleton and the number of charge on the porphyrin are the primary factors to influence their efficiency to bind with and cleave DNA. Since porphyrin **1** has the least hindrance and electronic effect compared to other cationic β-substituted porphyrins, it implies that the unfavor binding position between porphyrin

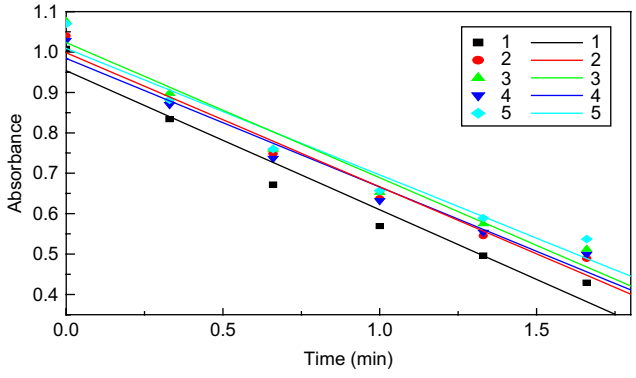


Figure 7. Decomposition of DPBF by compounds **1**, **2**, **3**, **4**, and **5**. Porphyrins (1 μM) and DPBF (100 μM) were irradiated in solution (3 mM Tris–HCl, 0.3 mM EDTA (pH 8.0), 2% DMF).

Table 2. The slopes (*S*) of the plots of bleached absorption of DPBF by photosensitization of **1**, **2**, **3**, **4**, and **5**

	1	**2**	**3**	**4**	**5**
S	−0.34	−0.33	−0.33	−0.32	−0.31

1 and DNA occurs, which induces the weakest ability to cleave DNA. Among porphyrins **2**, **3**, and **4**, all with one positive charge, porphyrin **3** with electron rich methyl groups induces more DNA cleavage under illumination although it has a weaker binding constant.

The number of charges on the porphyrin ring is another important factor to affect the cleavage ability of porphyrin. Obviously, the Columbic force between positive and negative charges strengthen the interaction of porphyrin and DNA.[48] Our experimental data indicates that, compared to other mono cationic porphyrins, dicationic porphyrin **5** upon DNA addition displays strong hypochromic shift in UV–vis absorbance spectra, novel hyperchromic shift in fluorescence emission spectrum and the best DNA cleavage ability. It may have the most efficient binding mode among these five porphyrins.

4. Conclusion

Five cationic porphyrins **1**, **2**, **3**, **4**, and **5** including four β-multisubstituted porphyrins **2**, **3**, **4**, and **5** have been synthesized and characterized. Their structures were optimized by semiempirical AM1 method. The results indicate that β-unsubstituted porphyrin has a planar ring structure while the substituents at β-position convert the porphyrin ring to distorted ones. The UV–vis absorption, fluorescence, CD, and DNA photocleavage evidence from the present study indicates that β-substituted cationic porphyrins bind to and photocleave DNA efficiently. CD spectra indicate that porphyrin **1** interacts with DNA in groove binding mode. Substituents on β-position of the porphyrins change their binding modes and intensify their interaction with DNA. Because of the differences on hypochromicity of Soret band by DNA addition and DNA photocleavage ability, octaphenyl substituents on β-position make porphyrin having a more efficient binding mode than the tetraphenyl substituted one. Our results also suggest that number of positive charge in the porphyrins plays an important role in the interaction with DNA. β-Octaphenyl dicationic porphyrin shows marked change both on UV–vis absorption and fluorescence intensity by adding calf thymus DNA and it therefore has the strongest interaction with DNA. Our further studies on the relationship of DNA site-specific binding with the β-substituted effects are undergoing.

5. Experimental

5.1. General experimental information

All chemicals were of reagent grade. $CHCl_3$ was washed by H_2O, followed by refluxing with CaH_2, distilled over CaH_2, and kept over 4-Å molecular sieves. NMR spectrum was recorded on a Varian Mercury-VX300 spectrometer at 300 MHz, MS was recorded on a Brucker Daltonics APE XII 47e and VG-707V-HF mass spectrometer. UV–vis spectra and binding constant measurement of DNA were carried out on UV–vis Spectrophotometer (Shimadzu). Fluorescence studies were performed on a JASCO fluorescence spectrometer. Circular Dichroism spectrum was recorded on a Jasco J-810 spectropolarimeter. In UV–vis spectra,

fluorescence, CD, and DNA photosensitized cleavage studies, 2–4% DMF was added to increase the solubility of porphyrins in water.

5.2. Synthesis of porphyrins

5.2.1. 2,3,12,13-Tetrabromo-5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (8). Porphyrin **7** (1 g, 1.08 mmol) was dissolved in chloroform and degassed by nitrogen for 10 min. Fuming nitric acid (0.87 mL, 1.3 g, 20.52 mmol) was added dropwise and the solution was stirred at 0–5 °C for 3 h. The reaction mixture was quenched by adding cold water and washed with H_2O. The organic layer was dried (Na_2SO_4) and evaporated to afford a crude product, which was purified by column chromatography on silica gel using $CHCl_3$–hexane (7:3) as eluent. Crystallization from CH_2Cl_2–MeOH gave porphyrin **8** in a yield of 24.3%. ^{1}H NMR ($CDCl_3$, 300 MHz): −2.80 (br s, 2H, pyrrole), 7.78–7.80 (m, 9H, 3,4,5-phenyl), 8.17 (d, J=5.4 Hz, 6H, 2,6-phenyl), 8.38 (d, J=8.4 Hz, 2H, 2,6-nitrophenyl), 8.65 (d, J=8.7 Hz, 2H, 3,5-nitrophenyl), 8.70–8.75 (m, 4H, β-pyrrole); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=439 (5.29), 505.5 (3.62), 535.5 (4.15), 644.0 (3.53), 687.0 (3.93). FABMS: $C_{44}H_{26}N_5O_2Br_4$ $[M+1]^+$: found 976, calcd 976.

5.2.2. 2,3,12,13-Tetraphenyl-5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (9). A 50-mL telfon-stoppered flask was charged with porphyrin **8** (113 mg 0.1 mmol), $PhB(OH)_2$ (141 mg, 1.1 mmol, 10 equiv), $(Ph_3P)_4Pd$ (54 mg, 0.05 mmol, 0.4 equiv), 2 M Na_2CO_3 (1.2 mL, 2.3 mmol, 20 equiv), and DMF (20 mL). The brown suspension was degassed by freeze-pump-thaw method (three cycles), and then was heated at 90–100 °C under N_2 for 12 h. The reaction mixture was worked up by extracting with 50 mL CH_2Cl_2 and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by column chromatography on silica gel using CH_2Cl_2 as eluent to give porphyrin **9** (80 mg, 0.08 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.14 (s, 2H, pyrrole), 6.76–6.80 (m, 20H, phenyl), 7.08–7.20 (m, 9H, 3,4,5-phenyl), 7.72 (s, 6H, 2,6-phenyl), 7.87 (d, J=7.5 Hz, 2H, 2,6-nitrophenyl), 7.94 (d, J=8.7 Hz, 2H, 3,5-nitrophenyl), 8.20–8.35 (m, 4H, β-pyrrole); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=436.0 (5.11), 498.5 (3.51), 529.0 (3.98), 642.5 (3.18), 683.5 (3.49); ESI HRMS for $C_{68}H_{46}N_5O_2$ $[M+H]^+$: found 964.3655, calcd 964.3646.

5.2.3. 2,3,12,13-Tetramethyl-5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (10). A 50-mL telfon-stoppered flask was charged with porphyrin **8** (105 mg 0.1 mmol), $MeB(OH)_2$ (65 mg, 1.1 mmol, 10 equiv), $(Ph_3P)_4Pd$ (50 mg, 0.04 mmol, 0.4 equiv), anhydrous K_2CO_3 (297 mg, 2.1 mmol, 20 equiv), and toluene (20 mL). The brown suspension was degassed by freeze-pump-thaw method (three cycles), and then was heated at 90–100 °C under N_2 for 3 days. The reaction mixture was worked up by extracting with 50 mL CH_2Cl_2 and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (200:1) as eluent to give porphyrin **10** (70 mg, 0.1 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.82 (s, 2H, pyrrole), 2.28–2.32 (m, 12H, CH_3), 7.58–7.68 (m, 9H, 3,4,5-phenyl), 7.98 (d, J=7.2 Hz, 6H, 2,6-phenyl), 8.13 (d, J=7.5 Hz, 2H, 2,6-nitrophenyl), 8.43 (d, J=7.5 Hz, 2H, 3,5-nitrophenyl), 8.16–8.39 (m, 4H, β-pyrrole); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=420.5 (5.22), 522.0 (3.95), 567.0 (3.08), 587.0 (3.51), 639.0 (3.18); ESI HRMS for $C_{48}H_{38}N_5O_2$ $[M+H]^+$: found 716.3009, calcd 716.3020.

5.2.4. 2,3,12,13-Tetraphenyl-5-(4-aminophenyl)-10,15,20-triphenylporphyrin (11) and 2,3,12,13-tetramethyl-5-(4-aminophenyl)-10,15,20-triphenylporphyrin (12). Nitroporphyrin (50 mg, 0.052 for **9**, 0.070 for **10**) was dissolved in concd HCl (10 mL) and degassed by nitrogen for 3 min followed by addition of $SnCl_2 \cdot H_2O$ (35 mg for **9**, 47 mg for **10**). The suspension was heated at 65–75 °C under N_2 for 3 h (over night for porphyrin **9**). The reaction mixture was quenched by cold water and concd aqueous ammonia (pH 8.0) and extracted by CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (100:1 for **9**, 100:2 for **10**) as eluent to give the aminoporphyrin **11** (yield 82.6%) and **12** (yield 87.2%).

5.2.5. 2,3,12,13-Tetraphenyl-5-(4-aminophenyl)-10,15,20-triphenylporphyrin (11). ^{1}H NMR ($CDCl_3$, 300 MHz): −1.99 (s, 2H, pyrrole), 6.53 (d, J=7.2 Hz, 2H, 3,5-aminophenyl), 6.84–6.89 (m, 20H, phenyl), 7.22 (d, J=11.7 Hz, 9H, 3,4,5-phenyl), 7.62 (d, J=7.8 Hz, 2H, 2,6-aminophenyl), 7.81 (d, J=6.6 Hz, 6H, 2,6-phenyl), 8.34 (s, 3H, β-pyrrole), 8.48 (s, 1H, β-pyrrole); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=439.0 (5.21), 514.5 (3.94), 533.0 (4.02), 643.5 (2.78), 688.0 (3.81); ESI HRMS for $C_{68}H_{48}N_5$ $[M+H]^+$: found 934.3896, calcd 934.3904.

5.2.6. 2,3,12,13-Tetramethyl-5-(4-aminophenyl)-10,15,20-triphenylporphyrin (12). ^{1}H NMR ($CDCl_3$, 300 MHz): −2.81 (s, 2H, pyrrole), 2.32 (s, 9H, CH_3), 2.43 (s, 3H, CH_3), 6.94 (d, J=8.1 Hz, 2H, 3,5-aminophenyl), 7.61 (d, J=7.2 Hz, 9H, 3,4,5-phenyl), 7.75 (d, J=8.1 Hz, 2H, 2,6-aminophenyl), 7.99 (d, J=5.7 Hz, 6H, 2,6-phenyl), 8.34 (s, 3H, β-pyrrole), 8.44 (s, 1H, β-pyrrole); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=422.5 (5.35), 503.5 (3.92), 521.0 (4.17), 588.0 (3.87), 650.0 (3.86); ESI HRMS for $C_{48}H_{40}N_5$ $[M+H]^+$: found 686.3277, calcd 686.3278.

5.2.7. 5-(*N*-trimethyl-4-aminiumphenyl)-10,15,20-triphenylporphyrin (1); 2,3,12,13-tetraphenyl-5-(*N*-trimethyl-4-aminiumphenyl)-10,15,20-triphenylporphyrin (2); and 2,3,12,13-tetramethyl-5-(*N*-trimethyl-4-aminiumphenyl)-10,15,20-triphenylporphyrin (3). Aminoporphyrin (40 mg, 0.079 for **21**, 0.043 for **11**, 0.058 for **12**) and CH_3I (1 mL) were dissolved in 10 mL of DMF. The reaction mixture was stirred at 45 °C for 5 h and then added 50 mL of CH_2Cl_2 followed by washing with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by crystallization from CH_2Cl_2–Et_2O to give salt **1** (yield 70.1%), **2** (yield 42.3%), and **3** (yield 61.6%).

5.2.8. 2,3,12,13-Tetraphenyl-5-(*N*-trimethyl-4-aminiumphenyl)-10,15,20-triphenyl porphyrin (2). Data provided in our previous paper.[27]

5.2.9. 2,3,12,13-Tetramethyl-5-(*N*-trimethyl-4-aminiumphenyl)-10,15,20-triphenyl porphyrin (3). ^{1}H NMR (DMSO-d_6, 300 MHz): −2.89 (s, 2H, pyrrole), 2.40 (s, 12H, CH_3), 3.88 (s, 9H, methyl), 7.75 (d, J=7.5 Hz, 9H, 3,4,5-phenyl), 7.90 (s, 2H, 3,5-aminophenyl), 8.00 (s, 6H, 2,6-phenyl), 8.30 (s, 4H, β-pyrrole), 8.37 (s, 2H, 2,6-aminophenyl); UV–vis (MeOH): λ_{max} (nm, log ε)=417.0 (5.20), 518.5 (3.90), 562.5 (3.26), 584.5 (3.51), 642.0 (3.30); ESI HRMS for $C_{51}H_{46}N_5$ [M^+-I^-]: found 728.3764, calcd 728.3748.

5.2.10. 2,3,7,8,12,13,17,18-Octabromo-5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (15). Porphyrin **13** (407 mg, 0.62 mmol) was dissolved in 100 mL of chloroform followed by the addition of $Cu(OAc)_2\cdot H_2O$ (617 mg, 3.1 mmol, 5 equiv). The reaction mixture was stirred at room temperature for 1 h. After complete conversion to copper porphyrin as detected by TLC, liquid Br_2 was added to the reaction mixture directly. The solution was continually stirred for about 1 day at room temperature. Aqueous solution of sodium thiosulfate was added to quench the reaction. After removal of the excessive bromine, the reaction mixture was washed by H_2O for several times and then 20 mL of perchloric acid was added to the solution. The reaction mixture was stirred for about 8 h for demetallization. The organic layer was separated and washed with aqueous and solution of sodium bicarbonate and water, and dried over anhydrous Na_2SO_4. The organic mixture was rotary evaporated to give a solid. The crude product was purified by column chromatography on silica gel using CH_2Cl_2 as eluent to give porphyrin **15** (512 mg, 0.4 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): 7.80–7.87 (m, 9H, 3,4,5-phenyl), 8.23 (d, J=6.0 Hz, 6H, 2,6-phenyl), 8.44 (d, J=8.1 Hz, 2H, 2,6-nitrophenyl), 8.62 (d, J=7.8 Hz, 2H, 3,5-nitrophenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=473 (5.17), 574 (3.84), 626 (4.00); ESI HRMS for $C_{44}H_{22}N_5O_2Br_8$ [M+H]$^+$: found 1291.5002 (4^{79}Br+4^{81}Br), calcd 1291.5158 (4^{79}Br+4^{81}Br).

5.2.11. 2,3,7,8,12,13,17,18-Octabromo-5,10-di(4-nitrophenyl)-15,20-triphenylporphyrin (16). Porphyrin **16** was synthesized by the same method as for porphyrin **15**. ^{1}H NMR ($CDCl_3$, 300 MHz): 7.70 (d, J=7.2 Hz, 6H, 3,4,5-phenyl), 8.20 (s, 4H, 2,6-phenyl), 8.42 (d, J=6.9 Hz, 4H, 2,6-nitrophenyl), 8.64 (d, J=8.4 Hz, 4H, 3,5-nitrophenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=475 (5.10), 573 (3.84), 625 (3.92); ESI HRMS for $C_{44}H_{21}N_6O_4Br_8$ [M+H]$^+$: found 1336.4890 (4^{79}Br+4^{81}Br), calcd 1336.5009 (4^{79}Br+4^{81}Br).

5.2.12. 2,3,7,8,12,13,17,18-Octaphenyl-5-(4-nitrophenyl)-10,15,20-triphenylporphyrin (17). A 100-mL telfon-stoppered flask was charged with porphyrin **15** (100 mg 0.08 mmol), $PhB(OH)_2$ (190 mg, 1.5 mmol, 20 equiv), $(Ph_3P)_4Pd$ (72 mg, 0.06 mmol, 0.8 equiv), anhydrous K_2CO_3 (428 mg, 3.1 mmol, 40 equiv), DMF (5 mL), and toluene (50 mL). The brown suspension was degassed by freeze-pump-thaw method (three cycles), and then was heated at 90–100 °C under N_2 for 7 days. The reaction mixture was worked up by extracting with 50 mL CH_2Cl_2 and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (200:1) as eluent to give porphyrin **17** (55 mg, 0.04 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): 6.67–6.68(m, 40H, phenyl), 7.12 (s, 9H, 3,4,5-phenyl), 7.89 (d, J=8.4 Hz, 2H, 3,5-nitrophenyl), 7.93–7.99 (t, 6H, 2,6-phenyl), 8.06 (d, J=9.0 Hz, 2H, 2,6-nitrophenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=469 (4.99), 562 (3.83), 617 (3.81); ESI HRMS for $C_{92}H_{62}N_5O_2$ [M+H])$^+$: found 1268.4884, calcd 1268.4898.

5.2.13. 2,3,7,8,12,13,17,18-Octaphenyl-5,10-di(4-nitrophenyl)-15,20-triphenylporphyrin (18). Porphyrin **18** was synthesized by the same method as for porphyrin **17**. ^{1}H NMR ($CDCl_3$, 300 MHz): 6.68–6.83 (m, 46H, phenyl and 3,4,5-phenyl), 7.53 (d, J=8.7 Hz, 4H, 3,5-nitrophenyl), 7.59 (d, J=7.5 Hz, 4H, 2,6-phenyl), 7.65 (d, J=8.7 Hz, 4H, 2,6-nitrophenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=493 (4.89), 586 (3.15), 642 (3.56); ESI HRMS for $C_{92}H_{61}N_6O_4$ [M+H]$^+$: found 1313.4755, calcd 1313.4749.

5.2.14. 2,3,7,8,12,13,17,18-Octaphenyl-5-(4-aminophenyl)-10,15,20-triphenyl porphyrin (19). Porphyrin **17** (30 mg, 0.02 mmol) was dissolved in 6 mL of a mixed solvent (DMF–concd HCl=1:5). The mixture was degassed by nitrogen for 3 min followed by addition of $SnCl_2\cdot H_2O$ (16 mg, 0.07 mmol). The suspension was heated to 80 °C under N_2 for 3 h. The reaction mixture was quenched by adding cold water and concd aqueous ammonia (pH 8.0) and extracted by CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by column chromatography on silica gel using CH_2Cl_2–MeOH (100:1) as eluent to give porphyrin **19** (18 mg, 0.01 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz): 6.06 (d, J=8.1 Hz, 2H, 3,5-aminophenyl), 6.68–6.84 (m, 49H, phenyl and 3,4,5-phenyl), 7.38 (d, J=8.1 Hz, 2H, 2,6-aminophenyl), 7.59 (d, J=6.9 Hz, 6H, 2,6-phenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=490 (4.88), 576 (3.11), 642 (3.51); ESI-MS for $C_{92}H_{63}N_5$ [M]: found 1238.7, calcd 1238.5; ESI HRMS for $C_{92}H_{64}N_5$ [M+H]: 1238.5156, calcd 1238.5158.

5.2.15. 2,3,7,8,12,13,17,18-Octaphenyl-5,10-di(4-aminophenyl)-15,20-triphenylporphyrin (20). Porphyrin **20** was synthesized by the same method as for porphyrin **19**. ^{1}H NMR ($CDCl_3$, 300 MHz): 6.05 (d, J=7.5 Hz, 4H, 3,5-aminophenyl), 6.68–6.74 (m, 46H, phenyl and 3,4,5-phenyl), 7.35 (d, J=7.5 Hz, 4H, 2,6-aminophenyl), 7.55 (d, J=6.6 Hz, 4H, 2,6-phenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=501 (4.89); ESI HRMS for $C_{92}H_{64}N_6$ [M+H]$^+$: found 1253.5320, calcd 1253.5224.

5.2.16. 2,3,7,8,12,13,17,18-Octaphenyl-5-(*N*-trimethyl-4-aminiumphenyl)-10,15,20-triphenylporphyrin (4). Porphyrin **19** (18 mg, 0.01 mmol) and CH_3I (1 mL) were dissolved in 10 ml DMF. The reaction mixture was stirred at 45 °C for 1 day and then added 50 mL of CH_2Cl_2 followed by washing with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and rotary evaporated to dryness. The crude product was purified by crystallization using DMF–CH_2Cl_2–Et_2O as eluent to give the salt **4** (17 mg, 0.01 mmol). ^{1}H NMR (DMSO-d_6, 300 MHz): 3.54–3.59 (m, 9H, methyl), 6.66–6.99 (m, 40H, phenyl), 7.06–7.21 (m, 9H, 3,4,5-phenyl), 7.31–8.48 (m, 10H, phenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=498 (4.97), 648 (3.82); ESI

HRMS for $C_{95}H_{70}N_5$ [M−I]: found 1280.5626, calcd 1280.5616.

5.2.17. 2,3,7,8,12,13,17,18-Octaphenyl-5,10-di(*N*-trimethyl-4-aminiumphenyl)-15,20-diphenylporphyrin (5). Porphyrin **5** was synthesized by the same method as for porphyrin **4**. ^{1}H NMR (DMSO-d_6, 300 MHz): 3.50 (s, 9H, methyl), 3.54 (s, 9H, methyl), 6.69–6.86 (m, 40H, phenyl), 7.01–7.10 (m, 6H, 3,4,5-phenyl), 7.26–8.11 (m, 12H, phenyl); UV–vis ($CHCl_3$): λ_{max} (nm, log ε)=493 (4.94), 649 (3.92); ESI-MS for $C_{98}H_{78}N_6$ [(M−2I)/2]: found 669.8, calcd 669.8; ESI HRMS for $C_{98}H_{78}N_6$ [(M−2I)/2]: found 669.3138, calcd 669.3133.

5.3. UV–vis spectroscopy-titration and fluorescence-titration studies

Titration of the porphyrins (10 μM) with calf thymus DNA was performed at room temperature in a buffer (3 mL, pH 8.0, 3 mM Tris–HCl, 0.3 mM EDTA) containing 2% DMF (with 0.1 M NaCl for UV–vis). A stock solution of CT DNA was prepared and stored in distilled water. The total concentration of DNA in the system was from 1 μM to 20 μM.

5.4. Circular dichroism (CD) studies

The spectral bandwidth was 2 nm, and the cell length was 1 cm. Calf thymus DNA was added to the porphyrins (20 μM), and after an incubation period of 15 min, the samples were scanned in the visible region. *R* value, ratio of concentration of the porphyrins to concentration of DNA, was 0.5. Baseline was corrected using the same buffer before scanning the samples. The spectra were recorded in 3 mM Tris–HCl, 0.3 mM EDTA (pH 8.0), 2% DMF.

5.5. Gel electrophoresis

Photocleavage of supercoiled pBR322 (0.15 μg) was performed with the porphyrins in buffer (pH 8.0, 3 mM Tris–HCl, 0.3 mM EDTA, 2%DMF). Samples were exposed to a 50-W high-pressure mercury lamp, which was placed 20 cm away from the samples at room temperature for 60 min and loaded onto 0.9% agarose gel containing 0.1 μg/mL ethidium bromide. The agarose gels were run at 130 V in TAE buffer. The gels were observed and photographed under UV light.

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Preparation of thiocyanates and isothiocyanates from alcohols, thiols, trimethylsilyl-, and tetrahydropyranyl ethers using triphenylphosphine/2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)/*n*-Bu_4NSCN system

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Abstract—A combination of triphenylphosphine (PPh_3) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) provides a safe and easily available mixed reagent system for the conversion of 1° and 2° alcohols, thiols, trimethylsilyl-, and tetrahydropyranyl ethers to their corresponding thiocyanates and the 3° ones to isothiocyanates in good to high yields.

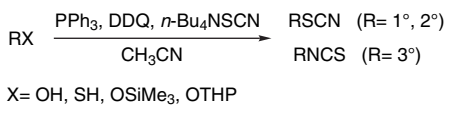
1. Introduction

The Mitsunobu reaction has been widely used for the displacement of alcohols by different nucleophiles.[1–3] In this reaction, in which a mixture of triphenylphosphine, diethyl azodicarboxylate (DEAD), and a nucleophile is used, the phosphine and the azodicarboxylate accepts the oxygen and the hydrogen atoms, respectively, to give triphenylphosphine oxide and a hydrazine derivative with the consequent replacement of the hydroxyl group by the nucleophile. Among these transformations, the conversion of a hydroxyl group to a thiocyanate functionality is widely used for the preparation of heterocycles.[4,5] Recently, we reported a new application for the Mitsunobu reaction and studied the reaction of alcohols, thiols, carboxylic acids, trimethylsilyl ethers, and carboxylates with PPh_3/DEAD/NH_4SCN.[6]

Prior to this work, we also reported on the use of in situ generated $PPh_3(SCN)_2$ for the conversion of alcohols,[7b] thiols,[8] and also trimethylsilyl ethers[9] to alkyl thiocyanates. Apart from the above mentioned methods, the use of $KSCN{\cdot}CuBr_2$,[10] solid-supported potassium thiocyanate,[11] and thiocyanate ion[12] preferably in the presence of phase-transfer agents[13–16] are widely used for the preparation of thiocyanates from alkyl halides.

2. Results and discussion

Recently we reported the use of DDQ as a suitable replacement for DEAD for the preparation of alkyl halides, nitriles, and azides from alcohols.[17] Very recently, the use of DDQ for the preparation of isocyanides from alcohols and thiols was also reported.[18] To reduce the problems encountered in thiocyanation reactions using the Mitsunobu reagent system[6] or $PPh_3(SCN)_2$,[7–9] e.g., DEAD is expensive, is not easily available, and may explode on heating and the poisonous $PPh_3(SCN)_2$ should be prepared freshly before use, we now report that the combination of PPh_3 and DDQ in the presence of *n*-Bu_4NSCN, which are readily available solid reagents, offers a simple and safe method for the conversion of primary and secondary alcohols as well as thiols, trimethylsilyl-, and tetrahydropyranyl ethers into their corresponding thiocyanates and tertiary systems into their isothiocyanates in good to high yields (Scheme 1).



Scheme 1.

We first carried out a comparative study on the possibility of using different electron-deficient reagents such as 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), 2,3,5,6-tetrachlorobenzoquinone (*p*-chloranil), tetracyanoethylene (TCNE), tetraphenylcyclopentadienone (tetracyclone), 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) as well as diethyl acetylenedicarboxylate (DEACD) in conjunction with PPh_3

Keywords: Thiocyanation; Isothiocyanation; Alcohol; Thiol; Trimethylsilyl ether; Tetrahydropyranyl ether; Triphenylphosphine; DDQ.
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and *n*-Bu_4NSCN for the conversion of benzyl alcohol to benzyl thiocyanate as a model reaction. The results obtained from this study (Table 1) show that the combination of PPh_3/DDQ/*n*-Bu_4NSCN and PPh_3/*p*-chloranil/*n*-Bu_4NSCN provides the most suitable reagent systems for this transformation and benzyl thiocyanate was formed in quantitative yield. Of these two reagent systems, the use of DDQ provides a much shorter reaction time (almost immediately) compared to using *p*-chloranil (8 h). The combination of PPh_3/DEAD/*n*-Bu_4NSCN produced only 20% of benzyl thiocyanate after 24 h and other reagent systems none. As we reported already,[6] this reaction occurs using the combination of DEAD, PPh_3, and NH_4SCN. However, apart from the problems encountered using DEAD, ammonia is also produced in the reaction, which could be harmful to some base-sensitive functional groups.

Table 1. Conversion of benzyl alcohol to benzyl thiocyanate using *n*-Bu_4NSCN in the presence of Ph_3P and different reagents in acetonitrile at room temperature

Entry	Reagent[a]	Time (h)	Conversion %[b]
1	DDQ	<1 min	100
2	*p*-Chloranil	8	100
3	DEAD	24	20
4	DEACD	24	0
5	TCNE	24	0
6	Tetracyclone	24	0
7	PTAD	24	0

[a] The stoichiometric ratio of PPh_3/DDQ/*n*-Bu_4NSCN/ROH is 2:2:2:1.
[b] GC yield using an internal standard.

We also used NH_4SCN instead of *n*-Bu_4NSCN but the reaction did not finished after 24 h and benzyl thiocyanate was formed only in 30% yield.

The order of addition of reagents is also very important. The mixture of PPh_3 and DDQ is first prepared in acetonitrile and *n*-Bu_4NSCN is added to this mixture followed by the addition of alcohol.

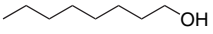
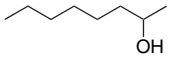
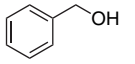
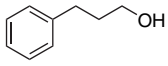
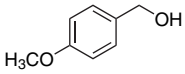
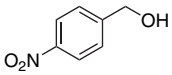
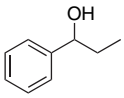
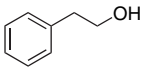
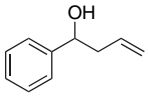
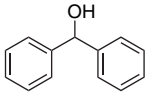
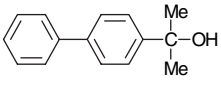
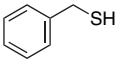
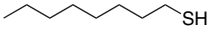
Using this method, primary alcohols are converted to their corresponding alkyl thiocyanates without the formation of any isothiocyanates, but secondary alcohols give also some minor amounts of isothiocyanates as a side-product. In the case of a tertiary alcohol, the isothiocyanate is formed as the major product together with small amounts of eliminated product.

^{13}C resonance of the –SCN and –NCS groups at ~111 and ~145 ppm, respectively, are very characteristic for thiocyanate and isothiocyanate functionalities.[7b,9,19] The formation of isothiocyanates does not occur through the isomerization of the corresponding thiocyanates since, when we heated the crude reaction mixture obtained from the reaction of 1-phenylpropanol at 25 °C to 60–70 °C, no change in the distribution of the isomeric products (RSCN, RNCS) was observed. Thus, their formation is due to the ambident nature of the NCS^- nucleophile and this is in agreement with the reported data in the literature.[20,21] Lowering the reaction temperature from 25 to 0 °C had also no significant effect on this distribution.

We then applied this method for the conversion of thiols to their corresponding thiocyanates. The results are shown in Table 2. In these reactions, thiocyanates were obtained in high yield without the formation of any disulfides through the dimerization of thiols.

Trimethylsilyl- and tetrahydropyranyl ethers are popular forms of protected hydroxyl groups, which are practical precursors for the preparation of many other compounds and their controlled transformation to other functional groups has been considered as an interesting goal in organic synthesis.[22,23]

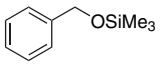
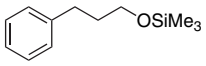
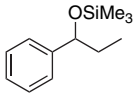
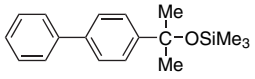
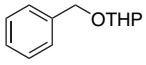
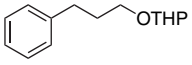
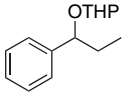
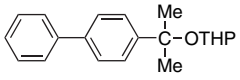
Table 2. Conversion of alcohols and thiols to alkyl thiocyanates or isothiocyanates

Entry	RX	RSCN/RNCS[a]	Time	Isolated yield % (ref)
1		100/0	5 min	90 (19a)
2		74/26	15 min	88[b] (20)
3		100/0	<1 min	95 (7b)
4		100/0	<1 min	92 (7b)
5		100/0	<1 min	92 (7b)
6		100/0	<1 min	90 (19)
7		61/39	<1 min	94[b] (7b)
8		100/0	<1 min	90 (7b)
9		60/10	<1 min	55[b,c] (19)
10		82/18	75 min	92[b] (7b)
11		0/80	20 h	68[c,d] (19)
12		100/0	45 min	90 (7b)
13		100/0	1 h	87 (19a)
14		80/20	90 min	90[b] (7b)

[a] GC and NMR yield using an internal standard.
[b] Mixture of thiocyanate and isothiocyanate was obtained.
[c] Eliminated product (15–20%) was also produced.
[d] This reaction was performed under reflux.

Trimethylsilyl- and tetrahydropyranyl ethers reacted efficiently with the same mixed reagent system and produced the corresponding thiocyanates or isothiocyanates in high yields. The results are shown in Table 3.

Table 3. Conversion of silyl- and THP-ethers to thiocyanates or isothiocyanates in acetonitrile[a]

Entry	RX	RSCN/RNCS[b]	Time	Isolated yield %
1		100/0	<1 min	91
2		100/0	<1 min	93
3		78/22	10 min	90
4		0/80	24 h	72[c,d]
5		100/0	13 h	92
6		100/0	15 h	88
7		77/23	20 h	90
8		0/85	40 h	78[c]

[a] The reactions of silyl ethers were performed at room temperature and THP-ethers under reflux conditions.
[b] GC and NMR yield using an internal standard.
[c] Eliminated products are also produced.
[d] Reflux condition.

Similar to the reaction of alcohols and thiols, primary silyl- and tetrahydropyranyl ethers produced exclusively the corresponding thiocyanates. In the case of secondary alcohols and thiols, thiocyanates were also formed as the major products together with minor amounts of the corresponding isothiocyanates, however, tertiary systems produced only isothiocyanates and small amounts of the eliminated product.

In conclusion, the use of DDQ instead of DEAD in conjunction with PPh_3 and *n*-Bu_4NSCN as safe, available, and easy handling reagents offers a simple, novel, and convenient method for the conversion of wide varieties of alcohols, thiols, trimethylsilyl-, and tetrahydropyranyl ethers to their corresponding thiocyanates or isothiocyanates.

3. Experimental

All the solvents and reagents were purchased from Fluka or Merck chemical Companies. The products were purified by column or prep. TLC techniques and identified by comparison of their spectral data with those of known compounds.[7b,9,19] FTIR spectra were recorded on a Shimadzu DR-8001 spectrometer. NMR spectra were recorded on a Brucker Avance DPX 250 MHz instrument. Mass spectra were recorded on a Shimadzu GC–MS-QP 1000PX.

3.1. Typical procedure for the conversion of benzyl alcohol to benzyl thiocyanate

A mixture of DDQ (2.0 mmol, 0.454 g) and PPh_3 (2.0 mmol, 0.524 g) in dry CH_3CN (5 ml), was stirred at room temperature for 5 min. Then, *n*-Bu_4NSCN (2.0 mmol, 0.6 g) was added and while the reaction mixture was stirring, benzyl alcohol (1.0 mmol, 0.108 g) was added. GC analysis showed the immediate completion of the reaction. The solvent was evaporated. Column chromatography of the crude mixture on silica-gel using *n*-hexane–ethyl acetate (3:1) as eluent gave benzyl thiocyanate as pale yellow crystals, 0.134 g, 95% yield (mp 40 °C, lit.[19a] mp 39–40 °C). IR (–SCN): 2155 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.3 (5H, s), 4.1 (2H, s); ^{13}C NMR ($CDCl_3$): δ (ppm) 134.8, 132.6, 129.6, 129.4, 112.2, 38.7.

3.2. Typical procedure for the conversion of trimethyl-(1-phenyl-propoxy)-silane to (1-thiocyanato-propyl)-benzene

To a flask containing a stirring mixture of DDQ (2.0 mmol, 0.454 g) and PPh_3 (2.0 mmol, 0.524 g) in dry CH_3CN (5 ml), was added *n*-Bu_4NSCN (2.0 mmol, 0.6 g) at room temperature. Then, trimethyl-(1-phenyl-propoxy)-silane (1 mmol, 0.2 g) was added to the mixture. The progress of the reaction was followed by GC. After completion of the reaction, solvent was evaporated. Column chromatography of the crude mixture on silica-gel using *n*-hexane–ethyl acetate (3:1) as eluent gave (1-thiocyanato-propyl)-benzene (Table 3, entry 3) as the main product with 75% yield. IR (–SCN): 2160 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 0.96 (3H, t), 2.1 (2H, m), 4.6 (1H, t), 7.2–7.4 (5H, m); ^{13}C NMR ($CDCl_3$): δ (ppm) 137.1, 127.8, 127.7, 124.7, 111.9, 54.2, 31.3, 11.1.

3.3. Typical procedure for the conversion of 2-(1-biphenyl-4-yl-ethoxy)-tetrahydro-pyran to 4-(1-isothiocyanato-1-methyl-ethyl)-biphenyl

To a refluxing solution of PPh_3 (2 mmol, 0.524 g), DDQ (2 mmol, 0.454 g), and *n*-Bu_4NSCN (2 mmol, 0.6 g) in dry CH_3CN (5 ml) was added 2-(1-biphenyl-4-yl-ethoxy)-tetrahydro-pyran (1 mmol, 0.296 g). After 40 h, the solvent was evaporated. Column chromatography on silica-gel using *n*-hexane–ethyl acetate (3:1) as eluent gave 4-(1-isothiocyanato-1-methyl-ethyl)-biphenyl (Table 3, entry 8) in 78% yield.

IR (–NCS): 2085 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 1.8 (6H, s), 7.1 (2H, d), 7.5 (5H, m), 8.3 (2H, d); ^{13}C NMR ($CDCl_3$): δ (ppm) 158.9, 145.9, 138.1, 132.2, 130.8, 129.7, 128.2, 124.9, 120.8, 56.0, 19.9.

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A DFT study of the Diels–Alder reaction between methyl acrolein derivatives and cyclopentadiene. Understanding the effects of Lewis acids catalysts based on sulfur containing boron heterocycles

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Abstract—The effects of Lewis acid catalysts based on sulfur containing boron heterocycles on the Diels–Alder reactions of two methyl acroleins with cyclopentadiene have been studied using DFT methods. These reactions take place along highly asynchronous concerted processes. While the reaction with crotonaldehyde leads to the expected *endo* adduct, the reaction with methacrolein leads to *exo* one in agreement with the experiments. The catalytic effect can be explained through the analysis of the electrophilicity index (ω) of the reagents, and the molecular structure of the corresponding transition structures.

1. Introduction

The Diels–Alder (DA) reaction is a powerful tool employed frequently in the synthesis of six-membered ring systems with excellent regio and stereoselective control.[1] The remarkable importance of the DA reaction in the synthesis of natural products and physiologically active molecules led to an upsurge in research activities aimed at developing newer methods to improve yields and selectivities of the [4+2] cycloaddition reactions.[1] In particular, the discovery in 1960 by Yates and Eaton,[2] that the Lewis acid (LA) $AlCl_3$ strongly accelerates the DA reaction encouraged the development of DA reactions of poorly reactive dienophiles[3] and several other carbon–carbon forming bond reactions.[4] LA catalyzed DA reactions can be considered one of the most investigated area in organic synthesis and many regio-, chemo-, diastereo-, and enantioselective DA reactions catalyzed by various LAs have been developed to date.[5]

The effect of LA catalysis in DA reactions has been the object of several theoretical[6–18] and experimental[19] studies employing semiempirical,[6,7,15,16] ab initio[8–12,17,18] and density functional theory (DFT) methods.[13,14a–f] They predict that the catalyst produces transition states (TSs) with significant zwitterionic character and it is related to the greater asynchronicity in the catalyzed reaction, as compared with the uncatalyzed one.[14a] Even, recent studies have also showed that cycloaddition reactions in the presence of LA can occur through a stepwise mechanism.[14b,c,f,16–18]

Carbonyl compounds can undergo DA reactions and the activation of ketones or aldehydes as poor dienophiles is achieved by coordination of LAs to oxygen atom of the carbonyl group, lowering the LUMO energy. Recently, some of us have reported that the ^{13}C NMR chemical shifts of the dienophile carbonyl groups show a deshielding effect of approximately 4–7 ppm, after the addition of LA catalyst on heterocycles containing boron, suggesting strong coordination and a concomitant activation effect in their chemical reactivity associated to DA reactions.[15] As a simple alternative to LA catalyst Rawal and Huang[20] have reported a significantly higher reaction rate for a hetero DA reactions involving aldehydes in Cl_3CH than in other aprotic organic solvents. To understand these solvent-assisted reactions, some of us have performed a theoretical investigation to assess how hydrogen bond-formation between Cl_3CH and the carbonyl oxygen atom influences the chemical reactivity of the hetero DA, making the carbonyl group a stronger hetero-dienophile.[21]

Keywords: Diels–Alder reactions; Lewis acid catalysts; Molecular mechanisms; Electrophilicity index; DFT analysis.
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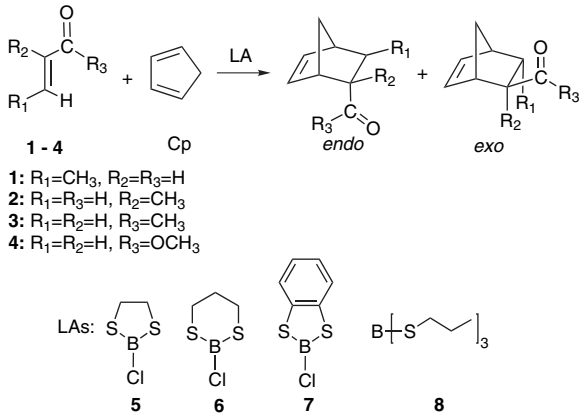
LAs catalysis based on catecholborane moiety have used in different types of DA reactions.[15,22b,c] In particular, Howarth et al.[22a] have carried out an extensive experimental study on the DA reactions between cyclopentadiene (Cp) with crotonaldehyde (**1**), methacrolein (**2**), methylvinyl ketone (**3**), and methylmethacrylate (**4**), that includes the presence of a wide range of LA catalysts based on heterocycles containing boron and sulfur atoms, **5**–**7**, and trialkythioboranes, **8** (see Scheme 1). Surprisingly, the DA reaction between methacrolein and methylmethacrylate lead to the unexpected *exo* adducts.[22a] These types of reactions are interesting, offering the possibility to carry out a complementary theoretical analysis to a better understanding about the key role of the LAs catalysts as well as challenging to study computationally due to the presence of competitive pathways, *endo* and *exo*. Thus, the present theoretical study has been undertaken to shed insight into the role of the sulfur containing boron heterocycle **5** as LA catalyst in the DA reactions between crotonaldehyde and methacrolein and Cp; in addition, the *endo* and *exo* reactive channels have been explored in order to explain the experimentally observed *endo*/*exo* stereoselectivity (see Schemes 2 and 3) using **5**, 2-chloro-1,3,2-dithioborolane, as LA catalyst. We have performed a systematic DFT study through the localization of the stationary points (reactants, TSs, and products) along the reaction pathway on the potential energy surface (PES) for these cycloadditions.



Scheme 1.

The article is structured as follows. The selected computational techniques and methodologies as well as the details of the model systems are listed in next section together with a brief theoretical background of the global electrophilicity index. Next, the results are presented and discussed on the basis of the generated trends in terms of global electrophilicity indexes and the analysis of stationary points on PES. This analysis allows to rationalize and to explain the experimental observations. Finally, in conclusions section, the net outcome of the work is summarized.

2. Computing procedures and model systems

Theoretical calculations based on DFT methods[23] have emerged as alternatives to traditional ab initio calculations in the study of structure and reactivity of chemical systems. DA reactions and related cycloadditions have been the object of several DFT studies showing that those that include gradient corrections and a hybrid functional, such as B3LYP,[24] with the 6-31G* basis set,[25] lead to activation energies in good agreement with the experimental results.[26]

The PESs have been calculated in detail to ensure that all relevant stationary points have been located and properly characterized. Stationary points on PES were characterized by frequency calculations. The optimization was carried out using the Berny analytical gradient optimization methods.[27–29] Finally, the nature of each stationary point was established by calculating analytically and diagonalizing the matrix of the second derivative of energy to determine the number of imaginary frequencies (zero for local minimum and one for TS). The transition vector (TV),[30] i.e., the eigenvector associated to the unique negative eigenvalue of the force constant matrix has been characterized.

The intrinsic reaction coordinate (IRC)[31] path was traced in order to check the energy profiles connecting each transition structure to the two associated minima of the proposed mechanism by using the second order González–Schlegel integration method.[32] The electronic structures of stationary points were analyzed by the natural bond orbital (NBO) method.[33] All calculations were carried out with the Gaussian 03 suite of programs.[34]

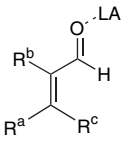
The values of the free energies have been calculated based on the total energies and the thermochemical analysis at the B3LYP/6-31G* level. The thermal contributions to the vibrational energy have been scaled by 0.96.[35] The free energies have been computed at −78 °C, and they were calculated with the standard statistical thermodynamic formulae.[25]

The solvent effects modeled as a continuum model have been analyzed by B3LYP/6-31G* single-point calculations on the gas-phase optimized geometries using a relatively simple self-consistent reaction field (SCRF)[36] based on the polarizable continuum model (PCM) of the Tomasi's group.[37] Since these cycloadditions are carried out in dichloromethane, we have selected its dielectric constant ε=8.93.

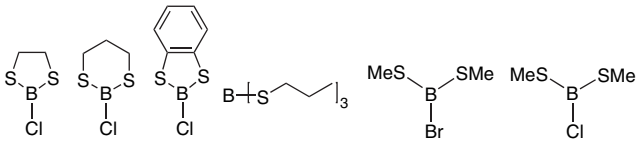
The global electrophilicity index,[38] ω, which measures the stabilization energy when the system acquires an additional electronic charge ΔN from the environment, has been given by the following simple expression,[38] $\omega=(\mu^2/2\eta)$, in terms of the electronic chemical potential μ and the chemical hardness η. Both quantities may be approached in terms of the one electron energies of the frontier molecular orbital HOMO and LUMO, ε_H and ε_L, as $\mu\approx(\varepsilon_H+\varepsilon_L)/2$ and $\eta\approx(\varepsilon_L-\varepsilon_H)/2$, respectively.[23a,39] Recent studies devoted to DA[40] and 1,3-dipolar cycloaddition[41] reactions have shown that the global indexes defined in the context of DFT[42] are a powerful tool to understand the behavior of polar cycloadditions. The difference of global electrophilicity between the reagent pair,[40] $\Delta\omega$, can be used to predict the polar character of the process and thereby the feasibility of the cycloaddition.

The DA reactions between Cp and crotonaldehyde (**1**), and methacrolein (**2**), in absence and in the presence of a LA catalyst, have been studied. The influence of the LA catalysts

Table 1. Global electrophilicity of the series of molecules included in this study



Lewis Acid (LA):



5 **6** **7** **8** **9** **10**

Entry	R^a	R^b	R^c	LA	ω (in eV)
1	H	H	H	**7**	5.66
2	Me	H	H	**7**	4.88
3	H	H	H	**10**	4.68
4	H	H	H	**9**	4.64
5	H	H	H	**5**	4.50
6	H	H	H	**6**	4.56
7	Me	H	H	**10**	4.12
8	Me	H	H	**9**	4.08
9	H	H	Me	**5**	4.10
10	H	Me	H	**5**	4.06
11	Me	H	H	**6**	4.01
12	Me	H	H	**5**	3.94
13	H	Me	Me	**5**	3.81
14	Me	H	Me	**5**	3.80
15	Me	H	H	**8**	3.67
16	Me	Me	H	**5**	3.54
17	H	H	H	—	1.84
18	H	Me	H	—	1.70
19	Me	H	H	—	1.65
20	Me	H	Me	—	1.59

The electrophilicity of Cp and 1,3-butadiene is 0.83 and 1.05 eV, respectively.[40]

has been modeled taking into account the formation of a complex between the boron atom of the LA catalyst **5** and the carbonyl oxygen atom of **1** or **2** (see **11** and **12** in Scheme 3 and Table 1).

3. Results and discussion

3.1. Global electrophilicity analysis

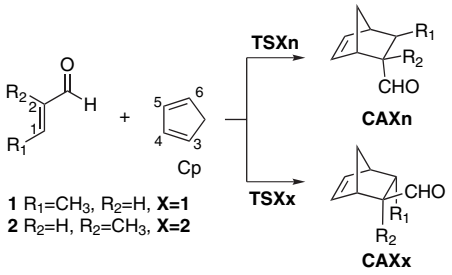
The global electrophilicity, ω, of crotonaldehyde **1**, methacrolein **2**, and a wide series of LA coordinated complexes studied by Howarth et al.[22a] are presented in Table 1. Acrolein, entry 17, has a global electrophilicity value of 1.84 eV and it may be classified as a strong electrophile within the electrophilicity scale.[40] Methyl substitution on the α and β positions of acrolein decreases the electrophilicity of crotonaldehyde **1** and methacrolein **2** to 1.65 and 1.70 eV, respectively (entries 19 and 18 in Table 1), as a consequence of the electron-releasing (ER) character of the methyl group. The presence of the methyl group on the β position of acrolein cause a large reduction of the electrophilicity.

Global electrophilicity scale was used to examine the effect of the LAs observed in the above reactions. Coordination of the boron based LAs, **5**–**10**, to the lone pair of the carbonyl oxygen atom of these acrolein derivatives increases notably the electrophilicity of the corresponding complex to 3.54–5.66 eV. Therefore, it is expected that these LA catalyzed DA reactions have a large polar character. Some conclusions can be obtained from the data given in Table 1. Electrophilicity of the complexes can be related to the electron-withdrawing (EW) ability of the corresponding LA, and the methyl substitution on the acrolein framework. Thus, the chloride derivative **10**, entries 3 and 7, has a more EW character than the bromide derivative **9**, entries 4 and 8, in clear agreement with more of the electronegative character of the chloride atom than the bromide one. The boron heterocycles **5** and **6** posses a lower EW effect than the LA **10** (see entries 3, 5, and 6), while the boron heterocycle derivative **7** possesses the largest EW effect of this series. Thus, coordination of the LA **7** to acrolein and crotonaldehyde raises the electrophilicity of the corresponding complexes to 5.66 and 4.88 eV, respectively (see entries 1 and 2). Finally, the trialkylthioborane **8** possesses a lower EW effect than the chloride derivative **10** as a consequence of the substitution of the chloride atom in **10** by the thioether in **8** (see entries 15 and 7).

In summary, the coordination of boron atom of LA catalysts, **5**–**10**, to the lone pair of the oxygen atom of the carbonyl group lowers the electron density at the oxygen atom and lowers the energy of the LUMO, the C=O π* orbital, with concomitant increases of the electrophilicity index, ω, activating the α,β-unsaturated carbonyl group towards nucleophilic attack. On the other hand, substitution on the acrolein by an ER methyl group decreases slightly the electrophilicity of these methyl derivatives; the substitution on the β position being a large incidence than at the α one.

3.2. Analysis based on the exploration of the PES

3.2.1. Study of the uncatalyzed cycloaddition between 1+Cp and 2+Cp. For the DA reactions **1**+Cp and **2**+Cp, two reactive channels are feasible depending on the relative approach of the diene system of Cp to the carbonyl group of these acrolein derivatives: in the *endo* mode, the π system of Cp is near to the carbonyl group, while in the *exo* it is far. The two approach modes have been considered (see Scheme 2). Analysis of the results renders that these cycloadditions take place along asynchronous concerted processes. Thus, two TSs: **TSXn** and **TSXx**, corresponding to the *endo* and *exo* approach modes of Cp to **1** and **2** (**X**=**1** for **1** and **X**=**2** for **2**), named as **n** and **x**, respectively, for each one of the two DA reactions considered were located and characterized. The total and relative energies corresponding to these reactions are summarized in Table 2.



Scheme 2.

The activation energies associated to these processes are: 21.36 (31.59) kcal/mol for **TS1n**, 20.89 (31.11) kcal/mol

Table 2. B3LYP/6-31G* total and free energies (E, E_{sol} in dichloromethane and G, in au), relative[a] (ΔE, ΔE_{sol} in dichloromethane and ΔG, in kcal/mol) for the stationary points involved in the boron based LA catalyzed Diels–Alder reactions between crotonaldehyde **1** and methacrolein **2** with cyclopentadiene

	E	ΔE	E_{sol}	ΔE_{sol}	G	ΔG
1+Cp	−425.335246		−425.340522		−425.189182	
TS1n	−425.301209	21.36	−425.306480	21.36	−425.138847	31.59
TS1x	−425.301953	20.89	−425.307152	20.94	−425.139612	31.11
CA1n	−425.356793	−13.52	−425.359091	−11.65	−425.189657	−0.30
CA1x	−425.356769	−13.51	−425.359099	−11.66	−425.189689	−0.32
2+Cp	−425.334607		−425.337096		−425.188503	
TS2n	−425.302951	19.86	−425.307402	18.63	−425.140719	29.98
TS2x	−425.305044	18.55	−425.309862	17.09	−425.142765	28.70
CA2n	−425.355757	−13.27	−425.357823	−13.01	−425.188431	0.05
CA2x	−425.355737	−13.26	−425.357959	−13.09	−425.188440	0.04
11+Cp	−1785.490026		−1785.512254		−1785.283995	
TS1Bn	−1785.471382	11.70	−1785.496789	9.70	−1785.249002	21.96
TS1Bx	−1785.471171	11.83	−1785.496291	10.02	−1785.248244	22.43
CA1Bn	−1785.509928	−12.49	−1785.526306	−8.82	−1785.282560	0.90
CA1Bx	−1785.509647	−12.31	−1785.526155	−8.72	−1785.282371	1.02
12+Cp	−1785.486882		−1785.505385		−1785.280854	
TS2Bn	−1785.473595	8.34	−1785.496799	5.39	−1785.250934	18.78
TS2Bx	−1785.474854	7.55	−1785.498392	4.39	−1785.252491	17.80
CA2Bn	−1785.508233	−13.40	−1785.523384	−11.29	−1785.281000	−0.09
CA2Bx	−1785.506821	−12.51	−1785.521017	−9.81	−1785.279853	0.63

[a] Relative to **1**+Cp, **2**+Cp, **11**+Cp or **12**+Cp.

for **TS1x**, 19.86 (29.98) kcal/mol for **TS2n**, and 18.55 (28.70) for **TS2x** (values in parenthesis are relative free energy). The discrepancy between both values is associated to the negative values of activation entropies while the present results are clear that both uncatalyzed reactions present a *exo* selective. The large activation energy associated to these uncatalyzed processes prevents the corresponding DA reaction instead that these cycloadditions are exothermic in ca. 13 kcal/mol.

The geometries of the TSs are given in Figure 1. The lengths of the C1–C3 and C2–C6 forming bonds at the TSs are: 2.091 and 2.408 Å (**TS1n**), and 2.089 and 2.429 Å (**TS1x**), and 2.024 and 2.531 Å (**TS2n**), 2.005 and 2.643 Å (**TS2x**), respectively. The extent of the asynchronicity on the bond-formation can be measured by means of the difference between the lengths of the two σ bonds that are being formed in the reaction, i.e., $\Delta r = d(\text{C2–C6}) - d(\text{C1–C3})$. These values are in the range 0.31–0.63; they indicate that these TSs correspond with asynchronous bond-formation processes, where the C1–C3 forming bond at the β position of acrolein derivative is being formed in a large extension as a consequence of the large polarization of the C1–C2 double bond. The TSs associated to the DA reaction between **2** and Cp are more asynchronous that those associated to reaction between **1** and Cp. The hindrance of the C2 methyl group on **2** could be responsible for the large asynchronicity. In addition the *exo* TSs are slightly more asynchronous than the *endo* ones.

The analysis of the atomic motion at the unique imaginary frequency of the TSs (see the corresponding values in Fig. 1) indicates that they are mainly associated to the movement of the C1 and C3 atoms and the C2 and C6 along the C1–C3 and C2–C6 bond-formation. At these asynchronous TSs, the two forming bond processes are coupled.

The extent of bond-formation along a reaction pathway is provided by the concept of bond order (BO).[43] The BO values of the C1–C3 and C2–C6 forming bonds at the TSs are: 0.45 and 0.27 (**TS1n**), and 0.44 and 0.27 (**TS1x**), and 0.49 and 0.23 (**TS2n**), 0.49 and 0.21 (**TS2x**), respectively. These values indicate that at these asynchronous bond-formation processes the C1–C3 bond-formation is more advanced than the C2–C6 one.

The natural population analysis (NPA) allows us to evaluate the charge transfer (CT) along the cycloaddition of these acrolein derivatives. The natural charges at the TSs appear

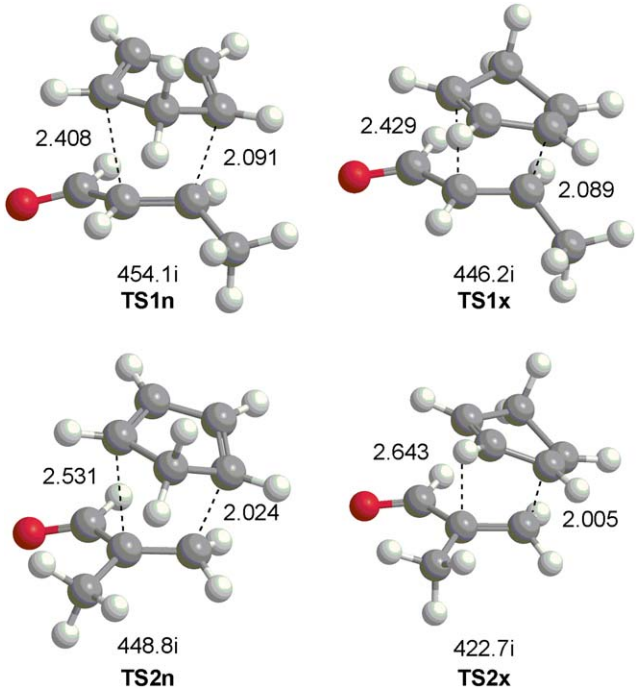
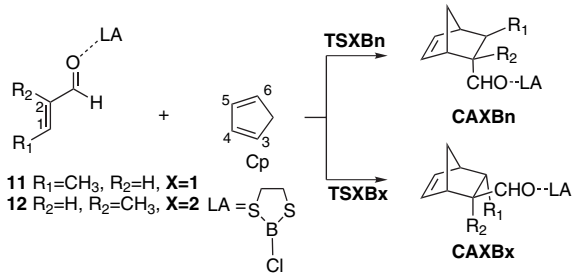


Figure 1. Geometries of the transition structures involved in the Diels–Alder reactions between crotonaldehyde **1** and methacrolein **2** with cyclopentadiene. The distances are given in Å. The unique imaginary frequencies in cm^{-1} are also given.

shared between the donor Cp and the acceptor methyl acrolein derivatives. The CT from Cp to **1** or **2** at the TSs are: 0.14 e at **TS1n**, **TS1x**, **TS2n**, and 0.13 e at **TS2x**. This values are slightly larger than that obtained at the TS associated to the DA reaction between butadiene/acrolein, 0.11 e.[40] Although acrolein is slightly more electrophile than the methyl derivatives **1** and **2**, see Table 1, Cp is better nucleophile than butadiene; note that $\Delta\omega$ for **1**+Cp and **2**+Cp DA reactions, 0.87 and 0.82 eV, respectively, are large than that for butadiene+acrolein DA reaction, 0.70 eV.[40]

3.2.2. Study of the Lewis acid catalyzed cycloaddition between 1+Cp and 2+Cp. The effect of LA catalyst could be confirmed with energetics of these cycloadditions. The *endo* and *exo* approach modes have been considered for the LA catalyzed DA reactions **1**+Cp and **2**+Cp (see Scheme 3). A complete exploration of the PES for the formal [4+2] cycloadditions between the complexes **11** and **12** with Cp affords that these reactions take place along highly asynchronous concerted processes, associated to the nucleophilic attack at the end of the 1,3-diene system of Cp to the β position of these LA coordinated acrolein derivatives. Therefore, these reactions can be considered a formally Michael-type addition. Thus, two TSs: **TSXBn** and **TSXBx** and two cycloadducts: **CAXBn** and **CAXBx**, corresponding to the *endo* and *exo* approach modes of Cp to the complexes **11** and **12** (**X**=**1** for **11** and **X**=**2** for **12**), named as **n** and **x**, respectively, for each one of the two DA reactions considered were located and characterized. The stationary points corresponding to these LA catalyzed DA reactions are shown in Scheme 3 together with the atom numbering. The total and relative energies are summarized in Table 2. The geometries of the TSs are given in Figure 2.



Scheme 3.

The activation energies associated with these process are: 11.70 (21.96) kcal/mol for **TS1Bn**, 11.83 (22.43) kcal/mol for **TS1Bx**, 8.34 (18.78) kcal/mol for **TS2Bn**, 7.55 (17.80) kcal/mol for **TS2Bx**, (values in parenthesis are relative free energy). The inclusion of the LA catalyst **5** decreases of the activation energies associated to these DA reactions between 9–12 kcal/mol. For the DA reaction between complex **11** (LA **5** plus crotonaldehyde **1**) and Cp the difference between *endo*/*exo* energies is of 0.13 (0.48) kcal/mol (the *endo* pathway is slightly favored with respect *exo* channel). This value give a *endo*:*exo* proportion of 53:47 (62:38) that is in reasonable agreement with the experimental results of 87:13.[22a] On the other hand, for the DA reaction between the complex **12** (LA **5** plus methacrolein **2**), and Cp there is a change on the stereoselectivity; now **TS2Bx** is 0.79 (0.98) kcal/mol lesser in energy that **TS2Bn**, this value give a *endo*:*exo* proportion of 31:69 (27:73) in agreement with the experimental results of 8:92 (*endo*/*exo*).[22a] These LA catalyzed DA reactions are exothermic in 12–13 kcal/mol. However, the inclusion of thermal corrections to the energy and the entropic term makes the process endergonic in 0–1 kcal/mol.

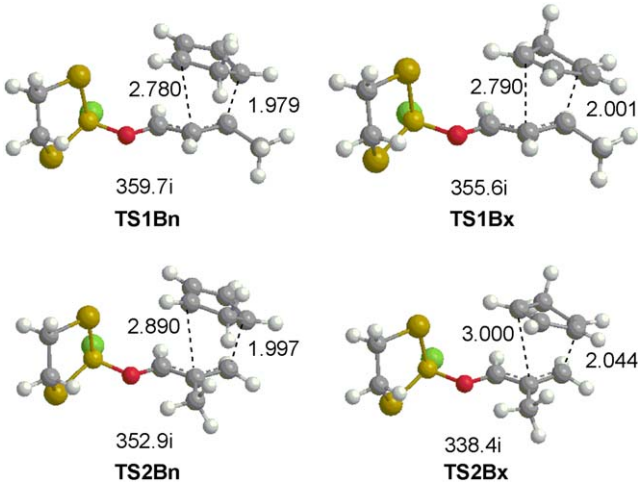


Figure 2. Geometries of the transition structures involved in the LA catalyzed Diels–Alder reactions between crotonaldehyde **1** and methacrolein **2** with cyclopentadiene. The distances are given in Å. The unique imaginary frequencies in cm^{-1} are also given.

The activation energy associated to the reaction of **12** with Cp is 4.15 kcal/mol lower than that associated to the reaction of **11** with Cp, while for the uncatalyzed processes this difference is 2.34 kcal/mol. Therefore, the presence of the ER methyl group on the β position of acrolein derivative **11** produces a lower catalytic effect on the activation energy value. Note that the methyl substitution produces an electrophilic deactivation of acrolein. This behavior can be also rationalized by means of the analysis of the ω values of **12** and **11**, 4.06 and 3.94 eV, respectively (see entries 10 and 12 in Table 1).

The geometries of the TSs are given in Figure 2. The lengths of the C1–C3 and C2–C6 forming bonds at the TSs associated to the nucleophilic attack of Cp to these LA coordinated acrolein derivatives are: 1.979 and 2.780 Å (**TS1Bn**), and 2.001 and 2.790 Å (**TS1Bx**), and 1.997 and 2.890 Å (**TS2Bn**), 2.044 and 3.000 Å (**TS2Bx**), respectively. The extent of the asynchronicity on the bond-formation, i.e., $\Delta r = d(C2–C6) - d(C1–C3)$, is in the range 0.79–0.96. Coordination of the LA to the carbonyl oxygen atom increases the asynchronicity of the process. These TSs correspond with highly asynchronous bond-formation processes, where the C1–C3 forming bond at the β position of acrolein derivative is being formed in a large extension as a consequence of the large polarization of the C1–C2 double bond.

An analysis of molecular structure of **TS2Bn** shows that along the *endo* approach of Cp to methacrolein **12**, the methylene group of Cp is positioned close to the methyl group of **12**; the C(methylene)–C(methyl) distance is 3.370 Å. The sum of the van der Waals radius of the two groups is ca. 4.08 Å. Therefore, while for the **11**+Cp reaction the attracting electrostatic forces at the *endo* TS are responsible for the *endo* selecivity,[14a] the non-bonding interactions between the

methylene group of Cp and the methyl group of methacrolein **12** are responsible for the *exo* selectivity experimentally observed.[22a]

The analysis of the atomic motion at the unique imaginary frequency of the TSs (see the corresponding values in Fig. 2) indicates that they are mainly associated to the movement of the C1 and C3 atoms along the C1–C3 bond-formation; the movement of the C2 and C6 atoms being negligible. This analysis reinforces the two-center interaction at these LA catalyzed DA reactions.

The BO values of the C1–C3 and C2–C6 forming bonds at the TSs are: 0.52 and 0.11 (**TS1Bn**), and 0.49 and 0.12 (**TS1Bx**), and 0.52 and 0.10 (**TS2Bn**), 0.46 and 0.09 (**TS2Bx**), respectively. These values indicate that at these asynchronous bond-formation processes the C1–C3 bond-formation is more advanced than the C2–C6 one. The *endo* TSs are slightly more asynchronous and more advanced than the *exo* ones.

To evaluate the CT along the nucleophilic attack of Cp to the LA coordinated acrolein derivatives, the natural charges at the TSs were shared between the donor Cp and the acceptor LA coordinated aldehyde **11** and **12**. The CT from Cp to the complexes **11** or **12** at the TSs are: 0.34 e at **TS1Bn**, 0.32 e at **TS1Bx**, at 0.33 e at **TS2Bn**, and 0.29 e at **TS2Bx**. These values indicate that these species have some zwitterionic character. These large values are in clear agreement with the increase of the difference of electrophilicity, $\Delta\omega$, between the reagents for these LA catalyzed DA reactions relative to the uncatalyzed ones. This behavior is a consequence of the large electrophile character of these LA coordinated acrolein derivatives and the CT at the *endo* TSs are slightly larger than that of the *exo* ones.

Finally, as these reactions were carried out in dichloromethane, and solvent effects can produce some alteration in both activation energies and stereoselectivity, they were considered by means of single-point calculations over the gas-phase optimized geometries using the PCM method. Table 2 reports the relative energies. Solvent effects stabilize all structures between 9–16 kcal/mol. The more stabilized species are the TSs as a consequence of their zwitterionic character. As a result, the electronic activation barriers decrease ca. 2 kcal/mol for the reaction of **11**, and ca. 3 kcal/mol for the reaction of **12**. A unlike behavior has the solvation on the stereochemistry of both DA reactions; while for the reaction between **11** and Cp, the *endo* TS is slightly more stabilized than the *exo* one, increasing the *endo* selectivity (*endo*:*exo* proportion of 58:42), for the reaction between **12** and Cp, the *exo* TS is slightly more stabilized, therefore increasing the gas-phase *exo* selectivity (*endo*:*exo* proportion of 27:73). These energetic results reinforce the gas-phase analysis. This value give a *endo*:*exo* proportion that is in reasonable agreement with the experimental results 87:13 and 8:92, respectively.[22a]

4. Conclusions

The present calculations using density functional theory, B3LYP/6-31G*, provide thermodynamic and kinetic information on the DA reaction between crotonaldehyde and methacrolein with Cp catalyzed by LAs based on sulfur containing boron heterocycles. The theoretical data obtained may thus provide a helpful tool for the interpretation of the experimental findings and a useful guide for understanding the mechanism of other analogous reactions. From the comprehensive study on these reaction pathways the following conclusions can be drawn:

(i) A DFT analysis based on the electrophilicity index, ω, of the reagents shows the large increase of the electrophilicity of crotonaldehyde and methacrolein with coordination of the sulfur containing boron heterocycles, as LA catalysts.

(ii) LAs were found to be extremely effective in promoting and accelerating the cycloaddition reactions. The large increase of $\Delta\omega$ values for these LA catalyzed DA reactions accounts for the polar character of these cycloadditions and the reduction of the corresponding activation energies. Then, these cycloadditions can be viewed as a nucleophilic attack.

(iii) The reaction pathways, *endo* and *exo*, associated with cycloaddition reactions of crotonaldehyde and methacrolein with cyclopentadiene (Cp) catalyzed by 2-chloro-1,3,2-dithioborolane as LA have been studied. The boron atom of LA catalysts is coordinated to the lone pair of the oxygen atom of the carbonyl group. This coordination lowers the electron density at the oxygen atom and increases ω value, activating the α,β-unsaturated carbonyl group towards nucleophilic attack.

(iv) These LA catalyzed cycloaddition reactions take place along highly asynchronous concerted bond-formation processes associated to the nucleophilic attack of Cp to the β position of the LA coordinated α,β-unsaturated carbonyl compounds. The DA reaction between crotonaldehyde and Cp leads the expected *endo* adducts, while the cycloaddition between methacrolein and Cp leads to the unusual *exo* adduct, in agreement with the experimental outcomes. While the attracting electrostatic forces at the ends of the zwitterionic *endo* TS associated to the reaction of crotonaldehyde with Cp are responsible for the *endo* selectivity, the hindrance between the methyl group of methacrolein and the methylene moiety of Cp in the *endo* TS is responsible for the *exo* selectivity found experimentally.

(v) Solvent effects of dichloromethane reinforce the gas-phase energetic results found for these polar cycloadditions.

(vi) The present computational study enhances our understanding on the reactivity of Cp with crotonaldehyde and methacrolein in sulfur containing boron heterocycles LA catalyzed DA reactions and would stimulate experimental interest. This efficient catalytic effect can be satisfactorily explained in terms of global electrophilicity scale as well as the analysis of the molecular structure of the corresponding transition structures.

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^{1}H and ^{13}C NMR assignments of the three dicyclopenta-fused pyrene congeners

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Abstract—Complete ^{1}H and ^{13}C NMR assignments of the (di-)cyclopenta-fused pyrene congeners, cyclopenta[*cd*]- (**2**), dicyclopenta[*cd*,*fg*]- (**3**), dicyclopenta[*cd*,*jk*]- (**4**) and dicyclopenta[*cd*,*mn*]pyrene (**5**), respectively, are achieved using two-dimensional (2D) NMR spectroscopy. The experimental ^{13}C chemical shift assignments are compared with computed ab initio CTOCD-*PZ2*/6-31G** ^{13}C chemical shifts; a satisfactory agreement is found. Substituent-induced chemical shifts in the pyrene core induced by annelation of cyclopenta moieties are discussed. Effects of dicyclopenta topology on electronic structure are illustrated for **3**–**5**.

1. Introduction

The (di-)cyclopenta-fused congeners of pyrene (**1**), viz. cyclopenta[*cd*]- (**2**),[1] dicyclopenta[*cd*,*fg*]- (**3**), dicyclopenta-[*cd*,*jk*]- (**4**) and dicyclopenta[*cd*,*mn*]pyrene (**5**),[2,3] belong to an important sub-class of the polycyclic aromatic hydrocarbons (PAHs) known as cyclopenta-fused PAHs (CP-PAHs) (Fig. 1).

The independent synthesis of CP-PAHs **2**–**5** has been instrumental for their identification as significant constituents of the nonpolar fraction of combustion exhausts derived from organic matter, viz. fossil fuels.[4–6] Compounds **3**–**5** have also been put forward as undesired side-products that are formed during thermal treatment of pyrene-contaminated soil.[7] Since CP-PAHs like **2**–**5** may exhibit mutagenic and carcinogenic properties,[8] they represent a potential human health concern.[9–15] Only recently the bacterial mutagenicity response of **3**–**5** was assayed employing the standard Ames-assay (*Salmonella typhimurium* strain TA98 with and without exogenous metabolic activation (±S9-mix)). CP-PAHs **3**–**5** were found to be highly active metabolic-dependent mutagens. Interestingly, congeners **3** and **5** were found to act as direct mutagens, that is, they are potent mutagens even in the absence of exogenous metabolic activation.[16]

From a fundamental perspective the nonalternant CP-PAHs **3**–**5** also possess unusual physico-chemical properties, such as high electron affinities (low one-electron reduction potentials),[17–20] characteristic upfield-shifted ^{1}H NMR

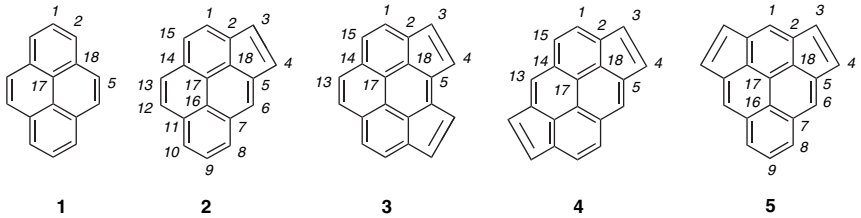

Figure 1. Pyrene (**1**), cyclopenta[*cd*]- (**2**), dicyclopenta[*cd*,*fg*]- (**3**), dicyclopenta[*cd*,*jk*]- (**4**) and dicyclopenta[*cd*,*mn*]pyrene (**5**). A generalized carbon atom numbering scheme is used to facilitate the comparison of related positions in compounds **1**–**5**.

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chemical shifts,[2,3,21] and photophysical properties, viz. UV–vis spectra that are strongly modulated by the number and topology of the annelated cyclopenta moieties[2,20] and anomalous fluorescence.[22] As a consequence, CP-PAHs **3**–**5** represent interesting probe molecules in the study of the energy and magnetic criteria of aromaticity in π-conjugated polycyclic systems. For **1**–**5** it has been shown both experimentally and theoretically that the number and topology of the annelated cyclopenta moieties markedly affects the global and local (distinct rings) aromatic character of **1**–**5**.[23–25] In another connection, **3**–**5** have been proposed as potential intermediates in the formation of fullerenes under flame conditions.[5,26–28]

The complete ^{1}H and ^{13}C NMR assignments of **3**–**5**, so far unreported, are the subject of this paper. Although the ^{1}H and ^{13}C NMR assignments of cyclopenta[*cd*]pyrene (**2**, $C_{18}H_{10}$) have been reported previously,[29,30] the assignment of some of its quaternary carbon atoms (C*14* and C*17*, see Fig. 1) remains ambiguous. Hence, **2** is also reinvestigated. Furthermore, a comparison of the experimental ^{13}C assignments with computed ab initio CTOCD-*PZ2*/6-31G** ^{13}C chemical shifts are carried out. Hence, conclusions can be drawn as to the effect of cyclopenta annelation to a pyrene perimeter on the ^{1}H[23,25] and ^{13}C chemical shifts (substituent-induced chemical shifts, SCS; $\Delta\delta$ with **2** as the reference compound for **3**–**5**, respectively) can be drawn.

2. Results and discussion

The ^{1}H and ^{13}C NMR assignments of **2**–**5** were achieved using two-dimensional (2D) NMR techniques. Nuclear Overhauser Effect Spectroscopy (NOESY) was used for the assignment of proton signals by evaluation of *through-space* dipolar interactions. Heteronuclear Chemical Shift Correlation (HETCOR) was applied to identify those carbon atoms bearing hydrogen. Long-Range (LR)-HETCOR reflecting one to three bond C–H couplings ($^{1}J_{C-H}$–$^{3}J_{C-H}$) was used to make a definitive assignment of the quaternary carbon atoms. Since not all expected $^{2}J_{C-H}$ and/or $^{3}J_{C-H}$ couplings could be observed in a single LR-HETCOR experiment, different experiments employing different long-range C–H coupling constants (range 4–10 Hz), were executed in order to visualize all the different correlations. In some cases, long-range C–H interactions were still not discernible, presumably because their $^{n}J_{C-H}$ values deviate from the common long-range coupling constants used in LR-HETCOR experiments (4, 6, 8 or 10 Hz). Note that $^{3}J_{C-H}$ values normally fall in the range of 4–10 Hz while $^{2}J_{C-H}$ and $^{4}J_{C-H}$ values are typically less than 4 and 2 Hz, respectively.[31]

2.1. Experimental NMR spectra

2.1.1. Cyclopenta[*cd*]pyrene (2). ^{1}H and ^{13}C NMR assignments of **2** (C_s symmetry) were first reported by Jans et al.[29] However in a more recent study,[30] in which partially ^{13}C-labelled **2** was used, the original assignment of the quaternary carbon atoms C*2*, C*7*, C*14*, C*16* and C*17* was questioned (Figs. 1 and 2). This prompted us to also take **2** into consideration. Since all ^{1}H NMR resonances of **2** have been assigned unambiguously (Table 1),[29,30,32] the carbon atoms of this molecule that bear hydrogen were readily identified using HETCOR. The ^{13}C resonances at δ 133.48 and 130.52 show a cross peak with δ 7.47 (H*3*) and 8.38 (H*8*), respectively, and thus originate from C*3* and C*8*, respectively. Following similar reasoning the ^{13}C resonances at δ 128.54, 127.81, 126.94, 126.93, 126.57, 126.48, 124.34 and 122.68 correspond to C*10*, C*4*, C*9*, C*12*, C*13*, C*6*, C*1* and C*15*, respectively, (see Fig. 2). These results are all in line with those previously reported.

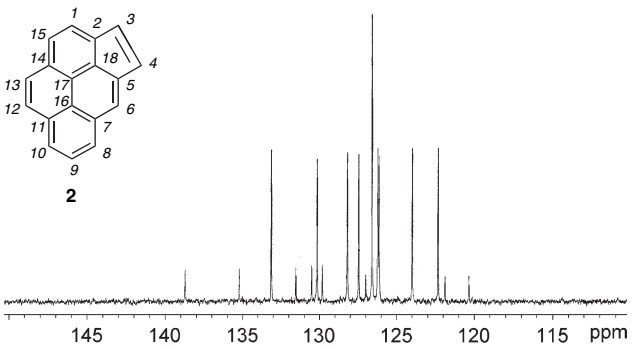

Figure 2. 1D ^{13}C NMR spectrum (solvent $CDCl_3$) of cyclopenta[*cd*]pyrene (**2**, see also Table 1).

Next, various LR-HETCOR experiments, in which different long-range $^{n}J_{C-H}$ coupling constants were used, were performed (Table 1 and see Section 4.1) in order to assign the eight quaternary carbon atoms of **2**. Since with LR-HETCOR two- ($^{2}J_{C-H}$) and three-bond ($^{3}J_{C-H}$) long-range couplings between quaternary carbon atoms and distant hydrogens are most likely to be observed[33] only these types of contributions are considered. The hydrogens H*6*, H*3* and H*4* (Fig. 1) show a cross peak with the ^{13}C resonance at δ 139.07, which can only correspond to either C*5* or C*18*. The ^{13}C resonance at δ 135.58 shows cross-peaks with H*1* and H*4*, implying that this resonance originates either from C*2* or C*18*. H*3* and H*4* also show a cross peak with the ^{13}C resonance at δ 127.34 , which may then correspond to C*2*, C*5* or C*18*. To distinguish between these three carbon atoms, the proton-decoupled 1D ^{13}C NMR spectrum of **2** (Fig. 2) is carefully examined. The ^{13}C resonance at δ 127.34 possesses the lowest intensity. This suggests that it belongs to a carbon atom located within the pyrene core, viz. C*18*. Owing to its increased distance from the hydrogens of the pyrene perimeter, its relaxation will be slower, leading to a reduced intensity.[34] If the assignment of C*18* (δ 127.34) is correct, it implies that the ^{13}C resonances at δ 139.07 and 135.58 correspond to C*5* and C*2*, respectively. The cross-peaks found between the ^{13}C signal at δ 130.87 and H*12* and H*13*, indicate that this ^{13}C resonance must originate either from C*11* or C*14*. The δ 131.90 signal can correspond only to C*7*, since it exhibits only cross-peaks with H*6* and H*9*. In analogy, the ^{13}C resonance at δ 122.23 shows interactions with H*6*, H*8*, H*10* and H*13*. This means that it can arise only from C*16*. With the identification of C*16*, the ^{13}C resonance at δ 130.18 can be assigned to C*11* due to its cross-peaks with H*10* and H*12*. The available assignments now permit the identification of C*14* at δ 130.87. By elimination, the ^{13}C resonance at δ 120.69 belongs to C*17*. The complete ^{1}H and the ^{13}C assignments of **2** are summarized in Table 1.

2.1.2. Dicyclopenta[*cd*,*fg*]pyrene (3). Since dicyclopenta[*cd*,*fg*]pyrene (**3**, $C_{20}H_{10}$) possesses C_{2v} symmetry, its

Table 1. Cross-peaks observed in the HETCOR and LR-HETCOR spectra of cyclopenta[*cd*]pyrene (**2**)[a,b]

Nucleus	δ (^{13}C) ppm	H*8*	H*6*	H*10*	*H1*	H*15*	H*12*	H*9*	H*13*	H*3*	H*4*
		8.38 (d, $^3J_{H-H}$ 7.70 Hz)	8.36 (s)	8.28 (d, $^3J_{H-H}$ 7.60 Hz)	8.12 (m)	8.08 (m)	8.03 (m)	8.00 (m)	7.98 (m)	7.47 (d, $^3J_{H-H}$ 5.10 Hz)	7.27 (d, $^3J_{H-H}$ 5.10 Hz)
C*17*	**120.69**					b,c,d					
C*16*	**122.23**	a	d	c					c,d		
C*15*	122.68					HT					
C*1*	124.34				HT	b					
C*6*	126.48		HT					c,d			
C*13*	126.57						c		HT		
C*12*	126.93		a				HT				
C*9*	126.94							HT			
C*18*	**127.34**									d	d
C*4*	127.81		a,d								HT
C10	128.54		b	HT				d			
C*11*	**130.18**			b			d				
C*8*	130.52	HT	a,d	c							
C*14*	**130.87**						d		b		
C*7*	**131.90**		a,c					b,d			
C*3*	133.48									HT	a
C*2*	**135.58**				d						c,d
C*5*	**139.07**		a							d	a

[a] See Figure 1 for the structure and atom numbering of **2**. In Figure 2 the experimental 1D ^{13}C NMR spectrum of **2** is shown. Quaternary carbon atoms are typeset in boldface and the multiplicity of the ^{1}H chemical shifts is indicated between parentheses.

[b] HETCOR cross-peaks ($^1J_{C-H}$ coupling constant 160 Hz, H relaxation delay 1 s) are indicated as HT. Independent LR-HETCOR experiments gave the cross-peaks indicated as observed with long-range coupling constants of 4 Hz (a), 6 Hz (b), 8 Hz (c) and 10 Hz (d) (relaxation delay 4 s).

^{1}H and ^{13}C NMR spectrum contains five and ten distinct resonances, respectively, (1D ^{13}C NMR, Fig. 3). In its ^{1}H NMR spectrum, the singlet at δ 7.45 (2H) is readily assigned to hydrogen H*13*. In addition, the vicinal hydrogen pairs H*1* and H*15*, and, H*3* and H*4*, can be distinguished on the basis of their different $^3J_{H-H}$ coupling constants, as the hydrogens of the externally fused cyclopenta-rings (H*3* and H*4*) possess a characteristic $^3J_{H-H}$ coupling constant of ca. 5 Hz. The ^{1}H assignments of **3** are completed by an NOESY experiment. The presence of cross-peaks between the doublet at δ 7.63 (2H, $^3J_{H-H}$ 7.60 Hz) and 7.03 (2H, $^3J_{H-H}$ 5.30 Hz) reveals that the former corresponds to H*1* and the latter to H*3*, respectively. Consequently, the doublets at δ 6.49 (2H, $^3J_{H-H}$ 5.30 Hz) and 7.72 (2H, $^3J_{H-H}$ 7.60 Hz) belong to H*4* and H*15*, respectively.

The unequivocal ^{1}H assignments of **3** now allow the identification of all the carbon atoms bearing hydrogen (HETCOR; see Fig. 4 for an illustrative example). The ^{13}C resonances positioned at δ 137.31, 127.88, 127.07, 127.03 and 123.45 belong to C*3*, C*4*, C*15*, C*13* and C*1*, respectively, (Table 2).

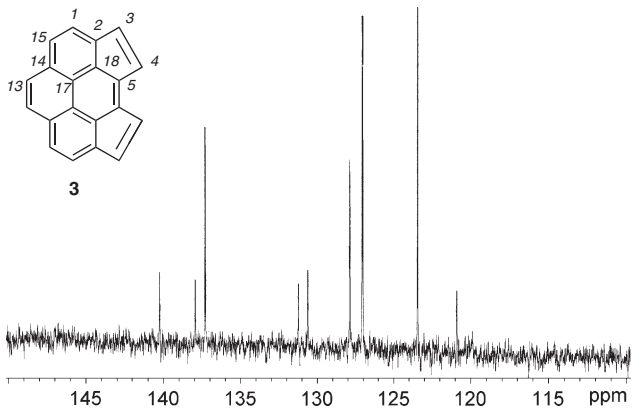

Figure 3. Experimental 1D ^{13}C NMR spectrum of dicyclopenta[*cd*,*fg*]-pyrene (**3**, see also Table 2).

Independent LR-HETCOR experiments optimized for different long-range $^nJ_{C-H}$ coupling constants (Table 2 and see Section 4.1) enabled the assignment of all the quaternary carbon atoms of **3** (see Fig. 4 for an illustrative example). Only $^nJ_{C-H}$ long-range interactions with n=2 or 3 between hydrogens and the quaternary carbon atoms are taken into consideration (vide supra). Hydrogen H*1* shows two cross-peaks with the ^{13}C resonances at δ 130.62 and 131.23, which may then correspond to either C*14* ($^3J_{C-H}$), C*2* or C*18* ($^2J_{C-H}$). Hydrogen H*13* shows two cross-peaks with the ^{13}C resonances at δ 130.62 and 120.91, which may thus arise from C*14* ($^2J_{C-H}$) or C*17* ($^3J_{C-H}$). Since the ^{13}C (δ 130.62) resonance was also found to interact with hydrogen H*1*, it can only correspond to C*14*. Consequently, the δ 120.91 ^{13}C signal belongs to C*17*. Hydrogen H*15* shows cross-peaks at δ 120.91 (C*17*) and 140.24, which may originate from C*2* ($^3J_{C-H}$) and C*14* ($^2J_{C-H}$). Since C*14* (δ 130.62) has already been assigned, the ^{13}C resonance at δ 140.24 has to correspond to C*2*. Thus, the ^{13}C signal at δ 131.23 belongs to C*18*. By elimination, the ^{13}C resonance at δ 137.94 then corresponds to C*5*. This is confirmed by the observation of cross-peaks at δ 137.94 with *H3* ($^3J_{C-H}$) and H*4* ($^2J_{C-H}$). The complete ^{1}H and ^{13}C NMR assignments of **3** are summarized in Table 2.

2.1.3. Dicyclopenta[*cd*,*jk*]pyrene (4). In the case of dicyclopenta[*cd*,*jk*]pyrene (**4**, $C_{20}H_{10}$), which possesses C_{2h} symmetry, five and ten distinct resonances, respectively, are found in the ^{1}H and ^{13}C NMR spectra (Fig. 5). The total ^{1}H assignment was accomplished by an NOESY experiment. The cross peak between the singlet at δ 7.50 (2H), which corresponds to H*13*, and the doublet at δ 6.62 (2H, $^3J_{H-H}$ 5.21 Hz) indicates that the latter arises from H*4* (cf. the characteristic $^3J_{H-H}$ coupling constant of ca. 5 Hz for the vicinal olefinic hydrogens in the annelated five-membered rings).

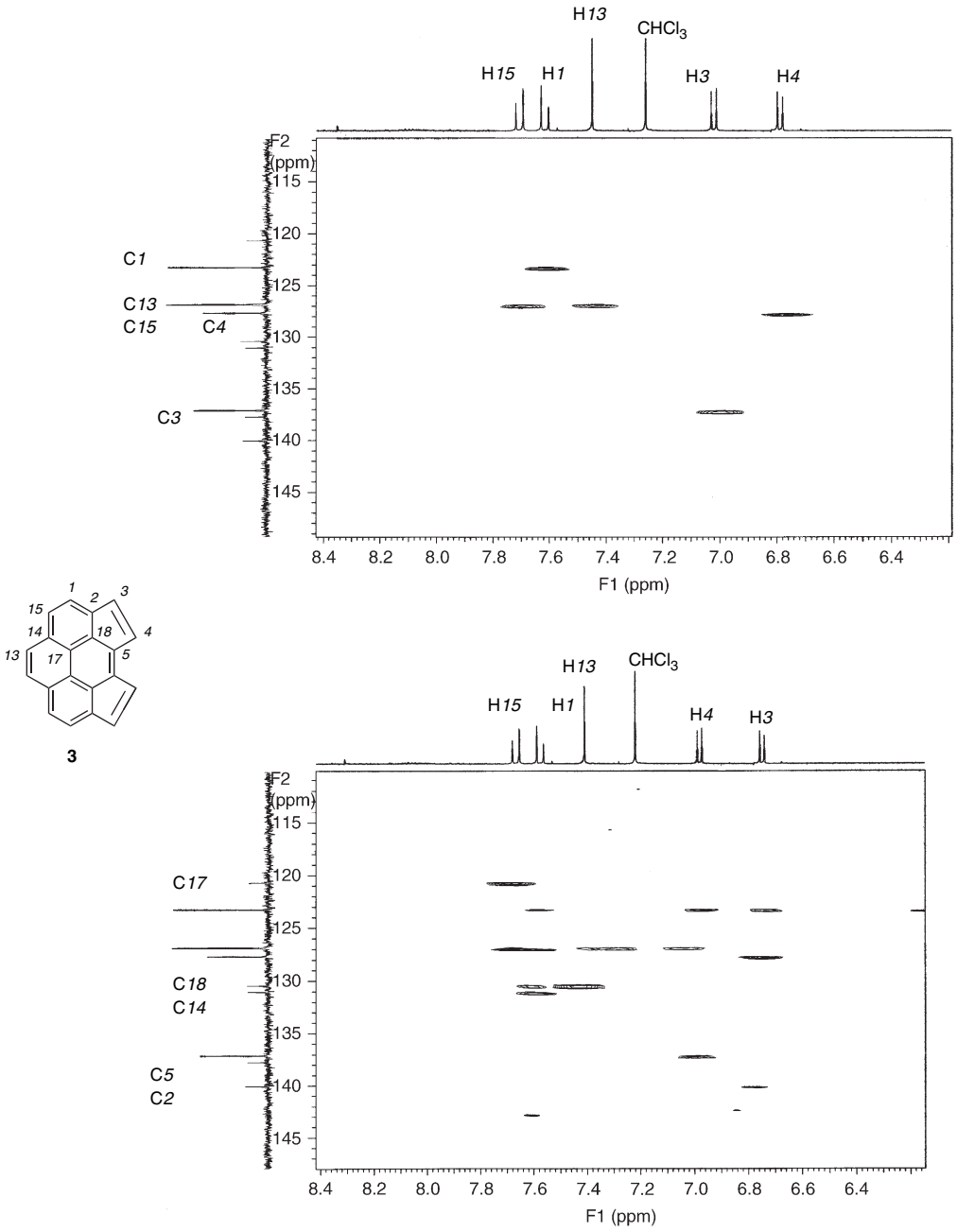

Figure 4. HETCOR ($^{1}J_{C-C}$ 160 Hz, top) and LR-HETCOR ($^{1}J_{C-C}$ 160 Hz and $^{n}J_{C-H}$ 8 Hz, bottom) spectra of dicyclopenta[*cd*,*fg*]pyrene (**3**).

Another interaction is found between the doublet at δ 7.47 (2H, $^{3}J_{H-H}$ 7.69 Hz) and the doublet at δ 6.71 (2H, $^{3}J_{H-H}$ 5.21 Hz). Thus, H*1* corresponds to δ 7.47 and H*3* to 6.71. Since all ^{1}H NMR signals of **4** have been now identified, those of its carbons atoms bearing a hydrogen can be assigned using HETCOR: δ 133.49 corresponds to C*3*, δ131.63 to C*4*, 129.63 to C*15*, 125.72 to C*13* and 122.40 to C*1* (Fig. 4 and Table 3).

The LR-HETCOR experiments with different long-range C–H coupling constants were used (Table 3 and see Section 4.1) in order to assign the quaternary carbon atoms of **4**. Hydrogen H*1* shows two cross-peaks with the ^{13}C resonances at δ 130.66 and 132.07, which can arise from C*14*, C*18* ($^{3}J_{C-H}$) and C*2* ($^{2}J_{C-H}$). H*15* exhibits interactions with the ^{13}C resonances at δ 141.00 and 121.44, which may then correspond to C*2* and C*17* ($^{3}J_{C-H}$) or C*14* ($^{2}J_{C-H}$). The ^{13}C resonance at δ 141.00 gives another cross peak with H*4*. Thus, we assign δ 141.00 to C*2* ($^{3}J_{C-H}$), as C*14* and C*17* would present a four- ($^{4}J_{C-H}$) and a five-bond ($^{5}J_{C-H}$) coupling with H*4*, respectively, which is unlikely to be observed under the applied experimental conditions.[33] Since the cross-peaks for H*1* with δ 130.66 and 132.07 can now only arise from C*14* and C*18*, this leaves δ 121.44 for C*17*. By elimination, it is now possible to relate ^{13}C signal at δ 140.42 to C*5*. These assignments are supported by the behaviour of H*13*, which exhibits two cross-peaks at δ 121.44 (C*17*), and 130.66, which can arise from the interaction with C*18* ($^{3}J_{C-H}$) or C*14* ($^{2}J_{C-H}$). The final assignment for C*14* and C*18* is achieved by comparing their intensities in the 1D ^{13}C NMR spectrum of **4** (Fig. 5). The intensity of the ^{13}C signal at δ 132.07 is stronger than that at δ 130.66. This strongly

Table 2. Cross-peaks observed in the HETCOR and LR-HETCOR spectra of dicyclopenta[*cd*,*fg*]pyrene (**3**)[a,b]

Nucleus	δ (^{13}C) ppm	H*15*	H*1*	H*13*	H*3*	H*4*
		7.72 (d, $^3J_{H-H}$ 7.60 Hz)	7.63 (d, $^3J_{H-H}$ 7.60 Hz)	7.45 (s)	7.03 (d, $^3J_{H-H}$ 5.30 Hz)	6.49 (d, $^3J_{H-H}$ 5.30 Hz)
C*17*	**120.91**	a,b		a		
C*1*	123.45	c	HT		b	a
C*13*	127.03	b	b	HT		
C*15*	127.07	HT		b		
C*4*	127.88				b	HT
C*14*	**130.62**		a,b	a,b,c		
C*18*	**131.23**		a,b,c			
C*3*	137.31				HT	a
C*5*	**137.94**				c	c
C*2*	**140.24**	a				b,c

[a] See Figure 1 for the structure and atom numbering of **3**. In Figure 3 the 1D ^{13}C NMR spectrum and in Figure 4 a HETCOR (top) and an LR-HETCOR (bottom) 2D spectra of **3** are shown. Quaternary carbon atoms are typeset in boldface and the multiplicity of the ^{1}H chemical shifts is indicated between parentheses.

[b] HETCOR cross-peaks are indicated as HT. LR-HETCOR experiments gave the cross-peaks shown as observed with long-range coupling constants of 6 Hz (a), 8 Hz (b) and 10 Hz (c).

Table 3. Cross-peaks observed in the HETCOR and LR-HETCOR spectra of dicyclopenta[*cd*,*jk*]pyrene (**4**)[a,b]

Nucleus	δ (^{13}C) ppm	H*15*	H*13*	H*1*	H*3*	H*4*
		7.71 (d, $^3J_{H-H}$ 7.69 Hz)	7.50 (s)	7.47 (d, $^3J_{H-H}$ 7.69 Hz)	6.71 (d, $^3J_{H-H}$ 5.21 Hz)	6.62 (d, $^3J_{H-H}$ 5.21 Hz)
C*17*	**121.44**	b,c,d	b,c			
C*1*	122.40	b,c		HT		
C*13*	125.72	a,b	HT			
C*15*	129.63	HT	a,b,c	c,d		
C*18*	**130.66**		c,d	b		
C*4*	131.63					HT
C14	**132.07**			b,c		
C*3*	133.49		c		HT	a
C5	**140.42**					
C2	**141.00**	a,b				a

[a] See Figure 1 for the structure and atom numbering of **4**. In Figure 5 the 1D ^{13}C NMR spectrum of **4** is shown. Quaternary carbon atoms are typeset in boldface and the multiplicity of the ^{1}H chemical shifts are indicated between parentheses.

[b] HETCOR cross-peaks are indicated as HT. LR-HETCOR experiments gave the cross-peaks shown as observed with long-range coupling constants of 4 Hz (a), 6 Hz (b), 8 Hz (c) and 10 Hz (d).

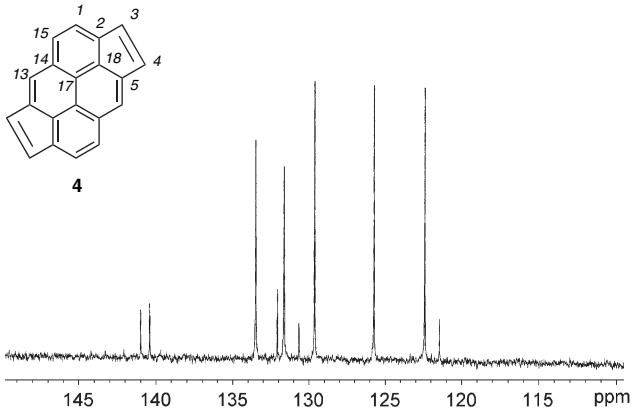

Figure 5. 1D ^{13}C NMR spectrum (solvent $CDCl_3$) of dicyclopenta[*cd*,*jk*]-pyrene (**4**, see also Table 3).

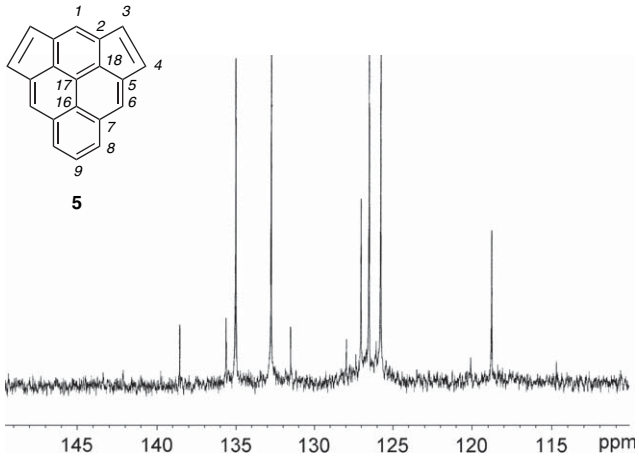

Figure 6. 1D ^{13}C NMR spectrum (solvent $CDCl_3$) of dicyclopenta[*cd*,*mn*]-pyrene (**5**, see also Table 4).

indicates that the ^{13}C resonances at δ 132.07 and 130.66 must originate from C*14* and from C*18*, respectively, since the latter carbon atom is more distant from the hydrogen containing pyrene perimeter.[34] The complete ^{1}H and ^{13}C NMR assignments of **4** are compiled in Table 3.

2.1.4. Dicyclopenta[*cd*,*mn*]pyrene (5). Dicyclopenta [*cd*,*mn*]pyrene (**5**, $C_{20}H_{10}$) possesses C_{2v} symmetry. This may give six and twelve distinct signals in the ^{1}H and ^{13}C NMR spectra (Fig. 6). Several of the ^{1}H resonances of **5** are readily assigned. The triplet at δ 8.08 (1H, $^3J_{H-H}$ 7.70 Hz) has to correspond to hydrogen H*9*. The singlets at δ 8.15 (1H) and 8.37 (2H) have to originate from H*1* and H*6*, respectively, and the doublet at δ 8.53 (2H, $^3J_{H-H}$ 7.70 Hz) belongs to H*8*. The doublets at δ 7.51 (2H, $^3J_{H-H}$ 5.10 Hz) and 7.26 (2H, $^3J_{H-H}$ 5.10 Hz) corresponds to H*3* and H*4* or vice versa (cf. the characteristic $^3J_{H-H}$ value of ca. 5 Hz for an olefinic hydrogens in a five-membered ring). An NOESY spectrum reveals cross-peaks between the singlet at δ 8.37 (H*6*) and the doublet at δ 7.26, on one hand, and the singlet at δ 8.15 (H*1*) and the doublet at δ 7.51, on the other hand. Thus, the doublets at δ 7.51 and 7.26 originate from H*3* and H*4*. As the complete ^{1}H NMR assignments of **5** have been achieved, its carbon atoms bearing hydrogen were identified using HETCOR (Table 2). The results show that the ^{13}C resonances located at δ 135.03, 132.76, 127.04, 126.53, 125.80 and 118.78 correspond to C*3*, C*8*, C*9*, C*4*, C*6* and C*1*, respectively.

The LR-HETCOR experiments with different long-range $^nJ_{C-H}$ coupling constants allow the assignments of the quaternary C atoms of **5** (Table 4 and see Section 4.1). For H*9* two cross-peaks are observed with the ^{13}C resonances at δ 120.10 and 127.96, which may correspond to C*7* and C*16*. Note, however, that for *C16* the interaction with H*9* has to be a long-range $^4J_{C-H}$ coupling. Hydrogen H*6* correlates with the ^{13}C signals positioned at δ 127.96, 131.52 and 138.56; possible assignments for these cross-peaks are C*7* and C*5* ($^2J_{C-H}$) or C*18* and C*16* ($^3J_{C-H}$). The ^{13}C resonances at δ 131.52 and 138.56 can only correspond to C*5* and C*18*, since the ^{13}C signal at δ 127.96 already has been assigned to either C*7* or C*16*. H*3* correlates with both the δ 131.52 and

Table 4. Cross-peaks observed in the HETCOR and LR-HETCOR spectra of dicyclopenta[*cd*,*mn*]pyrene (**5**)[a,b]

Nucleus	^{13}C (ppm)	H*8*	H*6*	H*1*	H*9*	H*3*	H*4*
		8.53 (d, $^{3}J_{H-H}$ 7.70 Hz)	8.37 (s)	8.15 (s)	8.08 (t, $^{3}J_{H-H}$ 7.70 Hz)	7.51 (d, $^{3}J_{H-H}$ 5.10 Hz)	7.26 (d, $^{3}J_{H-H}$ 5.10 Hz)
C*17*	**114.71**						
C*1*	118.78			HT			
C*16*	**120.10**				b		
C*6*	125.80	b	HT				
C*4*	126.53						HT
C*9*	127.04		a		HT		
C*7*	**127.96**		c		b		
C*18*	**131.52**		a			a	a
C*8*	132.76	HT	b				
C*3*	135.03					HT	c
C*2*	**135.63**					b	c
C*5*	**138.56**		b				b

[a] See Figure 1 for the structure and atom numbering of **5**. In Figure 6 the 1D ^{13}C NMR spectrum of **5** is shown. Quaternary carbon atoms are typeset in boldface and the multiplicity of the ^{1}H chemical shifts are indicated between parentheses.

[b] HETCOR cross-peaks are indicated as HT. LR-HETCOR experiments gave the cross-peaks shown as observed with long-range coupling constants of 10 Hz (a) 4 Hz (b) and 10 Hz (c).

135.63 signals. Consequently, these signals can be assigned to C*2*, C*5* and C*18*. Since δ 131.52 and 138.56 belongs to either C*5* or C*18*, the ^{13}C resonance at δ 135.63 has to originate from C*2*. Note that in the 1D ^{13}C NMR spectrum of **5**, the resonance at δ 138.56 is slightly more intense than that at δ 131.52 (Fig. 6). Hence, the resonance at δ 138.56 most likely corresponds to C*5*, since the more distant a carbon atom is from a molecular perimeter, the lower the intensity of the signal will be.[34] This leaves δ 131.52 for C*18*. In addition, the ^{13}C signal at δ 127.96 is more intense than that positioned at δ 120.10; this allows their assignment to C*7* and C*16*, respectively. By elimination, the ^{13}C resonance at δ 114.71 then belongs to C*17*. The complete ^{1}H and ^{13}C assignments of **5** are summarized in Table 4.

2.2. Computed ^{13}C chemical shifts

In a previous paper,[25] we have tentatively assigned the ^{1}H NMR resonances of CP-PAHs **1**–**5** using computed ^{1}H chemical shifts obtained with the *PZ2* (paramagnetic zero) variant[35] of the all electron ab initio continuous transformation of origin of current density (CTOCD) method with the 6-31G** basis as implemented in the Exeter version of SYSMO[36] using 6-31G optimized geometries (see Section 4.2). Although the computed ^{1}H NMR chemical shifts of **1**–**5** deviate by ca. 0.5 ppm from their experimental values, they satisfactorily reflect the trends found in the experimental ^{1}H NMR spectra (see also Ref. 23). These observations prompted us to compute the ^{13}C NMR chemical shifts of **1**–**5** using the same CTOCD-*PZ2*/6-31G**//6-31G approach (Table 5 for **3**–**5** and Table 6 for **1**–**2**). For ^{13}C NMR chemical shifts, which span a considerably wider range in parts per

Table 5. CTOCD-*PZ2*/6-31G**//6-31G computed ab initio absolute carbon nuclear shielding constants (σ_{av} in parts per million) of dicyclopenta[*cd*,*fg*]- (**3**), dicyclopenta[*cd*,*jk*]- (**4**) and dicyclopenta[*cd*,*mn*]pyrene (**5**) (see Fig. 1 for a generalized atom numbering scheme)[a]

Compound	Nucleus	σ_{out}	σ_{in}	σ_{av}	δ_{calcd}	δ_{exp}	$\Delta\delta$[b]
3	C*1*	185.3	−1.0	61.1	124.5	123.45	1.05
	C*2*	160.5	−12.2	45.4	140.2	140.24	−0.04
	C*3*	145.1	−4.3	45.5	140.1	137.31	2.79
	C*4*	153.3	6.8	45.5	130.0	127.88	2.12
	C*5*	146.1	−3.0	46.7	138.9	137.95	0.95
	C*13*	168.6	2.1	57.6	128.0	127.03	0.97
	C*14*	192.4	−15.0	54.1	131.5	130.62	0.88
	C*15*	177.4	−1.2	58.3	127.3	127.07	0.23
	C*17*	197.9	−3.2	63.8	121.8	120.91	0.89
	C*18*	169.0	−3.0	54.3	131.3	131.23	0.07
4	C*1*	185.2	0.8	62.3	123.3	122.40	0.90
	C*2*	160.1	−12.7	44.9	140.7	141.00	−0.30
	C*3*	146.5	0.7	49.3	136.3	133.49	2.81
	C*4*	149.4	2.3	51.3	134.2	131.63	2.57
	C*5*	147.8	−4.5	46.3	139.3	140.42	−1.12
	C*13*	167.0	2.9	57.6	128.0	125.72	2.28
	C*14*	186.7	−13.7	53.1	132.5	132.07	0.43
	C*15*	176.3	−4.6	55.7	129.9	129.63	0.27
	C*17*	198.1	−3.6	63.6	122.0	121.45	0.55
	C*18*	173.4	−2.5	56.2	129.4	130.66	−1.26
5	C*1*	183.3	1.6	62.2	123.4	118.78	4.62
	C*2*	159.8	−3.5	50.9	134.7	135.63	−0.93
	C*3*	148.6	−5.1	46.1	139.5	135.03	4.47
	C*4*	152.1	9.0	56.7	128.9	126.53	2.37
	C*5*	158.6	−7.6	47.8	137.8	138.56	−0.76
	C*6*	165.2	2.4	56.6	129.0	125.80	3.20
	C*7*	191.0	−14.1	54.3	131.3	127.96	3.34
	C*8*	178.5	−11.8	51.6	134.0	132.76	1.24
	C*9*	187.7	−3.7	60.1	125.5	127.04	−1.54
	C*16*	204.0	−8.8	62.1	123.5	120.10	3.40
	C*17*	209.1	2.3	71.3	114.3	114.71	−0.41
	C*18*	178.2	−7.6	47.8	129.8	131.52	−1.72

[a] σ_{out} is the component of the absolute shielding out-of-plane of the ring, σ_{in} is the mean shielding in-plane and σ_{av} the overall mean value. The corresponding δ values (expressed in parts per million) were obtained by the rule $\sigma_{av}\times10^{6}+\delta=185.6$ (see Section 4.2). [38]Quaternary carbon atoms are typeset in boldface.

[b] $\Delta\delta=\delta_{calcd}-\delta_{exp}$.

Table 6. CTOCD-*PZ2*/6-31G**//6-31G computed ab initio absolute carbon nuclear shielding constants (σ_{av} in parts per million) of pyrene (**1**) and cyclopenta[*cd*]pyrene (**2**, See Fig. 1 for a generalized atom numbering scheme)[a]

Compound	Nucleus	σ_{out}	σ_{in}	σ_{av}	δ_{calcd}	δ_{exp}	$\Delta\delta$[b]
1	C*1*	191.9	−7.5	58.9	126.7	125.78	0.92
	C*2*	177.9	0.8	59.8	125.8	124.87	0.93
	C*5*	167.4	2.7	57.6	128.0	127.32	0.68
	C17	200.5	−11.6	59.1	126.5	124.61	1.89
	C18	194.6	−16.8	53.7	131.9	131.08	0.82
2	C*1*	186.8	−2.5	60.6	125.0	124.34	0.66
	C2	163.7	−7.3	49.7	135.9	135.58	0.32
	C*3*	149.6	−1.7	48.7	136.9	133.48	3.42
	C*4*	152.9	6.5	55.3	130.3	127.81	2.49
	C5	154.9	−5.8	47.8	137.8	139.07	−1.27
	C*6*	164.3	3.0	56.7	128.9	126.48	2.42
	C7	190.9	−15.2	53.5	132.1	131.90	0.20
	C*8*	177.4	−6.9	54.5	131.1	130.52	0.58
	C*9*	189.9	−5.2	59.8	125.8	126.94	−1.14
	C*10*	178.9	−5.0	56.3	129.3	128.54	0.76
	C11	195.7	−15.0	55.2	130.4	130.18	0.22
	C*12*	167.9	2.1	57.4	128.2	126.93	1.27
	C*13*	170.6	2.1	58.3	127.3	126.57	0.73
	C14	193.4	−16.9	53.2	132.4	130.87	1.53
	C*15*	176.5	3.5	61.1	124.5	122.68	1.82
	C16	201.0	−9.3	60.8	124.8	122.23	2.57
	C17	203.5	−5.5	64.1	121.5	120.69	0.81
	C18	180.2	−4.0	57.4	128.2	127.34	0.86

[a] σ_{out} is the component of the absolute shielding out-of-plane of the ring, σ_{in} is the mean shielding in-plane and σ_{av} the overall mean value. The corresponding δ values (expressed in parts per million) were obtained by the rule $\sigma_{av}\times10^6+\delta=185.6$ (see Section 4.2). [38]Quaternary carbon atoms are typeset in boldface.
[b] $\Delta\delta=\delta_{calcd}-\delta_{exp}$.

million, the deviations between the computed and experimental values, which appear to be the most significant for the carbon atoms of the cyclopenta moieties (range 2.1–4.5 ppm), are still sufficiently small (for all carbon atoms: range 0.0–4.6 ppm, see $\Delta\delta$ values in Tables 5 and 6) to render the computations a material aid for ^{13}C assignments at least in π-conjugated polycyclics. This is to an extent a fortunate consequence of the combination of basis set (6-31G**) and the CTOCD-*PZ2* method, as the results for ^{13}C shifts in the 6-31G** basis can be closer to experiment than 'accurate' Hartree–Fock limiting results.[37]

For **1**–**5** the computed ^{13}C chemical shifts can thus be used to validate the experimental LR-HETCOR ^{13}C assignments (Tables 5 and 6). As shown by the data, the assignments in which the intensity of the ^{13}C signals (cf. compounds **2**, **4** and **5**, vide supra) was used are confirmed by the computed CTOCD-*PZ2*/6-31G**//6-31G ^{13}C chemical shifts.

A comparison of the absolute carbon nuclear shield constants (σ_{av}) of **1**–**5** and their out-of-plane (σ_{out}) and mean in-plane (σ_{in}) components reveal that all carbon sites possess a large diamagnetic, σ_{out} component. The in-plane principal components approximately cancel each other leaving a relatively small, usually paramagnetic, mean in-plane σ_{in} value (Table 5). Similar behaviour has been found for other CP-PAHs.[38]

2.3. Substituent-induced ^{13}C chemical shifts: $\Delta\delta$

To gain insight into the extent to which mono- and dicyclopenta annelation influences the ^{13}C NMR chemical shifts of related carbon positions Substituent-Induced Chemical Shifts (SCS; ^{13}C $\Delta\delta$ in parts per million) were calculated using either the experimental or computed ab initio CTOCD-*PZ2*/6-31G**/6-31G ^{13}C chemical shifts. For the calculation of SCS $\Delta\delta_{exp}$ and $\Delta\delta_{calcd}$ values for CP-PAHs **3**–**5**, the ^{13}C chemical shifts of cyclopenta[*cd*]pyrene (**2**), were taken as a reference ($\Delta\delta_{exp}$(**2**) vs $\Delta\delta_{calcd}$ (**2**), Table 7). The $\Delta\delta_{exp}$ (**2**) and $\Delta\delta_{calcd}$ (**2**) values consistently show that the five-membered ring *ipso* carbon atoms of **3** and **4** behave differently from those of **5** (Table 7). The *ipso* carbon atom C*2* (Fig. 1) of **3** and **4** are deshielded by ca. 4–5 ppm ($\Delta\delta_{exp}$ (**2**) and $\Delta\delta_{calcd}$ (**2**)), whereas the other *ipso* carbon C*5* is

Table 7. Computed CTOCD-*PZ2*/6-31G**//6-31G ab initio ($\Delta\delta_{calcd}$) and experimental ($\Delta\delta_{exp}$) ^{13}C substituent-induced chemical shifts ($\Delta\delta$ (**2**); relative to cyclopenta[*cd*]pyrene (**2**)) for dicyclopenta[*cd*,*fg*]- (**3**), dicyclopenta[*cd*,*jk*]- (**4**) and dicyclopenta[*cd*,*mn*]pyrene (**5**, see Fig. 1 and also Tables 5 and 6)

Nucleus	**3**		**4**		**5**	
	$\Delta\delta$ (**2**)		$\Delta\delta$ (**2**)		$\Delta\delta$ (**2**)	
	Calcd	Exp	Calcd	Exp	Calcd	Exp
C*1*	−0.50	−0.89	−1.70	−1.94	−1.60	−5.57
C2	4.30	4.66	4.80	5.41	−1.20	0.05
C*3*	3.20	3.83	−0.60	0.01	2.60	1.55
C*4*	−0.30	0.06	3.90	3.82	−1.40	−1.28
C5	1.10	−1.13	1.50	1.35	0.00	−0.51
C*6*	***C5***[a]	***C5***[a]	C*13*[a]	C*13*[a]	0.10	−0.69
C7	***C18***[a]	***C18***[a]	***C14***[a]	***C14***[a]	−0.80	−4.34
C*8*	***C2***[a]	***C2***[a]	C*15*[a]	C*15*[a]	2.90	2.24
C*9*	C*1*[a]	C*1*[a]	C*1*[a]	C*1*[a]	−0.30	0.10
C*10*	C*15*[a]	C*15*[a]	***C2***[a]	***C2***[a]	[a]C*8*	[a]C*8*
C*11*	***C14***[a]	***C14***[a]	***C18***[a]	***C18***[a]	***C7***[a]	***C7***[a]
C*12*	C*13*[a]	C*13*[a]	***C5***[a]	***C5***[a]	C*6*[a]	C*6*[a]
C*13*	0.70	0.46	0.70	−0.85	***C5***[a]	***C5***[a]
C14	−0.90	−0.25	0.10	1.20	***C18***[a]	***C18***[a]
C*15*	2.80	4.39	5.40	6.95	***C2***[a]	***C2***[a]
C*16*	***C17***[a]	***C17***[a]	***C17***[a]	***C17***[a]	−1.30	−2.14
C17	0.50	0.22	0.30	0.75	−7.20	−5.98
C18	1.20	3.89	1.80	3.32	1.60	4.18

[a] Positions equivalent by molecular symmetry; quaternary carbon atoms are typeset in boldface.

slightly shielded in **3** ($\Delta\delta_{exp}$ (**2**), −1.13 ppm) but deshielded in **4** ($\Delta\delta_{exp}$ (**2**), 1.35 ppm). The corresponding $\Delta\delta_{calcd}$ (**2**) values are ca. 1 ppm for C*5* in both **3** and **4**. In contrast, the related $\Delta\delta_{exp}$ (**2**) values for **5** are 0.05 (C*2*) and −0.51 (C*5*) (for comparison: $\Delta\delta_{calcd}$ (**2**), −1.20 (C*2*) and 0.00 (C*5*)). This confirms that the topology of the two annelated cyclopenta moieties affects the electronic structure of **3–5** (see also Ref. 19). This is further substantiated by the $\Delta\delta_{exp}$ (**2**) and $\Delta\delta_{calcd}$ (**2**) values found for some of the more distant carbon atoms (e.g., C*8*, C*10*, C*15*, C*17* and C*18*) in the series **3–5** (Table 7 and Fig. 1), in which all the carbon atoms show a different response.

3. Conclusions

Complete ^{1}H and ^{13}C NMR assignments for the nonalternant (di-) cyclopenta-fused pyrene congeners **3–5** are reported. The experimental assignments are corroborated by chemical shift calculations using the all electron ab initio CTOCD-*PZ2*/6-31G** method. A comparison of the SCS values, $\Delta\delta_{exp}$ (**2**) and $\Delta\delta_{calcd}$ (**2**), shows that for **3–5** with **2** as a reference the number and topology of the annelated cyclopenta moieties markedly affects their electronic structure.

4. Experimental

CP-PAHs **2–5** were synthesized using Flash Vacuum Thermolysis (FVT). The corresponding (1-choroethenyl)-substituted PAH precursors were prepared as described previously.[1–3] Pure CP-PAHs **2–5** were isolated from the pyrolysates by re-crystallization from *n*-hexane. Caution: CP-PAHs **2–5** have to be handled according to the NIH guidelines for carcinogens (see also Ref. 16).

4.1. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded at 25 °C (Varian Unity Inova Spectrometer operating at 300.13 and 75.47 MHz, respectively). The two-dimensional (2D) NMR experiments were also performed on the Varian Unity Inova Spectrometer at 25 °C. In all the experiments, saturated $CDCl_3$ solutions of the CP-PAH **1–5** were used. ^{1}H and ^{13}C NMR chemical shifts are reported in parts per million using residual $CHCl_3$ (δ 7.26 ppm) and $CDCl_3$ (δ 77.00 ppm), respectively, as an internal standard.

Prior to the Long-Range-Heteronuclear Chemical Shift Correlation LR-HETCOR experiments, the proton-coupled C–H ^{13}C spectra of **2–5** were collected to establish the magnitude of $^{1}J_{C-H}$ coupling constants. The ^{1}H decay time (T_1) was determined in order to optimise the relaxation delay in the 2D NMR experiments. Nuclear Overhauser Effect Spectroscopy (NOESY): the relaxation delay was set approximately at T_1 for each compound, mixing time 0.75 s, acquisition time 1.3 s, 2D width 700 Hz and number of increments 64. The recording time of the NOESY spectra was in the range 12–20 h. HETCOR and LR-HETCOR: in the HETCOR and LR-HETCOR experiments, a $^{1}J_{C-H}$= 160 Hz coupling constant was used. The relaxation delay was set as 1 s for HETCOR and as either 1 s or 4 s for LR-HETCOR in order to obtain the two- ($^{2}J_{C-H}$) and three-bond ($^{3}J_{C-H}$) C–H coupling constants. HETCOR and LR-HETCOR experiments were recorded with a proton and carbon sweep width of 500–600 and 2500–3000 Hz, respectively. The number of increments was 32. Recording times were ca. 24 h for the HETCOR spectra and 60–72 h for the LR-HETCOR spectra.

4.2. Computations

The absolute carbon nuclear shielding constants (σ_{av} in parts per million), their out-of-plane (σ_{out} in parts per million) and mean in-plane (σ_{in} in parts per million) components of CP-PAH **1–5** were computed using the *PZ2* (paramagnetic zero) variant[38] of the all electron ab initio continuous transformation of origin of current density (CTOCD) method as implemented in the Exeter version of SYSMO,[36] all within the 6-31G** basis. The σ_{av} values ($\sigma_{av}=1/3(\sigma_{out}+2\sigma_{in})$) were converted into δ values by the rule $\sigma_{av}\times10^{6}+\delta=185.6$.[37]

The molecular geometries of **1–5** were optimized at the restricted Hartree–Fock level in the 6-31G basis using the GAMESS-UK program[39] and were taken from a previous study.[25] All optimised geometries (**1** (D_{2h}), **2** (C_s), **3** (C_{2v}), **4** (C_{2h}) and **5** (C_{2v})) were characterized as genuine minima by Hessian calculations.

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Novel 24-nor-, 24-nor-2,3-*seco*-, and 3,24-dinor-2,4-*seco*-ursane triterpenes from *Diospyros decandra*: evidences for ring A biosynthetic transformations

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Abstract—Novel 24-nor-, 24-nor-2,3-*seco*, and 3,24-dinor-2,4-*seco*-ursane type triterpenes have been isolated along with one known compound from the stem bark of *Diospyros decandra*. The structures of these highly oxidized metabolites were established on the basis of extensive NMR and MS studies. They possibly represent intermediates in the biosynthetic transformation of ring A in an ursane triterpene. Some isolates showed mild anti-mycobacterial activity against *Mycobacterium tuberculosis* with MIC values ranging from 25 to 200 μg/mL.

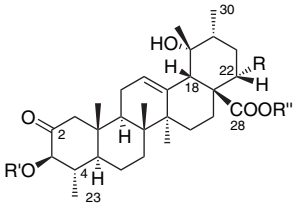
1. Introduction

Diospyros species are found in most part of tropics and subtropics and many of them have been used in traditional medicines. A considerable number of species has been investigated and the presence of quinones,[1–3] triterpenes,[1,4,5] flavonoids,[1] coumarin derivatives,[1,6] and phenolic glycosides[1,4] has been reported. In our ongoing search for bioactive compounds from Thai medicinal plants we have studied *Diospyros decandra* Lour. (syn. *Diospyros packmanii*) known in Thailand as 'Chan'. This plant is a tree in the Ebenaceae family, which grows to about 10–20 m in height. Its pale yellow flowers are small and fragrant. The round fruits are edible and fragrant when mature. The wood decoction is drunk as a blood tonic, anthelmintic, and also to relieve fever.[7]

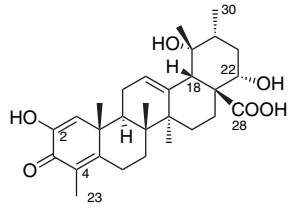
2. Results and discussion

Repeated column chromatography of the chloroform extract of the bark of *D. decandra* resulted in the isolation of five novel compounds, in addition to one known compound. The known compound was identified as betulinic acid.[8]

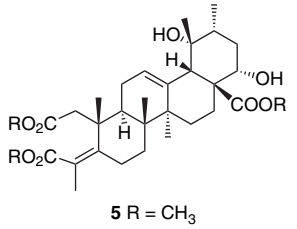
Compound **1** was obtained as colorless solid, mp 161–162 °C. The FTIR spectrum showed the presence of a carboxyl group at ν_{max} 3569 and 1709 cm^{-1} as well as the



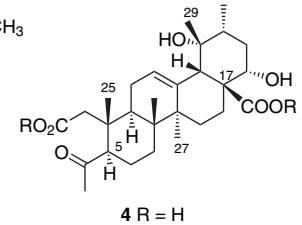
1 R = R' = R'' = H
1a R = R'' = H, R' = $COCH_3$
1b R = H, R' = $COCH_3$, R'' = CH_3
2 R = OH, R' = R'' = H
2a R = $OCOCH_3$, R' = $COCH_3$, R'' = H
2b R = $OCOCH_3$, R' = $COCH_3$, R'' = CH_3



3



5 R = CH_3



4 R = H
4a R = CH_3

C=C stretching at ν_{max} 1686 cm^{-1}. The HRFABMS negative ionization mode displayed a $[M-H]^-$ ion at *m/z* 471.3118 corresponding to $C_{29}H_{43}O_5$ or to M^+ of

Keywords: *Diospyros decandra*; Ebenaceae; 19-Hydroxy ursane triterpenes.
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$C_{29}H_{44}O_5$. The ^{13}C NMR spectrum showed 29 carbon signals comprising of six methyls, eight methylenes, seven methines, and eight quaternary carbons including one carboxyl, one keto, and one olefinic quaternary carbon. The detection of a secondary methyl signal at δ_H 0.91 (d, 6.8), and a trisubstituted olefinic proton signal at δ_H 5.32 [δ_C 128.5 (d) and 138.4 (s)] provide the most useful indications for an urs-12-ene triterpene skeleton.[9] The presence of a carboxyl group at C-28 and an OH group at C-19 was established from long range ^1H–^{13}C correlations between H-18 (δ_H 2.52)/C-12 (δ_C 128.5), C-16 (δ_C 25.4), C-17 (δ_C 47.6), C-19 (δ_C 73.0), C-20 (δ_C 41.1), C-28 (δ_C 182.0), and C-29 (δ_C 27.3). The placement of a keto group at C-2 (δ_C 210.3), an additional secondary hydroxyl group at C-3 (δ_H 3.62, d, J=10.2 Hz, δ_C 80.5), and only one methyl group connected to C-4 (δ_H 1.14, d, J=6.2 Hz, δ_C 16.6, q, assigned for H_3-23), instead of two methyl groups as commonly found in an ursane triterpene, was proven by the ^1H–^{13}C correlations between H-5 (δ_H 1.25)/C-1 (δ_C 52.2, t), C-3, C-23, and C-25 (δ_C 14.7), and between H-1(δ_H 2.03 and 2.52)/C-2, C-3, and C-25. A 3-β-hydroxy-24-nor-ursane backbone was established by examination of the NOE effects from the NOESY spectrum of **1** (Fig. 1). The NOE correlations between H-5/H-3, and a methyl proton doublet signal at δ_H 1.14, in conjunction with NOE interaction of H_3-25/H-4 indicated that both H-3 and a methyl group bonded to C-4 are α-oriented. The α-oriented methyl group at C-4 corresponds to H_3-23 of the ursane skeleton. The 'nor' methyl group is thus the H_3-24. The NOE effects between H-12/H-18, and H_3-29 clearly established ring E as cis-fused ring.[10] The remaining ^1H and ^{13}C resonances could be unambiguously assigned by the use of ^1H–^1H COSY, HMQC, and HMBC correlations spectra (Tables 1 and 2). Compound **1**, for which the trivial name diospyric acid A was proposed, was identified as 2-oxo-3β,19α-dihydroxy-24-nor-urs-12-en-28-oic acid. Further confirmation of the presence of a secondary hydroxyl and a carboxylic functional group was made by acetylating **1** with acetic anhydride in pyridine to produce **1a** and further methylation of **1a** with CH_2N_2 to produce the methyl ester **1b**. The ^1H NMR spectrum of **1a** indicated the presence of an acetyl group at δ_H 2.16 (s) and a downfield shift of the oxymethine proton at δ_H 4.80 (d, J=11.2 Hz, H-3). An additional OCH_3 signal at δ_H 3.56 (s) was observed in the ^1H NMR spectrum of **1b**. Full assignments of the ^1H and ^{13}C NMR chemical shifts of **1**, **1a**, and **1b** are shown in Tables 1 and 2.

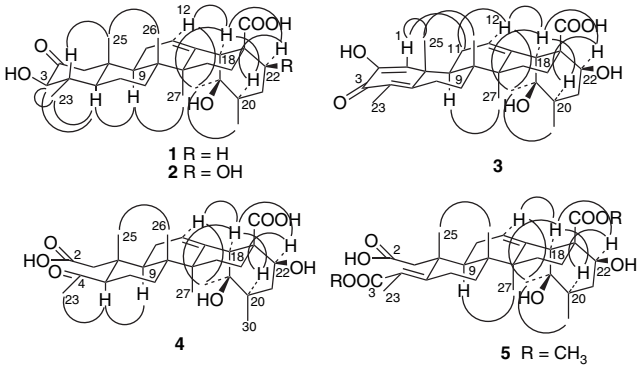


Figure 1. Important NOE effects in compounds **1**–**5**.

Table 1. ^1H NMR spectroscopic data of **1**–**2b** ($CDCl_3$)

Position	1	1a	1b	2	2a	2b
1	2.03 (d, 12.7) 2.52[a]	2.06 (d, 12.9) 2.47 (d, 12.9)	2.08 (α-H), 2.48	2.02, 2.48[e]	2.05 (d, 12.8) 2.50 (d, 12.8)	2.05 (d, 12.3) 2.46 (d, 12.8)
3	3.62 (d, 10.2)	4.80 (d, 11.2)	4.77 (d, 11.3)	3.61[f]	4.78 (d, 11.2)	4.78 (d, 11.2)
4	1.56	1.86[b]	1.90	1.48	1.85	1.86
5	1.25	1.34[c]	1.32	1.25	1.33	1.31
6	1.21, 1.70	1.18, 1.73	1.22, 1.72	1.21, 1.71	1.23, 1.74	nd
7	1.32, 1.53	1.36[c], 1.57	1.35, 1.57	1.32, 1.53	1.34, 1.56	1.34, 1.55
8	—	—	—	—	—	—
9	1.89	1.88[b]	1.88	1.87	1.92	1.90
10	—	—	—	—	—	—
11	1.95	1.97	1.95	1.84, 1.97	1.84, 1.96	1.85, 1.97
12	5.32 (br s)	5.33 (br s)	5.32 (br s)	5.30 (br s)	5.32 (br s)	5.31 (br s)
15	1.74, 2.09	1.04, 1.75	1.02, 1.60	1.23, 1.65	1.08, 1.83	1.06, 1.76
16	1.58, 2.52	1.55, 2.51[d]	1.58, 2.51	1.88, 2.22	1.96, 2.36 (ddd, 4.9, 8.4, 13.3)	1.96, 2.26 (ddd, 4.9, 8.5, 13.4)
18	2.52[a]	2.53[d]	2.57	2.46[e]	2.55 (br s)	2.61 (br s)
20	1.37	1.38	1.43	1.54	1.61	1.58
21	1.25, 1.72	1.26, 1.68	1.24	1.49, 1.75	1.44, 1.84	1.43, 1.84
22	1.63, 1.77	1.63, 1.78	1.59 (β-H), 1.72	3.64 (m)[f]	5.02 (dd, 4.4, 12.1)	5.00 (dd, 4.6, 12.3)
23	1.14 (d, 16.2)	1.04 (d, 6.3)	1.03 (d, 6.2)	1.13 (d, 5.7)	1.03 (d, 6.3)	1.03 (d, 6.3)
25	0.74 (s)	0.77 (s)	0.81	0.74[g]	0.76 (s)	0.78 (s)
26	0.72 (s)	0.70 (s)	0.68	0.74[g]	0.68 (s)	0.64 (s)
27	1.26 (s)	1.28 (s)	1.28	1.26	1.29 (s)	1.29 (s)
28	—	—	—	—	—	—
29	1.17 (s)	1.18 (s)	1.20 (s)	1.14	1.17 (s)	1.18 (s)
30	0.91 (d, 6.8)	0.92 (d, 6.7)	0.92 (d, 6.7)	0.93 (d, 6.5)	0.94 (d, 6.6)	0.95 (d, 6.7)
3-COCH_3	—	2.16 (s)	2.17 (s)	—	2.17 (s)	2.16 (s)
22-COCH_3	—	—	—	—	1.99 (s)	2.00 (s)
28-OCH_3	—	—	3.56 (s)	—	—	3.52 (s)

nd=not detected.
[a–g] Overlapping signals.

Table 2. ^{13}C NMR spectroscopic data of **1**–**2b** ($CDCl_3$)

Position	1	1a	1b	2	2a	2b
1	52.5 (t)	53.7 (t)	53.7 (t)	52.7 (t)	53.7 (t)	53.7 (t)
2	210.7 (s)	203.5 (s)	203.4 (s)	210.9 (s)	203.5 (s)	203.4 (s)
3	80.5 (d)	81.7 (d)	81.7 (d)	80.5 (d)	81.6 (d)	81.6 (d)
4	42.2 (d)[a]	38.2 (d)	38.2 (d)	38.9 (d)	38.1 (d)	38.2 (d)
5	50.4 (d)	51.1 (d)	51.2 (d)	50.4 (d)	51.1 (d)	51.1 (d)
6	20.8 (t)	21.0 (t)	21.0 (t)	20.7 (t)	21.0 (t)	21.0 (t)
7	31.6 (t)	31.6 (t)	31.6 (t)	31.6 (t)	31.4 (t)	31.4 (t)
8	40.0 (s)	40.0 (s)	39.9 (s)	39.9 (s)	40.1 (s)	40.1 (s)
9	44.6 (d)	44.5 (d)	44.4 (d)	44.5 (d)	44.3 (d)	44.3 (d)
10	42.2 (s)[a]	41.5 (s)[b]	41.4 (s)[d]	42.0 (s)[e]	41.4 (s)	41.4 (s)
11	24.0 (t)	24.0 (t)	24.0 (t)	24.0 (t)	24.0 (t)	24.0 (t)
12	128.5 (d)	128.6 (d)	128.4 (d)	128.5 (d)	129.3 (d)	129.2 (d)
13	138.4 (s)	138.3 (s)	138.5 (s)	138.0 (s)	137.4 (s)	137.7 (s)
14	41.5 (s)	41.5 (s)[b]	41.4 (s)[d]	42.0 (s)[e]	41.6 (s)	41.6 (s)
15	28.2 (t)	28.2 (t)	28.2 (t)	27.7 (t)	27.3 (t)	27.4 (t)
16	25.4 (t)	25.4 (t)	25.5 (t)	18.5 (t)	18.5 (t)	18.7 (t)
17	47.6 (s)	47.8 (s)	47.9 (s)	53.2 (s)	51.8 (s)	52.0 (s)
18	53.0 (d)	52.9 (d)	53.3 (d)	53.5 (d)	53.8 (d)	54.0 (d)
19	73.0 (s)	73.1 (s)	73.1 (s)	72.7 (s)	72.5 (s)	72.5 (s)
20	41.1 (d)	41.1 (d)	41.1 (d)	42.8 (d)	39.1 (d)	39.2 (d)
21	25.9 (t)	25.9 (t)	26.0 (t)	33.3 (t)	31.1 (t)	31.3 (t)
22	37.3 (t)	37.4 (t)	37.3 (t)	73.6 (d)	74.9 (d)	74.7 (d)
23	16.6 (q)	16.4 (q)[c]	16.4 (q)	16.5 (q)	16.4 (q)	16.4 (q)
25	14.7 (q)	14.5 (q)	14.5 (q)	14.7 (q)	14.5 (q)	14.2 (q)
26	16.3 (q)	16.4 (q)[c]	16.3 (q)	16.1 (q)	15.9 (q)	16.1 (q)
27	24.2 (q)	24.3 (q)	24.2 (q)	24.1 (q)	24.8 (q)	24.6 (q)
28	182.0 (s)	183.8 (s)	178.2 (s)	180.0 (s)	180.2 (s)	175.0 (s)
29	27.3 (q)	27.4 (q)	27.4 (q)	26.9 (q)	27.1 (q)	27.0 (q)
30	16.0 (q)	16.1 (q)	16.0 (q)	15.7 (q)	15.5 (q)	15.5 (q)
3-*CO*CH_3	—	170.4 (s)	170.4 (s)	—	170.4 (s)	170.5 (s)
3-CO*C*H_3	—	20.6 (q)	20.6 (q)	—	20.6 (q)	20.6 (q)[f]
22-CO*C*H_3	—	—	51.7 (q)	—	20.9 (q)	20.6 (q)[f]
22-*CO*CH_3	—	—	—	—	170.6 (s)	170.3 (s)
28-O*C*H_3	—	—	—	—	—	52.0 (q)

[a–f] Overlapping signals.

Compound **2** was obtained as a crystalline solid. The FTIR spectrum showed the presence of a carboxyl group at ν_{max} 3397 and 1711 cm^{-1}, and a C=C stretching at ν_{max} 1635 cm^{-1}. The HRFABMS negative ionization mode showed a $[M-H]^-$ ion at *m/z* 487.3067, which corresponded to $C_{29}H_{43}O_6$. The ^{13}C NMR spectrum displayed 29 carbon signals comprising six methyls, seven methylenes, eight methines, and eight quaternary carbons including one carboxyl, one keto and one olefinic quaternary carbon. The ^1H and ^{13}C NMR spectra showed a close resemblance to those of compound **1**, except for the presence of an additional secondary alcohol group (δ_H 3.64, m, and δ_C 73.6, d). The position of an extra hydroxyl group at C-22 was established from the ^1H–^{13}C long-range correlations between H-22/C-16 (δ_C 18.5), C-20 (δ_C 42.8), C-21 (δ_C 33.3), and C-28 (δ_C 180.0). Treatment of **2** with acetic anhydride-pyridine gave a di-acetate derivative (**2a**), which displayed two additional methyl singlet signals at δ_H 1.99 and 2.17 in the ^1H NMR spectrum, and as a consequence, acetylation also caused the downfield shift of the methine protons at C-3 and C-22 to δ_H 4.78 (d, *J*=11.2 Hz) and 5.02 (dd, *J*=12.1 and 4.4 Hz), respectively. The use of diazomethane to react with **2a** gave the corresponding mono-methyl ester (**2b**). The ^1H and ^{13}C NMR chemical shifts assignment of **2**, **2a**, and **2b** were achieved from ^1H–^1H COSY, HMQC, and HMBC NMR spectra (Tables 1 and 2). The relative stereochemistry of **2** in most of the molecule is identical to that of **1** as shown by NOESY spectra of **1**, **2**, and their derivatives. Additional NOE correlations between H-22/H-18 and H-20 indicated a relative stereochemistry of **2** as shown in Figure. 1. In the ^1H NMR spectrum of **2a** and **2b**, the signal at ~δ_H 5.00 (dd, *J*=12.1 and 4.4 Hz) was assigned to H-22, this is in accord with values reported for H-22 in methyl musangate A isolated from *Musanga cecropioides*,[10] and gave further support to the assignment of OH-22 of **2**, **2a**, and **2b** as α-equatorial with respect to ring E. Compound **2**, which was assigned a trivial name as diospyric acid B, and was identified as 2-oxo-3β,19α,22α-trihydroxy-24-nor-urs-12-en-28-oic acid.

Compound **3** was obtained as solid, mp 182–184 °C. The FTIR spectrum indicated the presence of a carboxyl (ν_{max} 3419 cm^{-1}) and a conjugated keto (ν_{max} 1697 cm^{-1}) function. The HREIMS negative ionization mode displayed a $[M-H]^-$ ion at *m/z* 483.2752 requiring $C_{29}H_{39}O_6$. The ^{13}C NMR spectrum showed 29 carbon signals comprising six methyls, six methylenes, six methines, and 11 quaternary carbons including one keto, one carboxyl, one oxygenated, and four olefinic carbons (Table 3). The ^1H NMR spectrum, which displayed a carbinolic proton at δ_H 3.64 (dd, *J*=11.6 and 4.2 Hz), an olefinic proton at δ_H 5.34 and a methyl signal at δ_H 0.91 (d, *J*=6.6 Hz) indicated that rings C–E part of the molecule is similar to compound **2**. The 2-hydroxy-$\Delta^{1,4}$-dien-3-one subunit was established from the key ^3J ^1H–^{13}C correlations of the less shielded olefinic proton at δ_H 6.24 (H-1)/C-9 (δ_C 44.7), the methyl carbon at δ_C 21.1(C-25), oxygenated C-2 (δ_C 144.3), and C-3 (δ_C 181.5). The long-range correlations from the vinyl methyl

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of **3–5** ($CDCl_3$)

Position	δ_H **3**	δ_H **4**	δ_H **4a**	δ_H **5**	δ_C **3**	δ_C **4**	δ_C **4a**	δ_C **5**
1	6.24 (s)	2.18 (d, 13.6) 2.44 (d, 13.6)	2.27 (d, 14.5) 2.37 (d, 14.5)	2.54 (d, 16.3) 2.79 (d, 16.3)	125.4 (d)	44.4 (t)	43.5 (t)	44.8 (t)
2	—	—	—	—	144.3 (s)	173.4 (s)	172.2 (s)	172.0 (s)
3	—	—	—	—	181.5 (s)	—	—	174.0 (s)
4	—	—	—	—	126.0 (s)	216.9 (s)	212.8 (s)	120.3 (s)
5	—	2.66 (dd, 12.7, 2.7)	2.88 (dd, 12.2, 3.0)	—	167.5 (s)	56.7 (d)	56.1 (d)	145.3 (s)
6	2.29, 2.70	1.71, 1.83	1.64, 1.72	2.31	25.2 (t)	21.7 (t)	21.9 (t)	25.7 (t)
7	1.57	1.25, 1.54	1.30 (dt, 13.2, 3.3), 1.56	1.19, 1.97	34.7 (t)	29.7 (t)	31.1 (t)	29.8 (t)
8	—	—	—	—	40.1 (s)	39.8 (s)	40.0 (s)	38.8 (s)
9	1.85	2.14 (dd, 6.4, 4.4)	2.07 (dd, 16.1, 11.0)[a]	2.68 (dd, 8.1, 9.2)	44.7 (d)	39.1 (d)	39.0 (d)	37.9 (d)
10	—	—	—	—	43.7 (s)	40.2 (s)	39.5 (s)	41.3 (s)
11	2.24	2.05, 2.32	2.06[a]	2.04 (dd, 3.5, 7.2)	25.8 (t)	23.5 (t)	23.5 (t)	25.1 (t)
12	5.34 (br s)	5.35 (obs t, 3.7)	5.34 (obs dd, 3.3, 2.6)	5.43 (t, 3.4)	128.6 (d)	129.0 (d)	129.0 (d)	130.6 (d)
13	—	—	—	—	138.1 (s)	137.2 (s)	137.6 (s)	137.3 (s)
14	—	—	—	—	42.8 (s)	42.4 (s)	42.3 (s)	43.2 (s)
15	1.08, 1.65	1.54	1.52	1.29, 1.53	28.2 (t)	27.8 (t)	27.8 (t)	27.6 (t)
16	2.22, 1.86	1.91, 2.30	1.95, 2.26	1.96, 2.26 (dt, 3.8, 1.4)	18.6 (t)	18.4 (t)	18.5 (t)	18.6 (t)[c]
17	—	—	—	—	53.4 (s)	53.6 (s)	53.9 (s)	54.0 (s)
18	2.47 (br s)	2.48 (br s)	2.53 (br s)	2.55 (s)	53.6 (d)	53.4 (d)	53.5 (d)	54.4 (d)
19	—	—	—	—	72.5 (s)	72.7 (s)	72.9 (s)	72.4 (s)
20	1.41	1.49	1.54[b]	1.51 (m)	38.9 (d)	38.9 (d)	39.0 (d)	39.0 (d)
21	1.48, 1.75	1.53 1.80 (q, 12.4)	1.54,[b] 1.75 (dd, 12.0, 11.3)	1.56 (ddd, 12.6, 4.1, 2.9), 1.79 (q, 12.2)	33.3 (t)	33.7 (t)	33.6 (t)	33.2 (t)
22	3.64 (dd, 11.6, 4.2)	3.71 (dd, 11.4, 4.4)	3.61 (dd, 12.0, 4.4)	3.64 (dd, 11.9, 4.3)	73.5 (d)	73.7 (d)	73.7 (d)	73.5 (d)
23	1.94 (s)	2.24	2.21 (s)	1.85 (s)	10.5 (q)	30.3 (q)	31.3 (q)	18.7 (q)[c]
25	1.23 (s)	1.04 (s)	1.09 (s)	1.21 (s)	21.1 (q)	18.5 (q)	17.8 (q)	23.2 (q)
26	1.02 (s)	0.82 (s)	0.72 (s)	0.80 (s)	16.3 (q)	16.6 (q)	16.4 (q)	19.0 (q)
27	1.09 (s)	1.23 (s)	1.23 (s)	1.35 (s)	23.6 (q)	24.0 (q)	24.1 (q)	23.5 (q)
28	—	—	—	—	178.9 (s)	181.0 (s)	176.4 (s)	177.2 (s)
29	1.11 (s)	1.17	1.18 (s)	1.20 (s)	26.8 (q)	27.1 (q)	27.2 (q)	27.2 (q)
30	0.91 (d, 6.6)	0.97 (d, 6.8)	0.96 (d, 6.6)	0.99 (d, 6.4)	15.7 (q)	15.8 (q)	15.6 (q)	15.7 (q)
2-OCH_3	—	—	3.64 (s)	3.63 (s)	—	—	51.0 (q)	51.2 (q)
3-OCH_3	—	—	—	3.71 (s)	—	—	—	51.5 (q)
28-OCH_3	—	—	3.65 (s)	3.67 (s)	—	—	51.9 (q)	52.0 (q)

[a–c] Overlapping signals.

proton (δ_H 1.94) to C-3, C-4 (δ_C 126.0), and C-5 (δ_C 167.5) confirmed the connectivity of this methyl group to C-4. The NOESY spectrum showed NOE effects as found in compound **2**, with additional interactions between H-1/H-11 and H_3-25 (Fig. 1). Compound **3**, which was given a trivial name as diospyric acid C, could be identified as 3-oxo-2,19α,22α-trihydroxy-24-nor-urs-1,4,12-trien-28-oic acid.

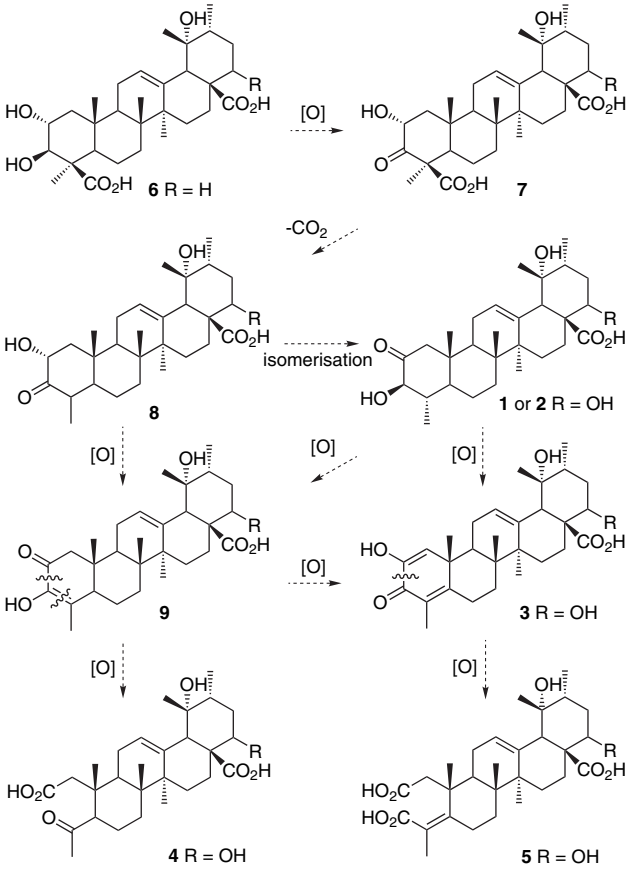
Compound **4** was obtained as solid. The FTIR spectrum showed the presence of a carboxyl group at ν_{max} 3554 and 1690 cm^{-1}. The HRFABMS negative ionization mode displayed a $[M-H]^-$ ion at *m/z* 489.2857 corresponding to the elemental formula of $C_{28}H_{41}O_7$. The ^{13}C NMR spectrum showed 28 carbon signals comprising six methyls, seven methylenes, six methines, and nine quaternary carbons including one keto and two carboxyl carbons. A singlet signal at δ_H 2.24, and a keto ^{13}C signal at δ_C 216.9, implied the presence of a CH_3CO moiety. The absence of the secondary methyl group doublet signal at ~δ_H 1.1 and a less shielded oxymethine proton doublet at ~δ_H 3.6, which were assigned as H_3-23 and H-3 of compounds **1** and **2**, respectively, indicated further loss of one more carbon atom of ring A. The ^{1}H–^{13}C correlations between H-5 (δ_H 2.66)/C-4 (δ_C 216.9), C-6 (δ_C 21.7), C-10 (δ_C 40.2), and C-25 (δ_C 18.5) requires the placement of a keto group at C-4 and a rupture of bond joining C-3 and C-4. The location of a carboxylic acid as well as its relationship to the B ring was established by ^{1}H–^{13}C correlations between H-1 (δ_H 2.18 and 2.44)/C-2 (δ_C 173.4), C-5 (δ_C 56.7), C-9 (δ_C 39.1), C-10 (δ_C 40.2), and C-25 (δ_C 18.5). The relative stereochemistry of **4** (Fig. 1) was deduced by NOESY and was very similar to the already described compounds **1** and **2**. Compound **4**, which was given a trivial name of diospyric acid D, was identified as 4-oxo-19α,22α-dihydroxy-3,24-dinor-2,4-*seco*-urs-12-en-2,28-dioic acid. Compound **4a**, which is the dimethyl ester of **4**, was obtained after reacting **4** with ethereal solution of diazomethane. The ^{1}H and ^{13}C NMR spectroscopic data of **4a** are also included in Table 3.

Compound **5** was obtained in a small quantity after methylation of the crude fraction containing **4** using diazomethane and purification by HPLC. The HREIMS of **5** showed a $[M]^+$ ion at *m/z* 560.3441 corresponding to $C_{32}H_{48}O_8$. The ^{1}H NMR spectrum revealed three methyl ester singlet signals at δ_H 3.63, 3.67, and 3.71, indicating the presence of three carboxyl groups in the parent compound. Comparison of the 1D and 2D NMR spectra of **5** with the respective data of **2** and **4** results the same configurations of rings B–E in **5**. The absence of a doublet signal at approximately δ_H 1.04–1.14 (assigned to H_3-23 in **2**), together with the presence of a low-field methyl signal at δ_H 1.84, assignable to CH_3–C═C, indicated differences in ring A compared to **2** and **4**. Long-range ^{1}H–^{13}C correlations of the signal at δ_H 1.84 (H_3-23) to ^{13}C NMR signals at δ_C 174.0 (s), 120.1

(s), and 145.0 (s) in particular, indicated a carboxyl group at C-3 and a double bond between C-4 and C-5. Heteronuclear long-range coupling between H-25/C-1, C-5, and C-10 as well as between the diastereotopic methylene proton signals at δ_H 2.54 and 2.78 (H_2-1) to the ^{13}C NMR signals at δ_C 171.9 (C-2, s), 145.0 (C-5, s), and 23.0 (C-25, q) indicated the presence of the second carboxyl group at C-2. Rupture of bond joining C-2 to C-3 was therefore envisaged. The third carboxyl group at C-28 could be detected from the HMBC correlations of signals at δ_H 2.55 (H-18) and 2.27 (H-16) to a carboxyl carbon signal at δ_C 177.2 (C-28, s). The ROESY spectrum indicated similar spatial arrangements of **5** to those of **4** (Fig. 1). All ^1H and ^{13}C chemical shifts derived from HMBC, HMQC, and dqf COSY NMR spectra are listed in Table 3. Compound **5** could thus be proposed as the trimethyl ester of the naturally occurring 19α,22α-dihydroxy-24-nor-2,3-*seco*-urs-12-en-2,3,28-trioic acid, which has been trivially named as diospyric acid E.

To the best of our knowledge, these are among the first examples of the occurrence of a 24-nor-ursane, as well as, a 24-nor-2,3-*seco*-, and 3, 24-dinor-2,4-*seco*-ursane type triterpenes in nature.

One plausible biogenetic pathway of these compounds could therefore be proposed (Scheme 1). Vismiaefolic acid, 2α,3β,19α,-trihydroxyurs-12-ene-24,28-dioic acid (**6**)[11] precipitated out of the MeOH extract,[12] was proposed as a precursor of 24-nor-ursane compounds (**1** and **2**). Oxidation of **6** at C-3-OH gave a corresponding 3-keto acid (**7**), from which further decarboxylation led to **8** and double bond isomerization in **8** led to compounds **1** or **2**. A 2,3-dicarbonyl compound (**9**) could be formed as a result of oxidation of **1** or **2** and **8** at ring A. Upon further oxidation of **9** with a loss of one molecule of CO_2 caused cleavage of bonds joining C-2/C-3 and C-3/C-4, could lead to **4**. Formation of compound **3** from **1** or **2** or **9** is probable through oxidation. Oxidative cleavage of bond joining C-2/C-3 in **3** lead to compound **5**. Although a compound of type **9** was not isolated in the present study, there has been a report of the isolation of an oleanane triterpene with 3-hydroxy-3,4-en-2-one subunit in ring A, as proposed for **9** from *Physena madagascariensis*.[13]



Scheme 1. Biogenetic pathways to the formation of compounds **1**–**5**.

Compounds **1**–**4** and betulinic acid were tested for their antitubercular, antifungal, and antimalarial activities. Betulinic acid and **1** showed moderate to weak anti-TB activity with MIC values of 25 and 200 μg/mL, respectively. Betulinic acid and **2** showed inhibition activity against *Candida albicans* with IC_{50} values of 27.2 and 42.6 μg/mL, respectively. All five compounds were inactive in an antimalarial assay. Due to the scarcity of pure compounds, only **1**–**3** were further tested for their cytotoxicity against the breast cancer (BC), nasopharyngeal carcinoma (KB) and human small cell lung NCI-H187 cell lines, and **3** exhibited very mild activity against NCI-H187 cell line with IC_{50} of 12.6 μg/mL, while **1** and **2** were inactive with all cell lines at 20 μg/mL.

3. Experimental

3.1. General

The specific rotations were measured by a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin Elmer Spectrum One FTIR spectrometer with universal ATR accessory. EIMS and HREIMS spectra were recorded on a Finnigan MAT 90 instrument. ^1H and ^{13}C spectra were obtained with a Bruker AVANCE 400 MHz and a Varian 500 MHz spectrometer with solvent signal as internal reference.

3.2. Plant material

The bark of *D. decandra* was collected from Suratthani Province, in 1995. The botanical identification was kindly made by Assoc. Professor Dr. Nijsiri Ruangrungsi, Department of Pharmacognosy, Faculty of Pharmaceutical Science, Chulalongkorn University. A voucher specimen (SSDD/1997) is deposited at the Chemistry Department, Faculty of Science, Ramkhamhaeng University, Bangkok.

3.3. Extraction and isolation

The pulverized dry bark (868.5 g) was defatted with hexane, then extracted using Soxhlet extractor with $CHCl_3$ and MeOH to yield $CHCl_3$ (18.92 g) and MeOH (176.02 g) extracts. The $CHCl_3$ extract was subjected to gradient column chromatography (silica gel, hexane to $CHCl_3$–MeOH 40:60) to obtain three major fractions. The most polar fraction, fraction 3 was purified using silica gel column chromatography (hexane–$CHCl_3$ 50:50 to $CHCl_3$–MeOH 80:20) to obtain three subfractions (3.1–3.3), further purification of subfraction 3.2 (2×, silica gel, hexane–$CHCl_3$ 65:35 to

$CHCl_3$–MeOH 10:90 then $CHCl_3$ to $CHCl_3$–MeOH 90:10) gave subfractions 3.2.1–3.2.4. Subfraction 3.2.3 was rechromatographed (silica gel, hexane–$CHCl_3$ 20:80 to $CHCl_3$–MeOH 10:90), and gave compound **1** (50 mg). Acetylation of **1** (20 mg) using acetic anhydride–pyridine gave **1a** (18 mg). Methylation of **1a** (10 mg) with CH_2N_2 gave **1b** (10 mg). Fraction 3.2.1 after purification using silica gel column chromatography (2×, hexane–EtOAc 80:20 to 50:50 then hexane–EtOAc 85:15) gave betulinic acid (8.8 mg). Column chromatography of subfraction 3.3 (silica gel, $CHCl_3$ to $CHCl_3$–MeOH 80:20) gave four subfractions (3.3.1–3.3.4). Subfraction 3.3.3 after purification (silica gel, hexane–EtOAc 50:50 to EtOAc–MeOH 80:20) gave subfractions 3.3.3.1–3.3.3.4. Subfraction 3.3.3.3 contained compound **2** (48 mg). Compound **2a** (18 mg) was obtained from **2** (20 mg) after acetylation. Methylation of **2a** (10 mg) gave the methyl ester **2b** (9.5 mg). Purification of subfraction 3.3.2 using column chromatography (silica gel, hexane–EtOAc 60:40 to EtOAc–MeOH 90:10) gave four subfractions (3.3.2.1–3.3.2.4). Compound **3** (12.7 mg) was obtained from subfraction 3.3.2.3 after chromatographic separation ($C_{18,}$ MeOH–H_2O 50:50 to MeOH then silica gel, hexane–EtOAc 70:30). Subfraction 3.3.2.2 after reversed phase column chromatography (C_{18}, MeOH–H_2O 50:50 to MeOH) gave four subfractions (3.3.2.2.1–3.3.2.2.4). Compound **4** (7.7 mg) was obtained after subfraction 3.3.2.2.2 was further purified (silica gel, CH_2Cl_2–MeOH 98:2). Methylation of subfraction 3.3.2.2.3 using CH_2N_2 and further purification using HPLC (RP-18 Lichrosphere 100×5 mm, MeOH–H_2O 70:30, flow rate 0.7 mL/min) gave **4a** (t_R=20.9 min, 1.5 mg) and **5** (t_R=30.2 min, 0.4 mg).

3.3.1. 2-Oxo-3β,19α-dihydroxy-24-nor-urs-12-en-28-oic acid (1). Colorless needles, mp 161–162 °C; R_f=0.45 (silica gel, hexane–EtOAc 60:40): $[\alpha]_D^{27}$ 14.4 (*c* 0.290, MeOH); IR (film) ν_{max}: 3569, 2925, 2860, 1709, 1686, 1623, 1461, 1385, 1375, 1266, 1218, 1158, 1107, 1044, 1018, 968, 935, 756 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC; H-1/C-2, C-3, C-4, C-5, C-9, C-10, C-25; H-3/C-2, C-4, C-5, C-23; H-4/C-3; H-5/C-1, C-3, C-9, C-23, C-25; H-6/C-8; H-7/C-5, C-26; H-9/C-1, C-5, C-8, C-11, C-14, C-25, C-26; H-11/C-9, C-12, C-13; H-12/C-9, C-11, C-14, C-18; H-15/C-16, C-17, C-27; H-16/C-15; H-18/C-12, C-13, C-16, C-17, C-19, C-20, C-28, C-29; H-21/C-17, C-19; H-22/H-17, C-20; H-23/C-3, C-4, C-5; H-25/C-1, C-4, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21, C-29; HRFABMS negative ionization mode *m/z* 471.3118 $[M-H]^-$ (calcd for $C_{29}H_{43}O_5$, 471.3111).

3.3.2. 2-Oxo-3β-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid (1a). Colorless needles, mp 260–261 °C: R_f=0.51 (silica gel, hexane–EtOAc 60:40, double runs): $[\alpha]_D^{27}$ 105.0 (*c* 0.14, MeOH); IR (film) ν_{max}:3504, 2936, 1726, 1676, 1453, 1247, 1227 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-9, C-10, C-25; H-3/C-2, *CO*CH_3-3, C-4, C-23; H-4/C-3, C-5, C-8, C-10, C-23; H-5/C-4, C-6, C-9, C-10, C-23, C-25; H-6/C-3, C-7, C-8; H-7/C-6, C-8, C-26; H-9/C-1, C-5, C-8, 11, C-25; H-11/C-8, C-9, C-12, C-13; H-12/C-9, C-11, C-14, C-18; H-15/C-8, C-14, C-16, C-17, C-27; H-16/C-14, C-15, C-17, C-18; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-20, C-28, C-29; H-20/C-18, C-21, C-29, C-30; H-21/C-17; H-22/C-16, C-17, C-18, C-20, C-21; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-C-18, C-19, C-20; H-30/C-19, C-20, C-21; CO*CH*$_3$-3/C-3, *CO*CH_3-3 HREIMS *m/z* 514.3294 $[M]^+$ (calcd for $C_{31}H_{46}O_6$, 514.3292).

3.3.3. 2-Oxo-3β-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid methyl ester (1b). R_f=0.55 (silica gel, hexane–EtOAc 80:20): $[\alpha]_D^{27}$ 57.2 (*c* 0.185, MeOH); IR (film) ν_{max}: 3516, 2930, 1720, 1452, 1375, 1283, 1251, 1234, 1192, 1154, 1096, 1074, 1050, 1030, 1013, 962, 769 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-6, C-9, C-10, C-25; H-3/C-2, CH_3*CO*-3, C-4, C-23; H-4/C-C-1, C-3, C-5, C-23; H-5/C-C-6, C-6, C-23, C-25; H-7/C-5, C-6, C-8, C-26; H-9/C-1, C-8, C-10, C-11, C-14, C-25, C-26; H-11/C-8, C-9, C-12, C-13, C-19; H-12/C-9, C-11, C-18, C-19; H-15/C-13, C-14, C-16, C-17, C-27; H-16/C-14, C-15, C-17, C-18, C-28; H-18/C-12, C-13, C-16, C-17, C-19, C-28, C-29; H-20/C-22; H-21/C-17; H-22/C-16, C-17, C-18, C-28; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; *CH*$_3$CO-3/C-3, CH_3*CO*-3; O*CH*$_3$-28/C-28; HRFABMS positive ionization mode *m/z* 529.3532 $[M+H]^+$ (calcd for $C_{32}H_{49}O_6$, 529.3529).

3.3.4. 2-Oxo-3β,19α,22α-trihydroxy-24-nor-urs-12-en-28-oic acid (2). R_f=0.43 (silica gel, hexane–EtOAc 50:50): $[\alpha]_D^{27}$ 19.5 (*c* 0.060, MeOH); IR (film) ν_{max}: 3397, 2916, 2850, 1711, 1635, 1451, 1378, 1259, 1216, 1165, 1087, 1051, 1011, 930 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-6, C-9, C-10, C-25; H-3/C-2, C-4, C-23; H-4/C-3, C-5, C-23; H-7/C-5, C-6, C-8, C-11, C-14; H-9/C-1, C-8, C-11, C-14, C-25, C-26; H-11/C-8, C-9, C-12, C-13, C-25; H-12/C-9, C-11, C-14, C-18; H-15/C-12, C-16, C-27; H-16/C-14, C-15, C-17, C-18, C-28; H-18/C-12, C-13, C-16, C-17, C-19, C-28, C-29; H-21/C-17, C-19, C-20, C-22, C-30; H-22/C-16, C-20, C-21, C-28; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; HRFABMS negative ionization mode *m/z* 487.3067 $[M-H]^-$ (calcd for $C_{29}H_{43}O_6$, 487.3060).

3.3.5. 2-Oxo-3β,22α-di-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid (2a). R_f=0.35 (silica gel, hexane–EtOAc 70:30, double runs): $[\alpha]_D^{27}$ 72.1 (*c* 0.55, MeOH); IR (film) ν_{max}: 3498, 2934, 2875, 1721, 1453, 1371, 1240, 1226, 1155, 1087, 1049, 1027, 969, 933, 885, 804, 760 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-6, C-9, C-10, C-19, C-25; H-3/C-2, C-4, C-23, *CO*CH_3-3; H-4/C-5, C-25; H-5/C-4, C-25; H-6/C-9; H-7/C-5, C-6, C-8, C-26; H-9/C-1, C-8; H-11/C-3, C-9, C-10, C-12, C-13; H-12/C-9, C-11, C-14, C-16, C-18, C-19; H-15/C-14, C-16, C-17, C-27; H-16/C-14, C-15, C-17, C-18, C-22, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-22, C-28, C-29; H-20/C-30; H-21/C-17, C-19, C-20, C-22, C-30; H-22/C-16, C-17, C-20, C-21, C-28, *CO*CH_3-22; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21,

C-22; *CH*$_3$CO-3/C-3, CH$_3$*CO*-3; *CH*$_3$CO-22/C-22, CH$_3$*CO*-22; HRFABMS positive ionization mode *m/z* 573.3429 [M+H]$^+$ (calcd for $C_{33}H_{49}O_8$, 573.3428).

3.3.6. 2-Oxo-3β,22α-di-*O*-acetyl-19α-hydroxy-24-nor-urs-12-en-28-oic acid methyl ester (2b). R_f=0.52 (silica gel, hexane–EtOAc 70:30): $[\alpha]_D^{27}$ 72.8 (*c* 0.15, MeOH); IR (film) ν_{max}: 3539, 2949, 2875, 2156, 1722, 1456, 1370, 1240, 1228, 1154, 1087, 1049, 1025, 970, 930, 886, 806, 770, 692 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Tables 1 and 2; HMBC: H-1/C-2, C-3, C-5, C-9, C-10, C-25; H-3/C-2, CH$_3$*CO*-3, C-4, C-23; H-4/C-1, C-3, C-5, C-25; H-5/C-4, C-6, C-25; H-9/C-5, C-8, C-10, C-11, C-25, C-26; H-11/C-9, C-10, C-12, C-13; H-12/C-9, C-11, C-18; H-15/C-8, C-14, C-16, C-27; H-16/C-15, C-17, C-18, C-22, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28, C-29; H-20/C-30; H-21/C-17, C-20; H-22/C-16, C-17, CH$_3$*CO*-22, C-28; H-23, C-3, C-4, C-5; H-25/C-7, C-8, C-9, C-14; H-27/C-13, C-14; *CH*$_3$CO-28/CH$_3$*CO*-28; H-29/C-19, C-20, C-21; *CH*$_3$CO-3/C-3, *CO*CH$_3$-3; *CH*$_3$CO-22/CH$_3$*CO*-22; *CH*$_3$CO-28/C-28; HRFABMS positive ionization mode *m/z* 587.3582 [M+H]$^+$ (calcd for $C_{34}H_{51}O_8$, 587.3584).

3.3.7. 3-Oxo-2,19α,22α-trihydroxy-24-nor-urs-1,4,12-trien-28-oic acid (3). Colorless solid, mp 182–184 °C; R_f=0.46 (silica gel, CH_2Cl_2/MeOH 92:8): $[\alpha]_D^{23}$ 45.9 (*c* 0.26, MeOH); IR (film) ν_{max}: 3419, 2930, 2881, 1697, 1623, 1456, 1429, 1376, 1239, 1167, 1058, 1018, 756, 517 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-3, C-5, C-9, C-10, C-25; H-6/C-4, C-7; H-9/C-1, C-5, C-7, C-8, C-10, C-11, C-25, C-26; H-11/C-8; H-12/C-9; H-15/C-8, C-14, C-16, C-27; H-16/C-15, C-18; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28; H-21/C-17, C-19, C-20, C-22; H-22/C-16, C-18, C-28; H-23/C-3, C-4, C-5; H-25/C-1, C-5, C-9; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; HREIMS negative ionization mode *m/z* 483.2752 [M−H]$^-$ (calcd for $C_{29}H_{39}O_6$, 483.2749)

3.3.8. 4-Oxo-19α,22α-dihydroxy-3,24-dinor-2,4-*seco*-urs-12-en-2,28-dioic acid (4). R_f=0.31 (silica gel, CH_2Cl_2/MeOH 95:5, double runs): $[\alpha]_D^{27}$ 28.7 (*c* 0.135, MeOH); IR (film) ν_{max}: 3554, 2951, 2922, 2850, 2653, 1690, 1456, 1375, 1362, 1259, 1158, 1082, 1029, 930, 890, 804 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-5, C-9, C-10, C-25; H-5/C-4, C-6, C-10, C-25; H-9/C-1, C-10, C-11, C-14, C-21, C-25, C-26; H-12/C-9, C-11, C-14; H-15/C-8, C-27; H-16/C-15, C-17; H-17, C-14; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28, C-29; H-20/C-18; H-21/C-17, C-20, C-22, C-30; H-22/C-16, C-18, C-21; H-23/C-4, C-5; H-25/C-1, C-2, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; HRFABMS negative ionization mode *m/z* 489.2857 [M−H]$^-$ (calcd for $C_{28}H_{41}O_7$, 489.2853).

3.3.9. 4-Oxo-19α,22α-dihydroxy-3,24-dinor-2,4-*seco*-urs-12-en-2,28-dioic acid dimethyl ester (4a). R_f=0.40 (silica gel, CH_2Cl_2/MeOH 98:2): $[\alpha]_D^{24}$ 65.6 (*c* 0.08, MeOH); ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-5, C-9, C-25; H-5/C-4, C-9, C-10, C-25; H-7/C-6, C-8, C-14, C-26; H-9/C-1, C-8, C-11, C-14, C-25, C-26; H-12/C-9, C-11, C-14; H-15/C-16, C-27; H-16/C-15, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28; H-21/C-18, C-20, C-22, C-30; H-22/C-16; H-23/C-4; H-25/C-1, C-2, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-9, C-13, C-14, C-15; H-29/C-18, C-19, C-20; H-30/C-19, C-20, C-21; *CH*$_3$CO-2/C-2; *CH*$_3$CO-28/C-28; HREIMS *m/z* 518.3307 [M]$^+$ (calcd for $C_{30}H_{46}O_7$, 518.3243).

3.3.10. 19α,22α-Dihydroxy-24-nor-2,3-*seco*-urs-12-en-2,3,28-trioic acid trimethyl ester (5). R_f=0.41 (silica gel, CH_2Cl_2/MeOH 98:2): IR (film) ν_{max}: 3508, 2941, 2918, 1721, 1456, 1433, 1373, 1350, 1266, 1216, 1193, 1158, 1102, 1089, 1014, 935, 735 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 3; HMBC: H-1/C-2, C-5, C-9, C-10, C-25; H-6/C-4, C-5, C-7, C-8; H-7/C-5, C-9, C-14, C-26; H-9/C-1, C-8, C-10, C-11, C-14, C-25, C-26; H-11/C-8, C-9, C-12, C-13; H-12/C-9, C-14, C-18; H-15/C-8; H-16/C-14, C-15, C-17, C-28; H-18/C-12, C-13, C-14, C-16, C-17, C-19, C-28; H-20/C-30; H-21/C-17, C-19, C-20, C-22, C-30; H-22/C-16, C-21; H-23/C-1, C-3, C-4, C-5, C-10, C-25; H-25/C-1, C-2, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-18, C-19; H-30, C-19, C-20, C-21; O*CH*$_3$-2/C-2; O*CH*$_3$-3/C-3; O*CH*$_3$-28/C-28; HREIMS *m/z* 560.3441 [M]$^+$ (calcd for $C_{32}H_{48}O_8$, 560.3349).

3.4. Bioassays

Antimalarial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) cultured continuously according to Trager and Jensen.[14] Quantitative determination of antimalarial activity in vitro was achieved by means of the microculture radioisotope technique based on Desjardins et al. described method.[14] The anti-mycobacterial activity (anti-TB) assay was performed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay.[15] Antifungal test was undertaken against *Candida albicans* (ATCC 90028) using tetrazolium/formazan assay method.[16] Cytotoxicity assays were evaluated using the colorimetric method.[17]

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Photomediated synthesis of β-alkylketones from cycloalkanes

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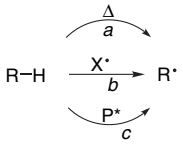
Abstract—β-Cycloalkylketones are prepared through a photomediated radical addition reaction onto enones starting from the corresponding alkanes (i.e., cyclopentane, -hexane, -heptane, -dodecane and adamantane). The alkyl radicals are generated via hydrogen abstraction by either an organic (benzophenone) or an inorganic (tetrabutylammonium decatungstate, TBADT) photomediator. Isolated yields vary in the range 30–80%. Benzophenone has to be considered as a reagent, since it is used in an equimolar amount with respect to enone and is completely consumed in the reaction. On the contrary, TBADT is shown to behave as a photocatalyst, which is active for at least 50 cycles. The potential of photomediated reactions for the generation of radicals from unusual precursors and the synthetic significance of this method are discussed.

1. Introduction

β-Alkylketones are prepared by conjugate addition starting from alkyl halides and α,β-unsaturated ketones under either anionic[1a] (by using organometallic species) or radical conditions.[1] An obvious improvement in terms of atom economy would be the substitution of alkanes for alkyl halides or other derivatives. Unfortunately, the direct activation of C–H bonds in alkanes still remains one of the 'Holy Grail in chemistry'.[2] Many efforts have been devoted to the activation of aromatic and olefinic hydrogens[3] by using metal (especially ruthenium[3d]) based complexes. Surprisingly, the activation of aliphatic C–H bonds has received much less attention despite the fact that saturated hydrocarbons constitute the main fraction of the available feedstock. This can be ascribed to the poor tendency of alkanes to become coordinated on metal centers. Furthermore, even when such activation has been achieved, the synthetic use has been mostly addressed to forming a C-heteroatom bond (e.g., in oxygenation reactions) rather than a C–C bond.[3,4]

The noncatalytic activation of alkanes generally requires rather harsh conditions and this leads to a poor selectivity. Thermolysis at a sufficient high temperature is a possibility (Scheme 1, path *a*) but the control of the reaction is difficult. Thus, the thermal synthesis of 4-cyclohexylbutanone (12% yield) from cyclohexane and 3-buten-2-one at a high temperature (ca. 400 °C) and pressure[5] is to our knowledge the only case where a β-cycloalkylketone has been prepared starting directly from the alkane and the enone. A method for the functionalization of alkanes under milder conditions than those of the above reaction is desirable as a green alternative.[6a] Electrophilic radicals such as hydroxy or acyloxy radicals ($X^{\bullet}$, path *b*) can be generated at a lower temperature and abstract a hydrogen, but again the reaction is difficult to control;[6b] the same radicals can be produced photochemically at room temperature, but the precursors, e.g., benzoylperoxide, absorb at too short a wavelength to make a photoreaction practical.



Scheme 1. Activation of C–H bonds in alkanes.

A range of compounds are known that absorb strongly in the near UV and in the excited state react with alkanes at a high rate either via hydrogen abstraction or via electron transfer and deprotonation[6c] (P*, path *c*). These can be used as photomediators for the activation of aliphatic C–H bonds (path *c*).[6c,d]

Indeed, we have demonstrated that radicals obtained through hydrogen abstraction from the corresponding cycloalkanes by benzophenone add to electrophilic olefins, viz, unsaturated nitriles,[7a] fumaramides[7b] (also bearing chiral auxiliaries), and ketene-*S*,*S*-dioxides[7c] and form new C–C bonds. A potentially useful variation is employing a heterogeneous sensitizer, easily recovered at the end of the reaction.

Keywords: Alkanes; Alkylation; C–H activation; Enones; Photochemistry; Radicals and radical reactions.
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A polymer-bound benzophenone has been reported to be effective for the functionalization of alkynes bearing electron-withdrawing groups.[8] There are a few further examples of this type in the literature.[9]

Furthermore, inorganic materials such as semiconductor oxides or sulfides (TiO_2, ZnS) or their soluble analogues, polyoxometallates are known to abstract hydrogen when excited from organic molecules, but they are generally used for the oxidative decomposition of organic contaminants and water depollution rather than for synthesis. There are a few preparative applications known,[10] although these mainly do not concern alkanes. There is, however, a recent report that tetrabutylammonium decatungstate (TBADT) can act as a photocatalyst in the synthesis of β-cycloalkylnitriles from cycloalkanes with essentially the same yield as benzophenone.[11]

Since the activation of aliphatic C–H bonds is a major target, further studies appeared worthwhile. Indeed, while the initial step, H abstraction by an excited state, has been thoroughly studied in the photochemical literature by using both organic compounds such as ketones and inorganic derivatives such as polyoxometallates, little is known on the subsequent reactions of the radicals and in particular on their trapping by electrophilic olefins and on the scope of the resulting photomediated reactions for the formation of C–C bonds.

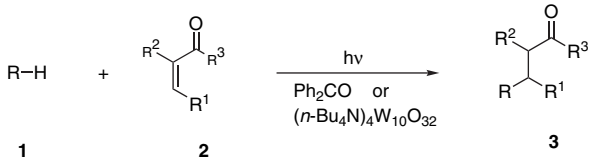
We presently report the synthesis of β-cycloalkylketones from cycloalkanes and α,β-unsaturated ketones. This was thought to represent a significant test because the enones may interfere with the aromatic ketones used as photomediators thus highlighting a possible difference with the use of polyoxometallates for that function. Furthermore α,β-unsaturated ketones have been less commonly used than other electrophilic alkenes in radical alkylation reactions, therefore this study may give some information about the synthetic scope of the method.

2. Results

Four cyclic alkanes (**1a–d**) and one bicyclic alkane (adamantane, **1e**) were explored in this work as precursors of the alkyl radicals. The unsaturated ketones used as radical traps included an open-chain enone (3-buten-2-one, **2j**), two cyclic enones (cyclopentenone **2k**, cyclohexenone **2l**), and one bicyclic enone (methylenenorbornanone **2m**). The synthesis of β-cycloalkylketones was carried out under two different conditions according to the photomediator used (either an aromatic ketones or TBADT).

2.1. Photochemical alkylation in the presence of an aromatic ketone (Method A)

The alkylation was carried out by using a solution of an enone **2** (0.05–0.1 M) in the presence of an equimolar amount of benzophenone (or xanthone) as the photomediator and an excess of an alkane **1** (see Table 1 and Scheme 2, Method A). Benzene was routinely used as the solvent due to its low hydrogen donating ability.[4] Less toxic solvents such as *t*-BuOH or dimethylcarbonate (DMC) were also used.



1a R = Cyclopentyl
b R = Cyclohexyl
c R = Cycloheptyl
d R = Cyclododecyl
e R = Adamantyl

2j $R^1 = R^2 = H$, $R^3 = CH_3$
k $R^2 = H$, R^1-R^3 = -$(CH_2)_2$-
l $R^2 = H$, R^1-R^3 = -$(CH_2)_3$-
m $R^1 = H$, R^2-R^3 = 1,3-cyclopentadiyl

Scheme 2. Photomediated synthesis of β-cycloalkylketones.

Table 1. Photomediated synthesis of β-cycloalkylketones **3** starting from cycloalkanes **1**

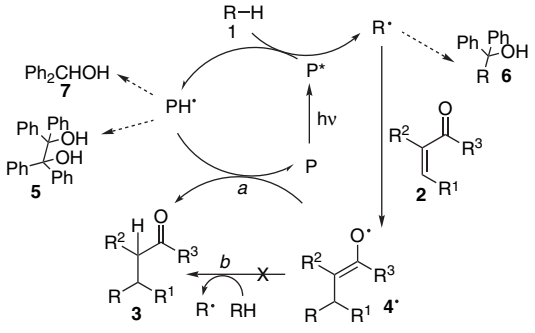
Enone	Product	Method A[a]			Method B[a]		
		Alkane (M)	% Yield[b]	Irradiation time (h)	Alkane (M)	% Yield[b]	Irradiation time (h)
2j	**3aj**	**1a** (3)	[c]	24	**1a** (0.5)	36	16
2j	**3bj**	**1b** (3)	[c]	24	**1b** (0.5)	55	16
2j	**3cj**	**1c** (3)	[c]	24	**1c** (0.5)	56	16
2k	**3ak**	**1a** (2)	46[d]	20	**1a** (0.5)	38	16
2k	**3bk**	**1b** (neat)	50	20	**1b** (0.5)	35, 43[e]	16
2k	**3ck**	**1c** (3)	66[d]	18	**1c** (0.5)	41	16
2k	**3dk**	**1d** (1)	48	15	**1d** (0.5)	20[f]	18
2k	**3ek**	**1e** (0.2)	65	20	**1e** (0.1)	15[f]	18
2l	**3al**	**1a** (3)	55	20	**1a** (0.5)	33	16
2l	**3bl**	**1b** (neat)	55	20	**1b** (0.5)	31	16
2l	**3cl**	**1c** (3)	52	26	**1c** (0.5)	43	16
2l	**3dl**	**1d** (1)	36	26	**1d** (0.2)	15[f]	18
2l	**3el**	**1e** (0.2)	40	26	**1e** (0.1)	21	18
2m	**3am**	**1a** (3)	[c]	20	**1a** (0.5)	45	16
2m	**3bm**	**1b** (3)	80[g]	20	**1b** (0.5)	49	16
2m	**3cm**	**1c** (4.1)	80[g]	20	**1c** (0.5)	45	16

[a] Method A: benzophenone (0.05–0.1 M) and enone (0.05–0.1 M) irradiated in a cycloalkane/benzene mixture as the solvent (unless otherwise stated). Method B: TBADT (2×10^{-3} M) and enone (0.1 M) irradiated in a cycloalkane/acetonitrile mixture as the solvent (unless otherwise stated).
[b] Isolated yields.
[c] No alkylation products were detected via GC analysis.
[d] *t*-BuOH as the solvent.
[e] Reaction carried out in an immersion-well apparatus.
[f] Benzene/acetonitrile 1:1 as the solvent.
[g] Dimethylcarbonate (DMC) as the solvent; xanthone as the photomediator (λ=366 nm).

In Table 1 the results with λ_{exc}=310 nm are reported. Blank tests were carried out, since at this wavelength part of the light used was absorbed by the enones. Thus, compounds **2j–m** were irradiated in the absence of benzophenone in order to assess the role of competing photoreactions resulting for their direct excitation. Actually, irradiation of both **2k** and **2l** in neat benzene led to the formation of a mixture of dimers as previously reported.[12] On the other hand, the same enones **2k** and **2l** have been reported to abstract hydrogen from alkanes and to form α- and β-alkylketones in variable amounts.[13] In our hands, however, irradiation at 310 nm of these enones in a cyclohexane/benzene 1:2 mixture for 24 h did not give the expected alkylated ketones (as determined by GC analysis), while, as indicated in Table 1, these were formed in moderate to good yield in the presence of an equimolar amount of benzophenone. Furthermore, the same products were formed in the presence of Ph_2CO upon irradiation at 360 nm, where **2k** and **2l** did not absorb.

The use of a lower molar amount of benzophenone (e.g., 50%) led to a less efficient alkylation. As an example, the yield of alkylated ketone **3bl** dropped from 55 to 33% under these conditions. On the other hand, increasing the benzophenone concentration above equimolecular did not improve the alkylation yield. Using neat alkane as the solvent mostly gave complex mixtures, including the desired alkylated ketones, although this choice gave good results in a couple of instances (see below).

In all of the reactions studied small variable amounts of cycloalkylbenzenes and of byproducts arising from benzophenone (e.g., alkyl diphenylmethanols) were detected by GC. As an example, in the synthesis of cycloalkyl derivatives **3bk–m**, benzopinacol (**5**, ca. 40%), benzhydrol (**7**, 10%), and cyclohexyldiphenylmethanol (**6**, 10%, R=cyclohexyl in Scheme 3) were determined by HPLC analysis.



Scheme 3. Photomediated generation and reactivity of alkyl radicals from alkane.

Other aromatic ketones could be used. Actually, with methylenenorbornanone **2m**, xanthone was preferred to benzophenone (λ=360 nm, DMC as the solvent), because byproducts from the former photomediator were better separated from the alkylated ketones by chromatography. However, this held for the cyclohexyl and cycloheptyl derivatives (**3bm** and **3cm**), which were isolated in ca. 80% yield while the cyclopentyl homologue **3am** was not obtained.

Furthermore, not all of the enones tested reacted as desired. In particular, the open-chain derivative **2j** was not alkylated under these conditions, either in benzene or in DMC or *t*-BuOH, as well as by using either benzophenone or xanthone (λ_{exc}=310 or 366 nm), while cyclic and bicyclic analogues reacted in moderate to good yields.

Alkanes were used at 2–4 M concentration, except for high-boiling compounds such as **1d–e**. These were used at a lower concentration due to both the limited solubility and the difficulty in the removal of excess from the raw photolyzate. With adamantane (**1e**, 0.2 M) the alkylation yields were satisfactory and only 1-adamantyl derivatives were obtained. Cyclododecane (**1d**) could be used in 1 M concentration, though the separation of the alkylated ketones required a careful separation by column chromatography.

In *t*-BuOH the reaction was less satisfactory, although yields comparable to those in benzene were obtained in some cases (see the synthesis of ketones **3ak** and **3ck**, in Table 1). In DMC the yields were in average 20–30% lower, except for the case of **3bm** or **3cm**, obtained in the same yield as in benzene. In the synthesis of **3bk** and **3bl**, the alkylation was most effective in neat alkane (yields ca. 50%) with no extensive formation of byproducts.

2.2. Photochemical alkylation in the presence of TBADT (Method B)

Differently from Method A, the photomediator was used in a much lower concentration (2×10^{-3} M) than to the unsaturated ketone (0.1 M) with acetonitrile as the solvent (in the reactions involving cyclododecane, a benzene/acetonitrile mixture was conveniently used). The salt is soluble in MeCN, not in less polar solvents, and photoinduced hydrogen abstraction from alkanes has been previously demonstrated to occur under these conditions.[11] During irradiation the intense blue color of reduced tungstate was apparent.

Both increasing and decreasing the concentration of TBADT lowered to some extent the alkylation yields. The photoinduced reaction was carried out, as in Method A, by external irradiation at 310 nm. Somewhat better yields were obtained by performing the reaction on a larger scale by internal irradiation (mercury arc) in an immersion-well apparatus (see the case of product **3bk** in Table 1). The relatively low solubility of the alkanes in MeCN (0.1–0.5 M) prevented their use in excess as large as in Method A. In general, isolated yields were modest, but the scope was wider (e.g., alkylation of enone **2j** was successful in this case) and workup was expeditious, viz, limited to evaporation of the solvent and separation of the alkylated products from the solid catalyst by bulb-to-bulb distillation.

2.3. Comparison of the methods

As it appears in Table 1, higher yields of cycloalkylketones could be obtained when using benzophenone rather than TBADT. However, in the former case the irradiation was carried out in benzene or in a moderately polar solvent such as DMC, which allowed a larger starting concentration of the alkanes (up to 3–4 M rather than 0.5 M). In order to compare the efficiency of aromatic ketones and tungstate as photomediators a further set of experiments was carried out under the same conditions (acetonitrile as the solvent,

Table 2. Comparison between aromatic ketones and TBADT as photomediators in the synthesis of β-cycloalkylketones[a]

Product	% Yield[b] (Method A)[c]	% Yield[b] (Method B)[d]
3ak	22	38
3bk	34	35
3ck	44	41
3al	7	33
3bl	9	31
3cl	16	43
3am	<2	45
3bm	10	49
3cm	15	45

[a] Reactions in acetonitrile, 0.5 M alkane irradiated at 310 nm for 16 h.
[b] GC determined yields.
[c] Benzophenone (0.1 M) as the photomediator.
[d] TBADT (2×10^{-3} M) as the photomediator.

0.5 M alkane, 16 h of irradiation). The results are gathered in Table 2.

This shows that the yields of β-cycloalkylketones are consistently about 40% when Method B was used, while with Method A, the yields largely depended on the structure of both the alkane and the enone and were generally lower than in the former case, except for the alkylation of cyclopentenone.

3. Discussion

As reported above, β-cycloalkylketones can be prepared under mild conditions by radical alkylation of enones, exploiting the photomediated generation of the key alkyl radicals by hydrogen abstraction directly from alkanes. Aromatic ketones and TBADT do not act as photosensitizer, i.e., the activation is not based on energy or electron transfer, but on a chemical reaction, H abstraction.[14] We prefer using the general term photomediator for such photoactivating species. When this is regenerated in a following thermal step (see below) the specific term photocatalyst will be used. The dependence of the reaction efficiency on experimental parameters and the possibility that the method has preparative significance are briefly discussed below.

The reaction can in principle be carried out in neat alkane when this is a low-boiling liquid and an organic photomediator is used. However, solubility is a limit with some photomediators (e.g., xanthone, $\leq 9\times10^{-3}$ M in cyclohexane) and side products are generally more abundant under these conditions, thus making this choice unpractical except than in a few cases (**3bk**, **3bl**). Accordingly, a cosolvent must be used that is transparent to the light used and is a poor hydrogen donor. With Method A, benzene gives good results, as previously observed in the alkylation of unsaturated nitriles,[7a] despite the fact that the benzophenone triplet lifetime is somewhat shorter in this solvent[15] and variable amounts of cycloalkylbenzenes byproducts are formed.[7a] More environmentally benign DMC and *t*-BuOH can be used, though the yields are as high as those obtained in benzene only in some cases (see Table 1). Acetonitrile is also a possibility and actually the only practical choice for an inorganic photomediator such as TBADT (Method B), although alkanes are poorly dissolved in this solvent.

Three specific limitations can be considered for the photomediated alkylation of enones differently from the case of other electrophilic olefins. First, the UV absorption of these compounds is more red-shifted than that of unsaturated nitriles or esters, and thus these absorb part of the light when a 310 nm lamp is used. However, blank tests showed that competitive absorption slows down the reaction, but leads to no significant alkylation on the time scale where the photosensitized reaction occurs.

Second, enones may quench the triplet of the photomediator and thus hinder hydrogen abstraction. Benzophenone and xanthone have a triplet energy of 287[7a] and 309 kJ mol^{-1},[7a] respectively. As for enones, cyclopentenone **2k** has a higher triplet (305 kJ mol^{-1})[16] with respect to homologue **2l** (263 kJ mol^{-1}).[17,18] Indeed, Table 2 shows that cyclopentenone **2k**, for which quenching of triplet benzophenone is endothermic, is more efficiently alkylated than **2l**. As shown in Table 1, however, when the irradiation time is increased, alkylated products are obtained also in the other cases. When occurring, triplet quenching is not involved in the alkylation. The short lifetime of enone triplets (from 8 to 30 ns)[19] limits their reactivity (see Scheme 3) and indeed no reaction occurs by direct irradiation of the enones as shown by blank tests (see the invariance of the product distribution when changing the exciting wavelength).

Finally, coplanar enones are effective electrophiles under these conditions, but alkylation of conformationally free 3-buten-2-one **2j** (see Table 1) appears to be too slow to occur with the low steady state radical concentration generated under benzophenone photomediation.[20] When the rate of formation is further limited by using a lower concentration of alkane (Table 2), the dependence on the enone coplanarity is apparent in the alkylation yield (cyclopentenone > cyclohexenone ≈ methylenenorbornanone).

The use of TBADT has a larger scope. The high molar extinction coefficient of this photomediator in acetonitrile ($\varepsilon_{323}=1.35\times10^4$ M^{-1} cm^{-1}) allows total absorption of the irradiation even when the decatungstate anion is for the most part present in the reduced form (see below). Apparently no physical quenching of the hydrogen-abstracting excited state by the enone occurs.[22a] The generation of the radicals proceeds at such a pace (see below) that the alkylation is efficient independently on the structure of the enone used.

The proposed overall mechanism is depicted in Scheme 3. This allows pointing out similarities and differences between using ketones and using the polyoxotungstate as photomediator (P).

Hydrogen abstraction is much faster with TBADT ($k_H=3.7\times10^7$ M^{-1} s^{-1} from cyclohexane in MeCN)[22b] than with Ph_2CO ($k_H=7.5\times10^5$ M^{-1} s^{-1} for the same case).[23] The relatively low rate of this step in the latter case explains the limited and conditions dependent efficiency of aromatic ketones as discussed above. H abstraction leads in any case to a persistent (P$^{\bullet}$, viz, TBADTH$^{\bullet}$[24] or $Ph_2C^{\bullet}OH$) and a reactive (R$^{\bullet}$) radical, the former one rapidly accumulating in solution. Indeed, the blue color of reduced decatungstate develops and spectrophotometric determination shows that ca. 90% of the initial amount is present in the reduced

form after 1–2 h.[25] A small portion of the salt remains in the oxidized form and carries on the reaction by absorbing at 310 nm. The ketyl radicals also accumulate, though not to such a high concentration, the limit being given by the dimerization rate constant ($k=7\times10^8\ M^{-1}\ s^{-1}$)[26] to form benzopinacol, indeed a major product.

The cosolvents used are known to be poorer hydrogen donors than the alkanes.[27] The alkyl radicals are trapped by enones **2** forming adduct (enol) radicals **4**$^{\bullet}$. The rate of this process can be estimated as about $k=10^6\ M^{-1}\ s^{-1}$.[28] The rate of addition of 1-adamantyl radical onto electron-poor olefin has been reported to be two orders of magnitude faster than that of cyclohexyl radical ($k\approx10^8\ M^{-1}\ s^{-1}$).[29,30] This explains that the adamantyl derivatives **3ek** and **3el** are obtained in a satisfactory yield despite the fact that this alkane can be used only at low concentration (0.1–0.2 M) and the selective formation of the 1-adamantyl derivatives, as already observed in other related photocatalyzed reactions.[7]

Another main difference between the two photochemical methods involves the fate of the radical adduct **4**$^{\bullet}$. Reduced TBADTH$^{\bullet}$ is subject to disproportionation but has no irreversible decay available. Importantly, it is oxidized back to TBADT by electron transfer to the radical adducts as previously demonstrated for the case of the addition to α,β-unsaturated nitriles.[11] In the present case:

$$\text{TBADTH}^{\bullet} + \mathbf{4}^{\bullet} \rightarrow \text{TBADT} + \mathbf{4}^{-} + \text{H}^{+} \rightarrow \text{TBADT} + \mathbf{3}$$

In other term, path *a* is 100% effective, i.e., the mediator is regenerated and functions as a nonconsumed catalyst (Scheme 3). This is different for the case of ketyl radicals $Ph_2C^{\bullet}OH$. These dimerize to **5** (and couple with R$^{\bullet}$, a less important process in view of the largely different steady state concentration of the two radicals). Thus benzophenone is not regenerated and indeed must be used in an equimolar amount. However, H transfer to **4**$^{\bullet}$ is negligible, in keeping with the high stabilization of the ketyl radical, known from various examples (e.g., $Ph_2C^{\bullet}OH$ does not transfer a hydrogen to PhCO$^{\bullet}$, while $Me_2C^{\bullet}OH$ transfers a hydrogen to Ph_2CO in a markedly exothermic process).[31] Furthermore, hydrogen abstraction from the alkanes by radical **4**$^{\bullet}$ (path *b* in Scheme 3) does not take place significantly.

The same characteristic had been previously noticed in the alkylation of unsaturated nitriles by alkanes. On the contrary when the radical precursor is a better hydrogen donor, e.g., a dioxolane, path *b* becomes accessible. In that case, formation of the end product regenerates an alkyl radical.[21] Thus a short chain may operate and indeed with dioxolane a lower amount (e.g., 20–40%) of the photomediator can be used.[32]

To summarize, in the alkylation by alkanes benzophenone acts as a reagent, while TBADT behaves as a catalyst and operates with a turnover number >50.[33]

4. Conclusion

The synthesis of β-cycloalkylketones was accomplished by photomediated reaction between cycloalkanes and enones, which is advantageous in term of atom economy. Both aromatic ketones and decatungstate salts can be used for the photochemical C–H activation of alkanes, although only the latter one is an actual photocatalyst. Yields of alkylated ketones are in most cases moderate, but the potential interest of the method is the use of an abundant and unexpensive starting material such as an alkane rather than an alkyl bromide or iodide. Furthermore, the use of the highly toxic stannanes typical of conventional radical alkylation is avoided. It is likely that a further improvement may be obtained by supporting the photocatalyst on a solid support, thus carrying out a more expeditious heterogeneous reaction. Work in this direction is in progress.

5. Experimental

All of the starting materials were of commercial origin. The starting enones were purified by distillation immediately before use. The photochemical reaction was carried out in quartz tubes by using a nitrogen-purged solution and irradiating in a multilamp reactor by using 6×15 W phosphor-coated lamps with emission maximum at 310 nm (in the presence of TBADT) or 366 nm (in the case of **2m** in the presence of xanthone). A larger scale synthesis of compound **3bk** was accomplished by irradiation in an immersion-well apparatus by using a 125 W high-pressure mercury arc through Pyrex while maintaining a nitrogen flux (see below).

The workup procedure consisted in concentration in vacuo of the photolyzed solution, followed by column chromatography (silica gel with various cyclohexane/ethyl acetate mixtures as the eluant) or, in the case of reactions with tungstates, by bulb-to-bulb distillation at reduced pressure.

The amount of compounds **5**–**7** formed was determined by HPLC (AQUASIL C18 (250×4.6 mm) column, MeCN/water from 70:30 to 90:10, flux 1 mL min^{-1}) with UV detection at λ=250 nm.

5.1. Preparation of tetrabutylammonium decatungstate (TBADT)

The preparation of TBADT described previously by Yamase[34] was modified as follows: tetrabutylammonium bromide (2.4 g) and sodium tungstate dihydrate (5 g, Aldrich) were dissolved each in 1 L of deionized water and kept at 90 °C. Concentrated hydrochloric acid was added dropwise to both solutions in order to adjust the pH at 2. The pH of the tungstate solution was carefully checked and maintained at pH 2 with additional hydrochloric acid, until the pH did not change appreciably. At this point, the two solutions were mixed and maintained at 90 °C for 30 min under stirring. A white suspension of TBADT was formed and filtered on a short silica gel column. The solid phase was washed initially with water and then with acetonitrile in order to dissolve all of the TBADT. After elimination of the solvent in vacuo, the raw decatungstate was purified by crystallization (from a water/acetonitrile mixture, 80% yield based on the content of tungsten). The end product showed a >90% of purity as evaluated by UV analysis ($\varepsilon_{323}=1.35\times10^4\ dm^3\ mol^{-1}\ cm^{-1}$ in acetonitrile[34]).

5.2. Photocatalyzed synthesis of β-cycloalkylketones. General procedures

Method A: Alkane **1** (from 0.1 M to neat), unsaturated ketone **2** (from 0.05 to 0.1 M), and benzophenone (xanthone in the case of **2m**, 0.1 M) were dissolved in the solvent chosen (benzene, *t*-BuOH or DMC), and the resulting solution was deareated and then irradiated until **2** was consumed. The workup procedure consisted in concentration in vacuo of the photolyzed solution followed by column chromatography (silica gel with cyclohexane/ethyl acetate mixtures as eluant).

Method B: A solution of alkane **1** (0.2–0.5 M in acetonitrile), unsaturated ketone **2** (0.1 M), and TBADT (2×10^{-3} M) was deareated and then irradiated until **2** was consumed. The workup procedure consisted in concentration in vacuo of the photolized solution followed by bulb-to-bulb distillation at reduced pressure. Chromatographic separation (see above) was used in the reactions in the presence of **1d** and **1e**.

5.2.1. 4-Cyclopentylbutan-2-one (3aj).[35] Method B: A solution of 850 μL (9 mmol, 0.5 M) of **1a**, 150 μL (1.8 mmol) of **2j**, and 120 mg of TBADT (36 μmol, 2×10^{-3} M) in 18 mL of acetonitrile was irradiated for 16 h and **3aj** (90 mg, 36%) was isolated as a colorless oil. ^{1}H NMR and infrared spectra were in accordance with literature data.[35] ^{13}C NMR ($CDCl_3$) δ 25.0 (CH_2), 29.7 (CH_3), 29.9 (CH_2), 32.5 (CH_2), 39.5 (CH), 43.0 (CH_2), 210 (CO). Anal. Calcd for $C_9H_{16}O$: C, 77.09, H, 11.50. Found: C, 76.8, H, 11.4.

5.2.2. 4-Cyclohexylbutan-2-one (3bj).[36] Method B: 1 mL (9 mmol, 0.5 M) of **1b**, 150 μL (1.8 mmol) of **2j**, and 120 mg of TBADT were dissolved in 18 mL of acetonitrile. The solution was then irradiated for 16 h and 150 mg of **3bj** (55%, colorless oil) were isolated. The spectroscopic data were in accordance with literature data.[36] Anal. Calcd for $C_{10}H_{18}O$: C, 77.87, H, 11.76. Found: C, 77.7, H, 11.6.

5.2.3. 4-Cycloheptylbutan-2-one (3cj).[37] Method B: 1.1 mL (9 mmol, 0.5 M) of **1c**, 150 μL (1.8 mmol) of **2j**, and 120 mg of TBADT were dissolved in 18 mL of acetonitrile. After 16 h of irradiation 165 mg (56%) of **3cj** were isolated as a colorless oil.

Compound **3cj**: ^{1}H NMR ($CDCl_3$) δ 1.05–1.2 (2H, m), 1.3–1.7 (13H, m), 2.1 (3H, s), 2.35–2.45 (2H, t, J=7 Hz). ^{13}C NMR ($CDCl_3$) δ 26.2 (CH_2), 28.3 (CH_2), 29.7 (CH_3), 31.7 (CH_2), 34.2 (CH_2), 38.7 (CH), 41.8 (CH_2), 209 (CO). IR (neat) ν/cm^{-1} 2920, 2853, 1720, 1500, 1360, 1170. Anal. Calcd for $C_{11}H_{20}O$: C, 78.51, H, 11.98. Found: C, 78.4, H, 12.1.

5.2.4. 3-Cyclopentylcyclopentanone (3ak).[38] Method A: 3.7 mL of **1a** (40 mmol, 2 M), 165 μL of **2k** (2 mmol, 0.1 M), and 365 mg of benzophenone (2 mmol, 0.1 M) were dissolved in 16 mL of *t*-BuOH and the solution was irradiated for 20 h. After chromatographic purification product **3ak** was obtained as a colorless oil (140 mg, 46%).

Method B: A solution of 1.1 mL (12 mmol, 0.5 M) of **1a**, 200 μL (2.4 mmol) of **2k**, and 160 mg of TBADT (48 μmol, 2×10^{-3} M) in 23 mL of acetonitrile was irradiated for 16 h and 140 mg (38%) of **3ak** were obtained.

The spectroscopic data of compound **3ak** were in accordance with literature data.[38] Anal. Calcd for $C_{10}H_{16}O$: C, 78.90, H, 10.59, O, 10.51. Found: C, 79.4, H, 10.1.

5.2.5. 3-Cyclohexylcyclopentanone (3bk).[36] Method A: To 20 mL of **1b** were added 165 μL of **2k** (2 mmol, 0.1 M) and 365 mg of benzophenone (2 mmol, 0.1 M). The resulting solution was irradiated for 20 h yielding 170 mg of **3bk** (50%) as a colorless oil.

Method B: 1.2 mL (11 mmol, 0.5 M) of **1b**, 200 μL (2.4 mmol) of **2k**, and 160 mg of TBADT were dissolved in 22 mL of acetonitrile. Irradiation of the resulting mixture (16 h) yielded 140 mg (35%) of **3bk**.

Compound **3bk** was obtained in a somewhat larger scale (650 mg, 43% yield) and in a shorter time (9 h) starting from 4.5 mL (4.5 mmol) of cyclohexane, 750 μL (9 mmol) of **2k**, and 590 mg (0.18 mmol) of TBADT in 90 mL of MeCN after irradiation in an immersion-well apparatus.

The spectroscopic data of compound **3bk** were in accordance with literature data.[36] Anal. Calcd for $C_{11}H_{18}O$: C, 79.46, H, 10.91, O, 9.62. Found: C, 79.0, H, 10.1.

5.2.6. 3-Cycloheptylcyclopentanone (3ck). Method A: 18.0 mL of **1c** (150 mmol, 3 M), 420 μL of **2k** (5.0 mmol, 0.1 M), and 910 mg of Ph_2CO (5.0 mmol, 0.1 M) were dissolved in 32 mL of *t*-BuOH and irradiated for 18 h. Compound **3ck** (66%, 595 mg) were obtained as a colorless oil.

Method B: 1.4 mL (12 mmol, 0.5 M) of **1c**, 200 μL (2.4 mmol) of **2k**, and 160 mg of TBADT were dissolved in 22 mL of acetonitrile. The solution was then irradiated for 16 h finally giving 176 mg (41%) of **3ck**.

3ck: ^{1}H NMR ($CDCl_3$) δ 1.1–2.1 (m, 16H), 2.1–2.35 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 26.2 (CH_2), 26.7 (CH_2), 27.6 (CH_2), 28.3 (CH_2), 31.6 (CH_2), 32.6 (CH_2), 38.9 (CH_2), 43.4 (CH), 43.6 (CH_2), 44.5 (CH), 219.6 (CO). IR (neat) ν/cm^{-1} 2920, 2853, 1741, 1460, 1403. Anal. Calcd for $C_{12}H_{22}O$: C, 79.06, H, 12.16. Found: C, 79.0, H, 12.0.

5.2.7. 3-Cyclododecylcyclopentanone (3dk). Method A: 10 g of **1d** (60 mmol, 1 M), 0.5 mL of **2k** (6.0 mmol, 0.1 M), and 1.09 g of benzophenone (6.0 mmol, 0.1 M) were dissolved in 60 mL of benzene. The solution was irradiated for 15 h and 709 mg of **3dk** (48%) were obtained as a glassy solid.

Method B: 1.7 g of **1d** (10 mmol, 0.5 M), 170 μL of **2k** (2 mmol), and 135 mg of TBADT (41 μmol, 2×10^{-3} M) were dissolved in 20 mL of benzene/acetonitrile 1:1. The solution was then irradiated for 18 h and after column chromatography on silica gel 100 mg of **3dk** (20%) were obtained.

3dk: ^{1}H NMR ($CDCl_3$) δ 1.1–1.6 (m, 24H), 1.7–1.9 (m, 1H), 2.0–2.5 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_2), 21.1 (CH_2), 22.6 (CH_2), 22.71 (CH_2), 22.75 (CH_2), 22.8 (CH_2), 24.9 (CH_2), 25.51 (CH_2), 25.54 (CH_2), 26.1 (CH_2), 27.2 (CH_2), 28.0 (CH_2), 38.9 (CH_2), 40.6 (CH), 41.1 (CH), 43.9 (CH_2), 219.9 (CO). IR (neat) ν/cm^{-1} 2929, 2868, 1743,

1470, 1445. Anal. Calcd for $C_{17}H_{30}O$: C, 81.54, H, 12.07. Found: C, 81.4, H, 11.9.

5.2.8. 3-(1-Adamantyl)-cyclopentanone (3ek). Method A: A solution of 1.36 g of **1e** (10 mmol, 0.2 M), 420 μL of **2k** (5.0 mmol, 0.1 M), and 910 mg of benzophenone (5.0 mmol, 0.1 M) in 50 mL of benzene was irradiated for 20 h. After chromatographic separation (silica gel, using first neat cyclohexane and then cyclohexane/ethyl acetate 97:3 as eluant) 680 mg of **3ek** (65%) were obtained as a glassy solid.

Method B: 272 mg of **1e** (2 mmol, 0.1 M), 170 μL of **2k** (2 mmol), and 135 mg of TBADT were dissolved in 20 mL of benzene/acetonitrile 1:1. The solution was then irradiated for 18 h and 65 mg of **3ek** (15%) were isolated after column chromatography on silica gel.

Compound **3ek**: ^{1}H NMR ($CDCl_3$) δ 1.1–2.1 (m, 18H), 2.1–2.4 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 22.4 (CH_2), 28.4 (CH), 29.0, 31.7 (CH_2), 36.2 (CH_2), 37.1 (CH_2), 38.7 (CH_2), 39.0 (CH_2), 39.9 (CH_2), 42.5 (CH_2), 46.0 (CH_2), 48.6 (CH), 48.7 (CH), 219.9 (CO). IR (neat) ν/cm^{-1} 2981, 2845, 1739, 1492, 1446. Anal. Calcd for $C_{15}H_{22}O$: C, 82.52, H, 10.16. Found: C, 82.6, H, 10.0.

5.2.9. 3-Cyclopentylcyclohexanone (3al).[39] Method A: 5.5 mL of **1a** (60 mmol, 3 M), 190 μL of **2l** (2 mmol, 0.1 M), and 365 mg of benzophenone (2 mmol, 0.1 M) were dissolved in 14 mL of benzene and the solution was irradiated for 20 h yielding **3al** as a colorless oil (178 mg, 54%).

Method B: A solution of 1.1 mL (12 mmol, 0.5 M) of **1a**, 232 μL (2.4 mmol) of **2l**, and 160 mg of TBADT in 22 mL of acetonitrile was irradiated for 16 h affording 130 mg (33%) of **3al**.

The spectroscopic data of compound **3al** were in accordance with literature data.[39]

5.2.10. 3-Cyclohexylcyclohexanone (3bl).[40] Method A: To 24 mL of **1b** were added 230 μL of **2l** (2.4 mmol, 0.1 M) and 437 mg of benzophenone (2.4 mmol, 0.1 M). The resulting solution was irradiated for 20 h. Compound **3bl** (55%, 238 mg) were isolated as a colorless oil.

Method B: A solution of 1.2 mL (12 mmol, 0.5 M) of **1b**, 230 μL (2.4 mmol) of **2l**, and 160 mg of TBADT in 22 mL of acetonitrile was irradiated for 16 h yielding 130 mg (33%) of **3bl**.

The spectroscopic data of compound **3bl** were in accordance with literature data.[40] Anal. Calcd for $C_{12}H_{20}O$: C, 79.94, H, 11.18. Found: C, 79.7, H, 10.8.

5.2.11. 3-Cycloheptylcyclohexanone (3cl). Method A: 18 mL of **1c** (150 mmol, 3 M), 480 μL of **2l** (5.0 mmol, 0.1 M), and 910 mg of benzophenone (5 mmol, 0.1 M) were dissolved in 32 mL benzene. Irradiation for 26 h of the solution formed afforded 505 mg of **3cl** (52%) as a colorless oil.

Method B: a solution of 1.5 mL (12 mmol, 0.5 M) of **1c**, 232 μL (2.4 mmol) of **2l**, and 160 mg of TBADT in 24 mL of acetonitrile was irradiated for 16 h giving 200 mg (43%) of **3cl**.

Compound **3cl**: ^{1}H NMR ($CDCl_3$) δ 1.1–1.8 (m, 16H), 1.8–2.4 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 25.6 (CH_2), 26.9 (CH_2), 27.0 (CH_2), 28.1 (CH_2), 30.9 (CH_2), 41.5 (CH_2), 43.6 (CH), 45.2 (CH_2), 45.6 (CH), 212.6 (CO). IR (neat) ν/cm^{-1} 2929, 2868, 1743, 1470, 1445. Anal. Calcd for $C_{13}H_{22}O$: C, 80.35, H, 11.41. Found: C, 80.3, H, 11.3.

5.2.12. 3-Cyclododecylcyclohexanone (3dl). Method A: 8.40 g of **1d** (50.0 mmol, 1 M), 480 μL of **2l** (5.0 mmol, 0.1 M), and 910 mg of benzophenone (5.00 mmol, 0.1 M) were dissolved in 50 mL of benzene. After irradiation for 26 h of the solution formed, 474 mg of **3dl** (36%) were obtained as a colorless solid (mp 166–168 °C).

Method B: A solution of 0.67 g of **1d** (4 mmol, 0.2 M), 190 μL of **2l** (2 mmol), and 135 mg of TBADT in 20 mL of benzene/acetonitrile 1:1 was irradiated for 18 h yielding 80 mg of **3dl** (15%).

Compound **3dl**: ^{1}H NMR ($CDCl_3$) δ 1.1–2.0 (m, 26H), 2.0–2.5 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 22.2 (CH_2), 22.3 (CH_2), 23.2 (CH_2), 23.3 (CH_2), 23.5 (CH_2), 23.6 (CH_2), 24.3 (CH_2), 25.2 (CH_2), 25.6 (CH_2), 25.7 (CH_2), 26.0 (CH_2), 26.1 (CH_2), 28.6 (CH_2), 38.0 (CH), 41.4 (CH_2), 41.5 (CH), 45.6 (CH_2), 212.6 (CO). IR (neat) ν/cm^{-1} 2931, 2868, 1711, 1468, 1445. Anal. Calcd for $C_{18}H_{32}O$: C, 81.75, H, 12.20. Found: C, 81.8, H, 12.1.

5.2.13. 3-(1-Adamantyl)-cyclohexanone (3el).[41] Method A: 436 mg of **1e** (3.2 mmol, 0.2 M), 150 μL of **2l** (1.6 mmol, 0.1 M), and 284 mg of benzophenone (1.6 mmol, 0.1 M) were dissolved in 16 mL of benzene, the solution was irradiated for 26 h. After column chromatography on silica gel, 65 mg of **3el** (40%) were obtained as a white solid (mp 58–60 °C, lit.[41] 60.2–61.6 °C).

Method B: 272 mg of **1e** (2 mmol, 0.1 M), 190 μL of **2l** (2 mmol), and 135 mg of TBADT in 20 mL acetonitrile were irradiated at 310 nm for 18 h. The solution was evaporated at reduced pressure and distilled in vacuo to give 97 mg of **3el** (0.42 mmol, 21%).

Compound **3el**: ^{1}H NMR ($CDCl_3$) δ 1.1–1.95 (m, 19H), 1.95–2.4 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 24.4 (CH_2), 25.5 (CH_2), 28.5 (CH), 34.2 (CH_2), 37.0 (CH_2), 39.2 (CH_2), 41.4 (CH_2), 41.9 (CH_2), 49.5 (CH), 213.2 (CO). IR (neat) ν/cm^{-1} 2920, 2848, 1711, 1492, 1446. Anal. Calcd for $C_{16}H_{24}O$: C, 82.70, H, 10.41. Found: C, 82.4, H, 10.1.

5.2.14. 3-Cyclopentylmethyl-bicyclo[2.2.1]heptan-2-one (3am). Method B: A solution of 1.1 mL (12 mmol, 0.5 M) of **1a**, 280 μL (2.4 mmol) of **2m**, and 160 mg of TBADT in 22 mL of acetonitrile was irradiated for 16 h affording 200 mg (45%) of **3am** as a colorless oil.

Compound **3am**: ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 20H), 2 (m, 1H), 2.5 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 21.2 (CH_2), 24.9 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 31.8 (CH_2), 32.2 (CH_2), 33.1 (CH_2), 37.0 (CH_2), 38.3 (CH), 38.4 (CH), 50.4 (CH), 53.0 (CH). IR (neat) ν/cm^{-1} 2950, 2873, 1740.

Anal. Calcd for $C_{13}H_{20}O$: C, 81.20, H, 10.48. Found: C, 81.0, H, 11.2.

5.2.15. 3-Cyclohexylmethyl-bicyclo[2.2.1]heptan-2-one (3bm).[42] Method A: 10 mL (90 mmol, 3 M) of **1b**, 280 μL (2.3 mmol, 0.08 M) of **2m**, and 440 mg of xanthone (2.3 mmol, 0.08 M) were dissolved in 20 mL of DMC. The solution was irradiated for 20 h and 380 mg of **3bm** (80%) were formed as a colorless syrup.

Method B: A solution of 1.2 mL (12 mmol, 0.5 M) of **1b**, 280 μL (2.4 mmol) of **2m**, and 160 mg of TBADT in 22 mL of acetonitrile was irradiated for 16 h giving 240 mg (49%) of **3bm**.

The spectroscopic data of compound **3bm** were in accordance with literature data.[42] MS (*m*/*z*) 206 (M^+, 15), 110 (32), 82 (100), 67 (46), 55 (28), 41 (27). Anal. Calcd for $C_{14}H_{22}O$: C, 81.50, H, 10.75. Found: C, 81.7, H, 10.5.

5.2.16. 3-Cycloheptylmethyl-bicyclo[2.2.1]heptan-2-one (3cm). Method A: 15 mL (0.12 mol, 4.1 M) of **1c**, 280 μL (2.3 mmol, 0.08 M) of **2m**, and 440 mg of xanthone (2.3 mmol, 0.08 M) were dissolved in 15 mL of DMC. The solution was irradiated for 20 h and 401 mg of **3cm** (80%) were isolated as a colorless syrup.

Method B: A solution of 1.4 mL (12 mmol, 0.5 M) of **1c**, 280 μL (2.4 mmol) of **2m**, and 160 mg of TBADT in 22 mL of acetonitrile was irradiated for 16 h giving 260 mg (45%) of **3cm**.

Compound **3cm**: ^{1}H NMR ($CDCl_3$) δ 0.7–1.95 (m, 24H), 2.1 (m, 1H), 2.6 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 21.1 (CH_2), 25.1 (CH_2), 26.0 (CH_2), 26.1 (CH_2), 28.2 (CH_2), 28.4 (CH_2), 32.8 (CH_2), 34.0 (CH_2), 35.4 (CH_2), 36.9 (CH_2), 37.1 (CH), 38.3 (CH), 50.4 (CH), 51.5 (CH). IR (neat) ν/cm^{-1} 2940, 2870, 1750, 1100. Anal. Calcd for $C_{15}H_{24}O$: C, 81.76, H, 10.98. Found: C, 81.6, H, 10.8.

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Synthesis and DNA binding studies of bis-intercalators with a novel spiro-cyclic linker

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Abstract—Threading polyintercalation has been demonstrated as a unique DNA binding mode in which a polyintercalating moiety threads back and forth through the DNA double helix. This binding topology necessitates linkers residing in both the minor and major grooves in an alternating fashion. In the present work, two novel, rigid, cis and trans oriented spiro-cyclic linkers were synthesized as potential groove binding elements in the context of threading bis-intercalation. Analysis of dissociation kinetics indicated that the cis oriented dimer has dramatically slower dissociation from poly(dGdC) and calf thymus (CT) DNA compared to the trans oriented dimer and a linear dimer control.

1. Introduction

Recognizing specific DNA sequences using synthetic molecules has been an exciting research area for over two decades due to the potential for controlling gene expression, creating new therapeutics, and exploration of molecular recognition in general. Several approaches to DNA recognition have been developed. A particularly successful strategy involves minor groove binding, exemplified by the naturally occurring netropsin, distamycin, beneril, and their analogues.[1] Based on these molecules, programmable sequence-specific binding has been achieved with the polyamides developed by Dervan and co-workers.[2] Importantly, molecules of this class have been shown to modulate gene function in vitro[3] and even in vivo,[4] although cellular uptake appears to be a considerable challenge. A second approach to programmable DNA recognition involves triplex formation in the major groove.[5]

Recently, our group has developed a novel threading polyintercalator approach based on the 1,4,5,8-naphthalenetetracarboxylic diimide (NDI) unit first identified as a threading intercalator by Wilson and co-workers.[6] Our multiple diimide polyintercalators are designed to bind specific DNA sequences, threading through both the major and minor grooves, an unprecedented feature for a DNA binding molecular scaffold.[7–9] It has been demonstrated that two different threading bis-intercalators can bind to specific DNA sequences with their linkers in the minor and major grooves, respectively.[7,8] Significantly, NMR structural analysis of a designed threading tetraintercalator was shown to have a predicted threading binding topology in which linkers alternated between the minor and major grooves, much as a snake might climb a ladder.[9] However, in this same analysis, the major groove binding linker exhibited significant disorder, leading to the conclusion that a properly designed, rigidified linker design could greatly improve binding specificity and affinity.

The present paper describes the design and synthesis of a rigidified linker intended to be a scaffold for future linker designs. Preliminary DNA binding studies are reported in the context of threading bis-intercalation.

2. Results and discussion

2.1. Linker design

The flexibility of our first generation linkers likely limits overall specificity as well as possible programmable recognition of different sequences of DNA. Specific recognition based on shape and/or hydrogen bonding complementarity may be compromised by the flexibility inherent in the unconstrained peptide chains. A conformationally restricted linker scaffold, of the proper size and geometry, should be able to improve not only specificity of DNA recognition, but overall binding affinity as well.[10] The important caveat here is that a rigidified scaffold should be readily synthesizeable and have the potential to be derivatized with various

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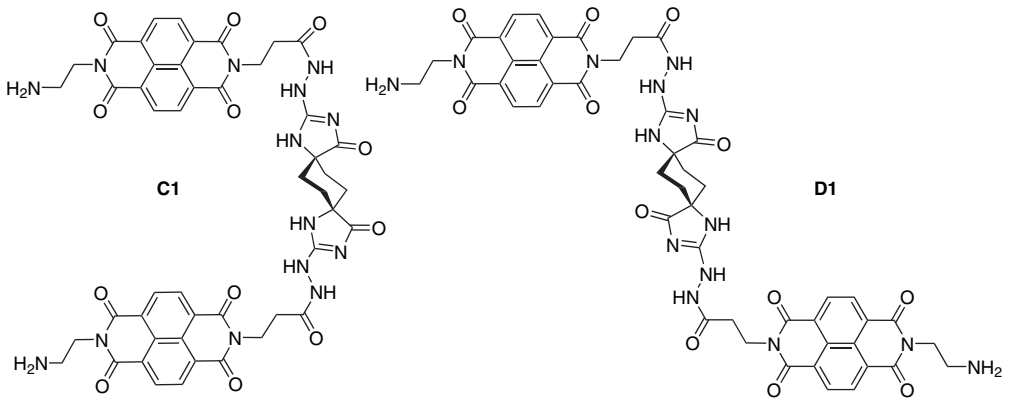


Figure 1. The structures of two dimers, **C1** and **D1**, containing cis and trans spiro-cyclic linkers, respectively.

functional groups capable of interacting with specific sites on the DNA bases.

Shown in Figure 1 are two novel rigid scaffolds.[11] The two heterocycles are intended to (1) lock the central cyclohexane ring into a chair conformation and (2) form hydrogen bonds with bases on the floor of a DNA groove. Next generation designs would involve derivatization of the central six-membered ring to achieve alternative specificities. Computer modeling indicates that these rigid scaffolds could be compatible with binding in either the minor or major grooves, increasing the potential applications of the design (Fig. 2). The same modeling indicated that the cis isomer should be more compatible with the curvature of DNA compared to the trans derivative.

2.2. Synthesis

2.2.1. trans-Spiro linker. A Bucherer–Bergs reaction gave the two bis-spirohydantoins shown (**1** and **2**) in excellent yields (Schemes 1 and 2).[12] Both structures were found to be in the trans geometry based on NOESY spectra (not shown). The kinetic and/or thermodynamic reasons for the exclusive formation of the trans geometry (i.e., no cis products were observed) are currently unknown. The *trans*-1, 4-diamino-1,4-dicarboxylic acid dimethyl ester (**3**) was synthesized in good yield. The structure of intermediate **5** was confirmed by X-ray crystallography (Fig. 3).

Scheme 3 describes the conversion of diamino dimethyl carboxylate **6** into the desired trans linker **10**. The easy removal of benzoyl group makes benzoyl isothiocyanate an ideal reagent to form a precursor to the *trans*,bis-spirothiohydantoin **8**. Using mild base, intermediate **8** was derived from cyclization in 75% yield. The structure of **8** was confirmed by X-ray crystallography (Fig. 4). Subsequent methylation and hydrazine substitution gave the desired trans linker **10** in good overall yield.

2.2.2. cis-Spiro linker for C1. Since the cis oriented bis-hydantoins could not be made via Bucherer–Bergs reaction when the two carbonyl groups of 1,4-cyclohexane were cyclized simultaneously, the two cyclization reactions were carried out separately. For this purpose, mono-protected 1,4-cyclohexanedione was used as shown in Scheme 4.

The Bucherer–Bergs reaction was used with the mono-protected 1,4-cyclohexanedione to give mono-spirohydantoin **11**. NMR analysis confirmed that **11** contained the NH group and CO group in axial and equatorial orientations, respectively. Alkylation gave compound **12**, whose geometry was confirmed by X-ray crystallography (Fig. 5). Acidic deprotection gave compound **13** in high yield. Based on the difference between *A*-values of $-NH_2$ and –CN, we anticipated that a Strecker reaction would give a cis and trans mixture of amino monohydantoins.[13] However, following a Strecker reaction on intermediate **13**, cyclization using chlorosulfonyl isocyanate and 1 M HCl gave us two bis-spirohydantoins **16** and **17**. Their NOESY spectra (not shown) suggested that both were of the trans geometry.

The fact that no cis isomer was found under the above reaction conditions might indicate that the reaction occurring on one carbonyl group directs the reaction pattern on the other. In addition, the unfavorable interaction between NH and NH_2 may contribute as well. Interestingly, in the case of an analogous five-member ring system, a cis and trans mixture was formed.[14]

A second attempt was made to obtain the cis structure based on a Diels–Alder strategy (Scheme 5) via key intermediate **24**. Starting from **18**, which can be made in a few steps,[15] DIBAl-H reduction at 0 °C and Diels–Alder reaction with DEAD gave the compound **20** in a modest yield due to competing side reactions such as aromatization of **19** and the ene reaction.[16] Enough material could be generated to continue the synthesis, but this step is nevertheless going to be optimized to hopefully improve the yield. Following protection of the hydroxyl groups, reductive cleavage using Na in ammonia gave compound **22** in high yield. The two hydroxyl groups were successfully oxidized using basic $KMnO_4$.

Shown in Scheme 6 are the final steps to make the cis linker **30** analogous to the procedures used in the trans linker synthesis. The cis geometry of bis-spirothiohydantoin intermediate **28** was confirmed by X-ray crystallography (Fig. 6).

The corresponding trans and cis NDI dimers **D1** and **C1** were synthesized in high yield using PyBop and HOBT as the coupling reagents (Scheme 7) to attach previously reported NDI derivatives to the trans and cis linkers.[17]

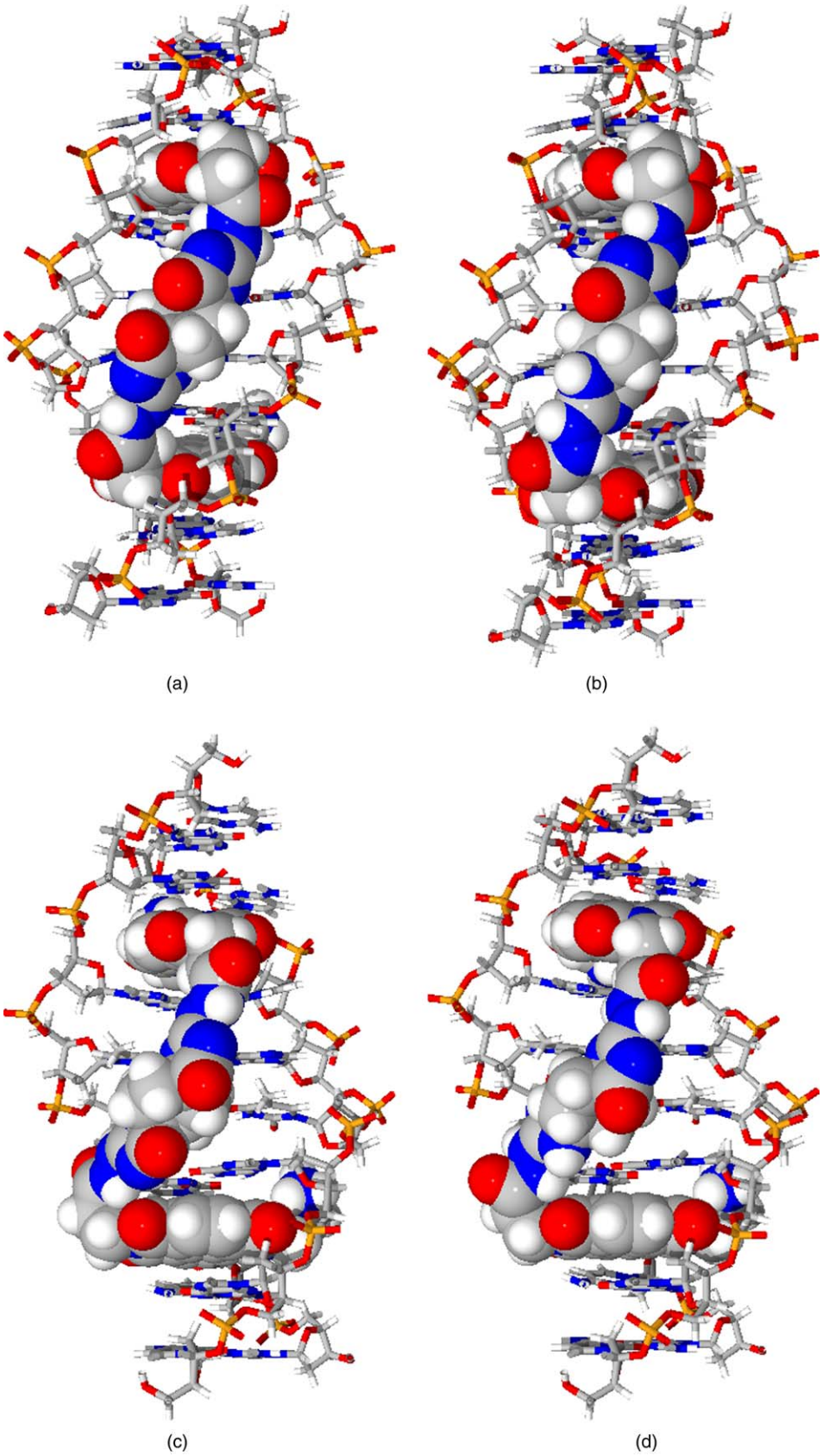
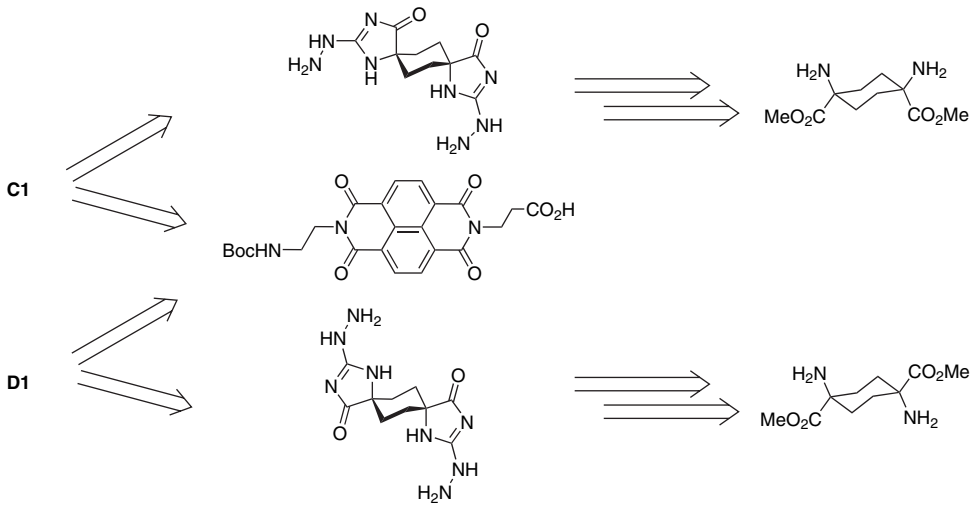
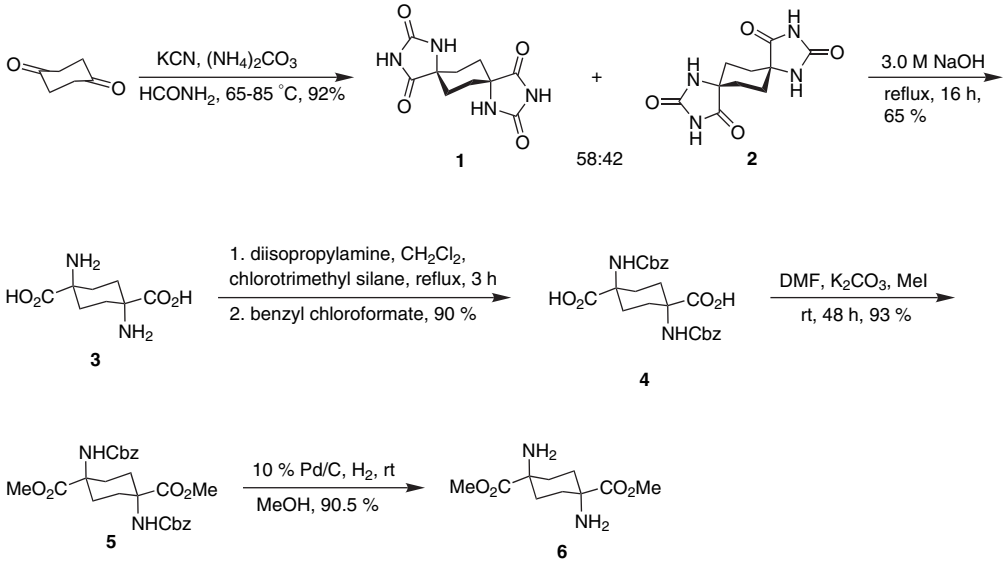


Figure 2. View from the minor grooves (a and b) and major grooves (c and d) of the **C1** (left) and **D1** (right) bis-intercalator/d(CGGTACCG)$_2$ complexes.



Scheme 1. Retrosynthetic analysis of bis-intercalators **C1** and **D1**.



Scheme 2.

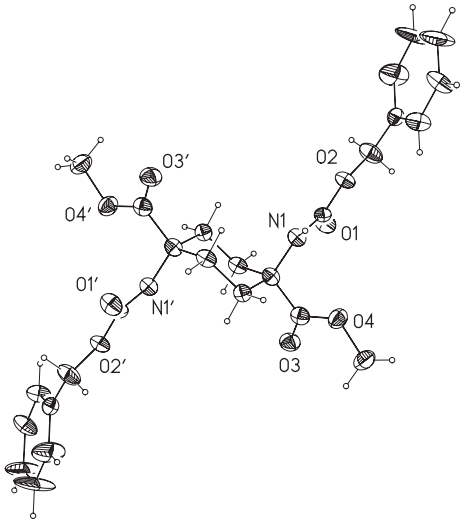
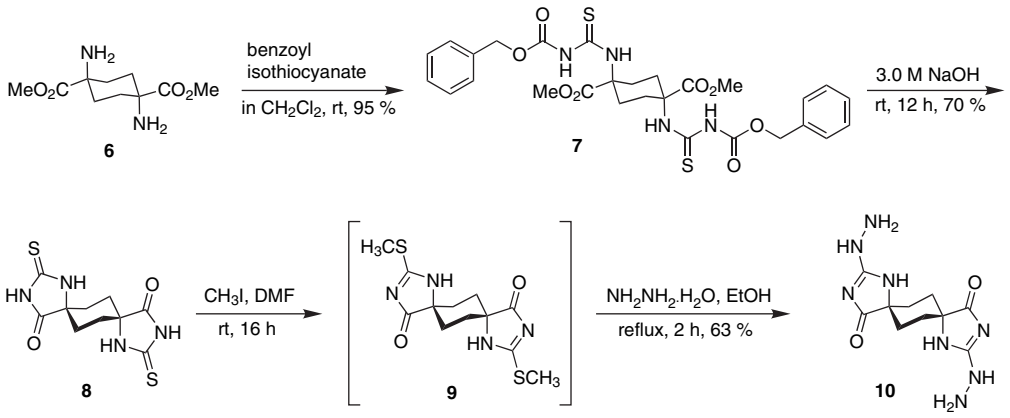


Figure 3. Displacement ellipsoid diagram for **5**.

2.3. Kinetics studies

As a preliminary investigation into the interaction of **D1** and **C1** with DNA, dissociation rates were determined for CT DNA, poly(dGdC) and poly(dAdT). These values were compared to similar data obtained with a previously reported dimer containing a flexible peptide linker (referred to a $\mathbf{G_3K}$).[8] For NDI bis-intercalators, dissociation rates are generally sufficiently slow to allow monitoring using a spectrophotometer without the need for a stopped-flow apparatus. Dissociation rate measurements were carried out with 2% SDS in the buffer to sequester the dissociated intercalators following dissociation from the DNA.[17] Control studies indicated that the dimers did not reassociate with the DNA under these conditions. All dissociation profiles were adequately described by one phase exponential decay, $A=a_0 \exp(-kt)+b_0$.[18]



Scheme 3.

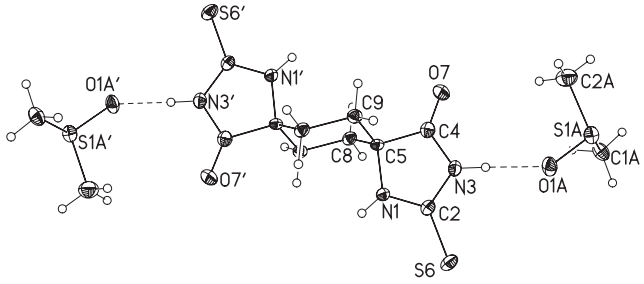


Figure 4. Displacement ellipsoid diagram for **8**·DMSO.

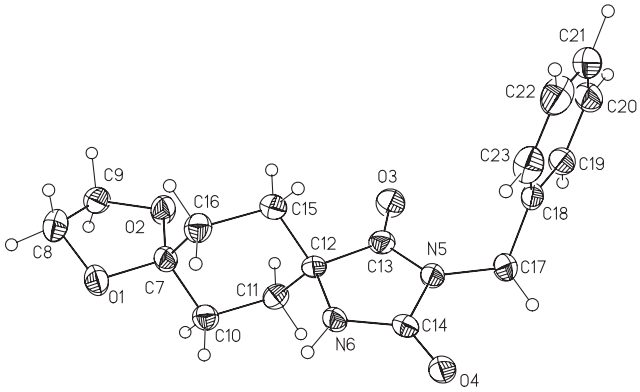
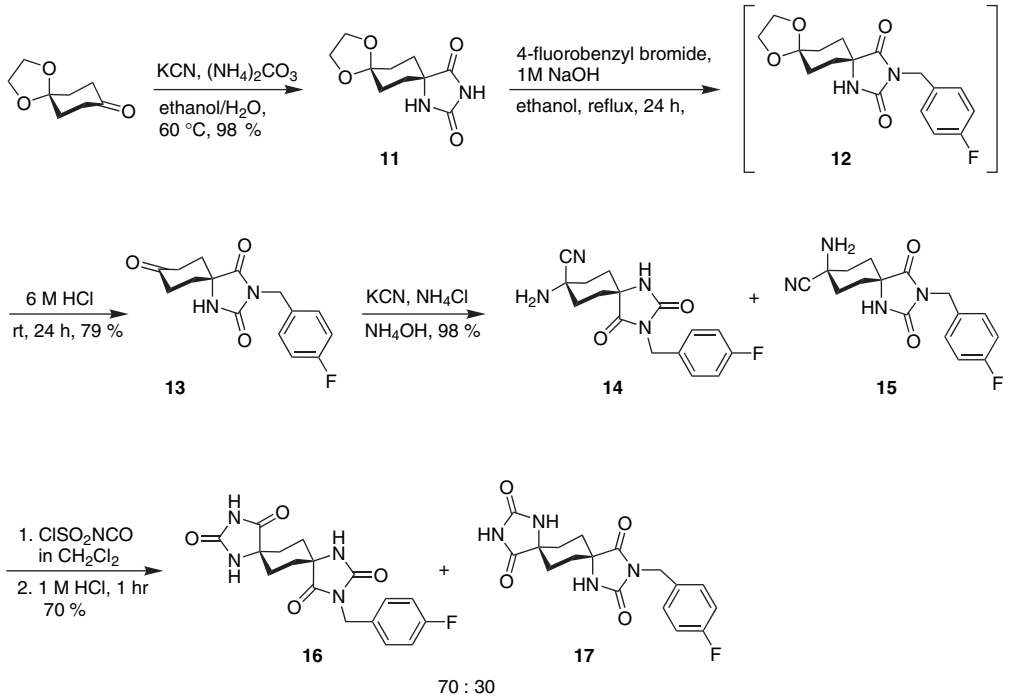
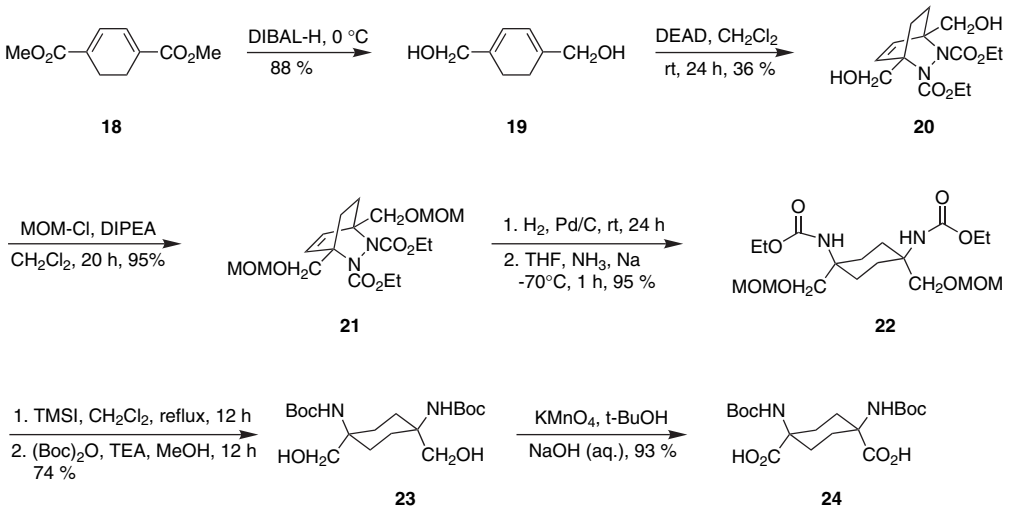


Figure 5. Displacement ellipsoid diagram for **12a** (an analogue of **12**).

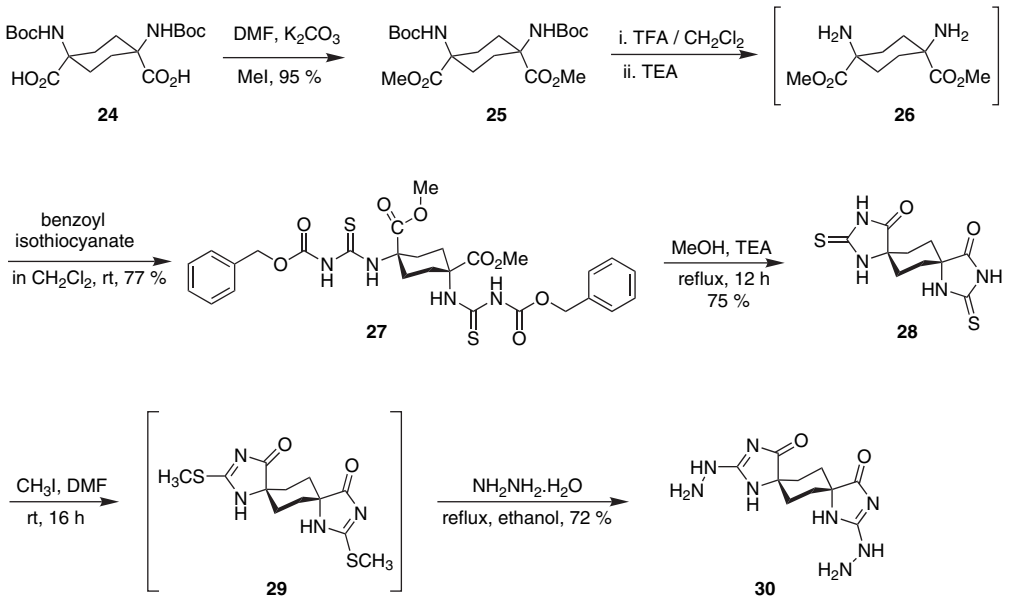
The dissociation rate data reveal that all dimers exhibit a dramatic preference for poly(dGdC) over poly(dAdT) (Table 1 and Supplementary data), a trend seen with previously reported NDI based intercalation.[17] The dissociation half-lives measured using poly(dGdC) for all of the dimers extend over 10 min, with **C1** exhibiting a 50 min half-life. In contrast, the dissociations of the three dimers from poly(dAdT) were almost complete within 1 min, with the cis dimer **C1**



Scheme 4.



Scheme 5.



Scheme 6.

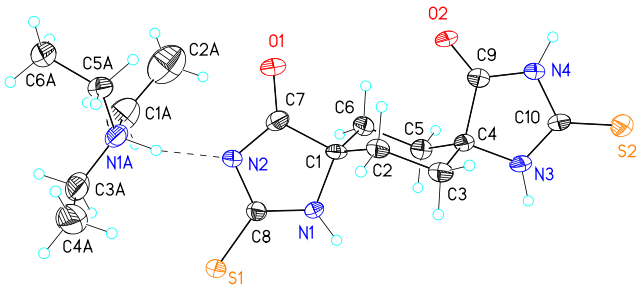
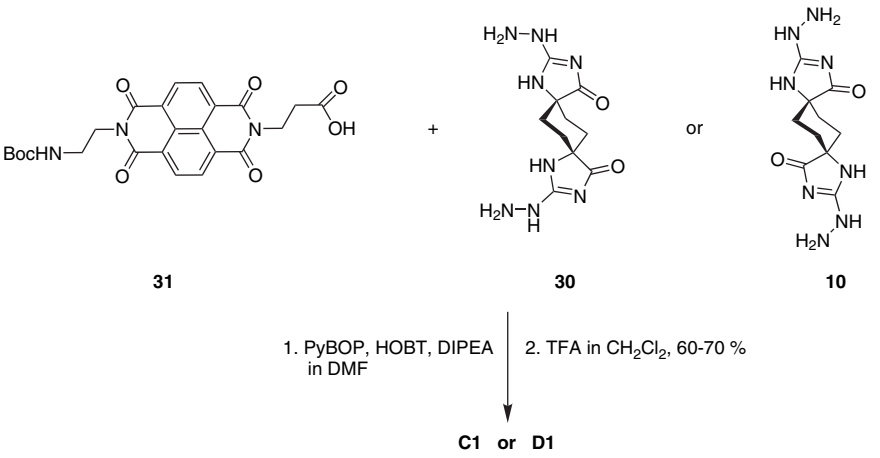


Figure 6. Displacement ellipsoid diagram for **28**·$N(CH_2CH_3)_3$.

exhibiting the shortest half-life. Taken together, our data indicate that the cis dimer **C1** binds with greatest overall affinity to poly(dGdC), and with the greatest overall ability to discriminate between poly(dGdC) and poly(dAdT).

3. Conclusion

Two novel threading bis-intercalators containing cis and trans oriented spiro-cyclic linkers were successfully synthesized, although preparation of the cis derivative proved to be a considerable challenge. Data from dissociation kinetics suggested that the DNA binding behavior of the cis oriented dimer **C1** is different from that of the trans oriented dimer **D1** and linear dimer **G_3K** based on studies with poly(dGdC), poly(dAdT), and CT DNA. The cis dimer **C1** shows the slowest off-rate toward poly(dGdC) and the fastest off-rate with poly(dAdT). The most straightforward interpretation of these results is that by constraining the rigidified linker in the appropriate cis geometry, both binding affinity and specificity have been enhanced compared to our flexible linker designs. DNAse I footprinting experiments and an NMR structural analysis are currently being carried out to identify the binding modes and specificities of **C1**.



Scheme 7. Solution phase dimer synthesis.

Table 1. Dissociation rates and half-life times

DNA type	D1		C1		G_3K	
	k (s^{-1})	t (min)	k (s^{-1})	t (min)	k (s^{-1})	t (min)
Poly(dAdT)	1.02	0.682	2.86	0.243	1.10	0.629
Poly(dGdC)	0.0528	13.1	0.0138	49.9	0.0471	14.7
CT DNA	0.51	1.4	0.13	5.3	0.37	1.9

4. Experimental

4.1. General

All chemicals were purchased from Aldrich, Acros, or Novabiochem. Dry solvents were either purchased or dried using common laboratory techniques. All chemical reactions were carried out in oven-dried glassware under an argon atmosphere. Silica gel 60 F_{254} glass-based plates (Merck) were used for TLC. Flash chromatography was carried out using ICN SiliTech 32-63D 60 Å silica gel. All NMR spectra were recorded on Varian 300 or 500 MHz instruments. $CDCl_3$ (δ_H=7.24 and δ_C=77.0 ppm) or DMSO-d_6 (δ_H=2.49 and δ_C=39.5 ppm) or D_2O (δ_H=4.67 ppm) were used as solvents. Assignment of ^{13}C signals is based on ^1H and ^{13}C-correlated 2D NMR spectra.

4.1.1. 1,3,9,11-Tetraaza-dispiro[4.2.4.2]tetradecane-2,4,10,12-tetraone (1,α) and (2,β). To a 40 mL Ace pressure tube were added 1,4-cyclohexanedione (1.12 g, 0.01 mol), potassium cyanide (1.43 g, 0.022 mol), and ammonium carbonate (4.8 g, 0.05 mol). Formamide (35 mL) was then added and the pressure tube was tightly closed. The tube was heated at 60 °C for 24 h, then at 85 °C for another 24 h with vigorous stirring. Vacuum filtration at room temperature gave a white solid after rinsing with cold water and ethanol. The filtrate was treated with enough mixed ion exchange resin (AG® 501-X8 (D) Resin, 20–50 mesh, BIO-RAD) to extract remaining cyanide anions. The crude solid was washed with CH_2Cl_2 (3×20 mL), giving a pure solid product (2.32 g, 92%): ^1H NMR (500 MHz, DMSO-d_6): δ 10.57 (br s, 2H), 8.53 (s, 1H, H1′ of **1**), 8.18 (s, 1H, H1′ of **2**), 2.10–2.05 (m, 4H, equatorial of **2**), 1.99–1.91 (m, 4H, equatorial of **1**), 1.68–1.63 (m, 4H, axial of **2**), 1.62–1.57 (m, 4H, axial of **1**); ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 177.4 (C4′ of **2**), 177.3 (C4′ of **1**), 155.8 (C2′ of **1**), 155.7 (C2′ of 2), 60.3 (C1 and C4 of **1**), 59.4 (C1 and C4 of **2**), 28.8 (C2, C3, C5, and C6 of **2**), 28.4 (C2, C3, C5, and C6 of **1**); HRMS-CI calcd for $C_{10}H_{13}N_4O_4$ $[M+H]^+$: 253.0937, found 253.0926.

4.1.2. *trans*-1,4-Diamino-cyclohexane-1,4-dicarboxylic acid (3). The dihydantoins **1** and **2** (1.16 g, 0.0046 mol) were suspended in 12 mL of 3 M NaOH aqueous solution and the mixture was heated at reflux (120 °C) while stirring for 16 h. The reaction mixture then was cooled to room temperature and filtered through a fritted glass filter. The filtrate was brought to pH 2 with 3 M HCl at 0 °C and a white precipitate formed. After 10 min at 0 °C, the mixture was filtered through a fine fritted glass filter. The solid was washed with copious cold deionized water and dried at 50 °C under vacuum oven over 12 h, yielding the desired product **3** (0.59 g, 65%): ^1H NMR (300 MHz, D_2O+NaOD): δ 1.75 (d, 4H, equatorial, J=9 Hz), 1.21 (d, 4H, axial, J=9 Hz); ^{13}C NMR (75.5 MHz, D_2O+NaOD, uncorrected): δ 184.8 (COOH), 57.5 (C1 and C4), 31.1 (C2, C3, C5, and C6). No useful mass data were available due to insolubility of this compound in common solvents.

4.1.3. *trans*-1,4-Bis-(benzyloxycarbonylamino)-1,4-cyclohexanedicarboxylic acid (4). To a 50 mL flask equipped with a magnetic stir bar and rubber septum was added diamino acid **3** (0.6 g, 0.003 mol). The solid was suspended in 20 mL of CH_2Cl_2. Diisopropylamine (1.72 mL, 0.0099 mol) was added with stirring. Chlorotrimethylsilane (1.71 mL, 0.0135 mol) was then added slowly, the rubber septum was replaced with a reflux condenser, and the solution was heated at reflux under nitrogen for 4 h. The condenser was removed and replaced with another rubber septum. The flask was cooled in an ice bath, and benzyl

chloroformate (Cbz-Cl) (0.81 mL, 0.006 mol) was added in one portion via syringe. The stirred solution was allowed to warm and stirred at room temperature overnight. The mixture was concentrated to a pink syrup, dissolved in 100 mL 1.0 M aqueous NaOH, and transferred to a separatory funnel. The aqueous solution was washed with ether (2×100 mL). The ether washes were combined and back extracted with water (2×200 mL). The aqueous layers were combined and acidified to pH 2 with 3 M HCl at 0 °C. White solid formed and was filtered through a fine fritted glass filter. The solid was washed with cold water then air-dried to give the desired product **4** (1.27 g, 90%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.40 (br s, 2H, COOH), 7.56 (s, 2H, 2NH), 7.36–7.31 (m, 10H, aromatic), 5.00 (s, 4H, 2CH_2Ph), 1.92–1.82 (m, 8H, 4CH_2 of central ring); ^{13}C NMR (75 MHz, DMSO-d_6): δ 176.0 (COOH), 155.5 (CONH), 137.0, 128.3, 127.8, and 127.7 (aromatic), 65.1 (CH_2Ph), 57.4 (C1 and C4), 26.4 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{24}H_{27}N_2O_8$ [M+H]$^+$: 471.1767, found 471.1774.

4.1.4. *trans*-1,4-Bis-(benzyloxycarbonylamino)-1,4-dimethoxycarbonyl-cyclohexane (5). Dicarboxylic acid **4** (1.955 g, 4.1 mmol) was dissolved in dry DMF (33 mL) in a 100 mL round bottom flask, and then anhydrous K_2CO_3 (1.72 g, 12.4 mmol) was added with vigorous stirring. MeI (0.65 mL, 10 mmol) was added slowly by syringe and the reaction was stirred at room temperature for 48 h. H_2O (120 mL) was added at the end of the reaction and the product precipitated. Filtration in a fine fritted glass filter followed by washing with cold water gave the desired product (1.9 g, 93%, R_f=0.25 in 1/49 CH_3OH/CH_2Cl_2): ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.76 (s, 2H, 2CONH), 7.37–7.30 (m, 10H, aromatic), 5.01 (s, 4H, 2CH_2Ph), 3.56 (s, 6H, 2CH_3), 1.92–1.82 (m, 8H, 4CH_2 of central ring); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 174.7 (CO_2CH_3), 155.5 (CONH), 137.0, 128.4, 127.8, and 127.7 (aromatic), 65.2 (CH_2Ph), 57.6 (C1 and C4), 52.0 (CH_3), 26.4 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{26}H_{31}N_2O_8$[M+H]$^+$: 499.2080, found 499.2075.

4.1.5. *trans*-1,4-Diamino-1,4-dimethoxycarbonyl-cyclohexane (6). Ester **5** (0.5 g, 1 mmol) was suspended in 20 mL methanol in a 50 mL three-neck round bottom flask, which was pre-saturated with dry Ar. Pd/C (10%, 0.15 g) was added slowly. The solution was degassed, placed under hydrogen atmosphere (10 psi), and stirred for 1.5 h. The reaction mixture was filtered through Celite on a fine fritted glass filter. The filtrate was evaporated at room temperature and then dried in vacuo. The dried solid was used directly for the next step without further purification (0.21 g, 90.5%, R_f=0.33 in 2/3 CH_3OH/CH_2Cl_2): ^{1}H NMR (300 MHz, pyridine-d_5): δ 3.59 (s, 6H, 2CH_3), 2.51–2.45 (m, 4H, equatorial), 2.03 (br s, 4H, 2NH_2), 1.63–1.57 (m, 4H, axial); ^{13}C NMR (75.5 MHz, pyridine-d_5): δ 178.6 (CO_2CH_3), 56.9 (C1 and C4), 51.8 (CH_3), 30.5 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{10}H_{19}N_2O_4$ [M+H]$^+$: 231.1345, found 231.1347.

4.1.6. *trans*-1,4-Bis-((*N*-benzoyl(thiocarbamoyl)amino)-1,4-dimethoxycarbonyl-cyclohexane (7). Diamine **6** (0.115 g, 0.5 mmol) was dissolved in dry CH_2Cl_2 (10 mL) in a 50 mL round bottom flask. Benzoyl isothiocyanate (0.15 mL, 1.1 mmol) was added by syringe with stirring. The solution became cloudy after stirring for a few hours. The reaction was terminated after 24 h by adding another 10 mL of CH_2Cl_2 to dilute the mixture and the solid was isolated by filtration using a fine fritted glass filter (0.26 g, 95%, R_f=0.35 in 1/1 CH_3OH/CH_2Cl_2): ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.56 (s, 2H, 2(C═O)NH(C═S)), 11.43 (s, 2H, 2NH(S═C)), 7.99–7.93 (m, 4H, aromatic), 7.68–7.64 (m, 2H, aromatic), 7.58–7.54 (m, 4H, aromatic), 3.61 (s, 6H, 2CH_3), 2.57–2.52 (m, 4H, equatorial), 1.97–1.93 (m, 4H, axial); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 179.8 (C═S), 172.0 (CO_2Me), 169.0 (CONH), 133.3, 131.9, 128.8, and 128.5 (aromatic), 70.1 (CH_2Ph), 60.0 (C1 and C4), 52.3 (CH_3), 26.4 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{26}H_{29}N_4O_6S_2$ [M+H]$^+$: 557.1529, found 557.1525.

4.1.7. *trans*-Cyclohexane-1, 4-bis-spiro-5′,5″-(2′,2″-thiohydantoin) (8). Isothiocyanate **7** (0.51 g, 0.9 mmol) was dissolved in 10 mL of 3 M NaOH aqueous solution. After stirring for 5 min, white precipitate formed in the flask. Stirring was continued for 16 h. The reaction was terminated by adding 1.0 M HCl to bring the solution pH to neutral. After being stirred for additional 1 h the mixture was filtered and rinsed with cold water to give the crude product. The product was purified by crystallization in DMSO (0.18 g, 70%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.65 (br s, 2H, 2(C═O)NH(C═S)), 10.70 (br s, 2H, 2(C═S)NH), 2.02 (d, 4H, equatorial, J=9.3 Hz), 1.69 (d, 4H, axial, J=9.3 Hz); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 181.4 (C═S), 178.0 (C═O), 63.8 (C1 and C4), 27.7 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{10}H_{13}N_4O_2S_2$ [M+H]$^+$: 285.0480, found 285.0488.

4.1.8. *trans*-2,10-Dihydrazino-1,3,9,11-tetraaza-dispiro[4.2.4.2]tetradeca-1,9-diene-4,12-dione (10). Thiohydantoin **8** (1.0 g, 3.5 mmol) was dissolved in anhydrous DMF (10 mL) with gentle heating in 25 mL round bottom flask. Methyl iodide (0.88 mL, 14 mmol) was then added via syringe. The flask was clear and light yellowish for the first few hours, and then some pale yellowish precipitate formed inside. After stirring the mixture for 12 h at room temperature, the solvent was removed at room temperature in vacuo. The dry solid was used directly for the next step due to its presumed moisture sensitivity. Solid methylsulfanyl hydantoin **9** was dissolved in 30 mL of ethanol in a round bottom flask. Hydrazine monohydrate (0.85 mL, 17.6 mmol) was added dropwise to the mixture. The clear solution was heated at reflux for 2 h, forming a white precipitate. The flask was cooled down to room temperature and vacuum filtration gave a white powder that was further dried in vacuo (0.62 g, 63%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.90 (br s, 2H, 2NH), 8.28 (s, 2H, 2NHNH_2), 4.49 (s, 4H, 2NH_2), 1.99 (d, 4H, equatorial, J=8.6 Hz), 1.34 (d, 4H, axial, J=8.6 Hz); ^{13}C NMR (75.5 MHz, DMSO-d_6+TFA): δ 178.0 (C═O), 158.9 (NH–C═N), 64.0 (C1 and C4), 29.1 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{10}H_{17}N_8O_2$ [M+H]$^+$: 281.1474, found 281.1465.

4.1.9. 9,12-Dioxa-1,3-diaza-dispiro[4.2.4.2]tetradecane-2,4-dione (11). To a 40 mL pressure tube, 1,4-cyclohexanedione monoethylene acetal (1.56 g, 0.01 mol), potassium cyanide (0.65 g, 0.01 mol), and ammonium carbonate (2.4 g, 0.025 mol) were added followed by 35 mL ethanol/water

(v/v=1/1). The reaction tube was tightly capped and heated at 65 °C for 24 h with stirring. The reaction mixture was cooled down to 0 °C using an ice bath. Approximately half of the solvent was removed by vacuum evaporation and the solid was isolated by vacuum filtration. The filtrate was treated with mixed ion exchange resin (AG® 501-X8 (D) Resin, 20–50 mesh, BIO-RAD) to extract the remaining KCN. The solid product was rinsed with dichloromethane and dried in vacuo (2.21 g, 98%): ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.55 (br s, 1H, (C=O)NH(C=O)), 8.44 (s, 1H, C=ONH–C), 3.86 (s, 4H, O(CH_2)$_2$O), 1.87–1.54 (m, 8H, central ring); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.2 (C4′), 156.3 (C2′), 106.8 (C1), 63.7 and 63.6 (OCH_2-CH_2O), 61.0 (C4), 31.3 (C2 and C6), 29.8 (C3 and C5); HRMS-CI calcd for $C_{10}H_{15}N_2O_4$ $[M+H]^+$: 227.1032, found 227.1028.

4.1.10. 3-(4-Fluoro-benzyl)-1,3-diaza-spiro[4.5]decane-2,4,8-trione (13). Hydantoin **11** (1.13 g, 0.005 mol) was suspended in 1.0 M NaOH solution (5 mL) and ethanol (5 mL) and the mixture was heated at reflux for 15 min. To this solution was added 4-fluorobenzyl bromide (0.59 mL, 0.0048 mol) dropwise through the top of a reflux condenser. The mixture was heated at reflux for 24 h and allowed to cool in ice bath. The resulting precipitate was washed with water and recrystallized from ethanol to give pure product **12**. Compound **12** was then dissolved in 3 M HCl (10 mL) and ethanol (10 mL). The clear solution was stirred at room temperature for 16 h. The reaction was terminated by adding 3 M NaOH to adjust the pH of the solution to 7.5. CH_2Cl_2 (4×40 mL) was used to extract the product from the mixture. The CH_2Cl_2 layer was dried (Na_2SO_4), followed by vacuum evaporation to yield the crude product. Flash silica gel chromatography (1/19 CH_3OH/CH_2Cl_2) gave the product **13** (1.14 g, 79%, R_f=0.33 in 1/19 CH_3OH/CH_2Cl_2): ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.07 (s, 1H, NH), 7.31–7.26 (m, 2H, aromatic), 7.18–7.12 (m, 2H, aromatic), 4.54 (s, 2H, CH_2Ph), 2.55–2.48 (m, 2H, axial on C2 and C6), 2.38–2.32 (m, 2H, equatorial on C2 and C6), 2.17–2.08 (m, 2H, axial on C3 and C5), 1.95–1.89 (m, 2H, equatorial on C3 and C5); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 208.0 (CO), 175.8 (C1′), 161.0 (d, aromatic C–F, $^1J_{CF}$=242 Hz), 155.5 (C3′), 132.9 (aromatic), 129.3 (d, aromatic *C*–(C–C–F), $^3J_{CF}$=8.8 Hz), 115.3 (d, aromatic *C*–(C–F), $^2J_{CF}$=21.5 Hz), 59.8 (C4), 40.4 (*C*H_2Ph), 36.3 (C2 and C6), 32.9 (C3 and C5); HRMS-CI calcd for $C_{15}H_{16}N_2O_3F$ $[M+H]^+$: 291.1145, found 291.1141.

4.1.11. 8-Amino-3-(4-fluoro-benzyl)-2,4-dioxo-1,3-diaza-spiro[4.5]decane-8-carbonitriles (14 and 15). To a 100 mL round bottom flask were added 4-fluorobenzyl hydantoin cyclohexanone **13** (0.6 g, 2.06 mmol), potassium cyanide (0.28 g, 4.3 mmol), ammonium chloride (0.24 g, 4.4 mmol), ammonium hydroxide (15 mL), and ethanol (30 mL). The flask was stirred at room temperature for 16 h. The clear reaction solution was then transferred to a separatory funnel and extracted with CH_2Cl_2 (4× 50 mL). The aqueous layer was treated with mixed ion exchange resin (AG® 501-X8 (D) Resin, 20–50 mesh, BIO-RAD) to absorb KCN. The organic layer was dried over Na_2SO_4, followed by vacuum evaporation to give the desired product (0.64 g, 98%, **14**—77%, **15**—23%, R_f(**14**)=0.16, R_f (**15**)=0.22 in 1/19 CH_3OH/CH_2Cl_2); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.86 (s, 1H, NH, **14**), 8.78 (s, 1H, NH, **15**), 7.32–7.10 (m, 8H, aromatic, **14** and **15**), 4.51 (s, 2H, CH_2Ph, **14**), 4.48 (s, 2H, CH_2Ph, **15**), 2.68 (br s, 2H, NH_2, **14**), 2.54 (br s, 2H, NH_2, **15**), 2.01–1.68 (m, 16H, central ring, **14** and **15**); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 176.1 (C1′), 163.0 and 159.8 (d, C–F, *J*=243 Hz), 155.4 (C3′ of **14**), 155.3 (C3′ of **15**), 133.0 and 132.9 (aromatic), 129.3 and 129.2 (aromatic of **14**), 128.4 and 128.3 (aromatic of **15**), 125.2 (CN of **15**), 124.0 (CN of **14**), 115.9 and 115.2 (aromatic of **14**, *J*=21.0 Hz), 114.9 and 114.6 (aromatic of **15**, *J*=20.9 Hz), 62.1 (C1 of **15**), 59.7 (C1 of **14**), 50.2 (C4 of **14**), 47.9 (C4 of **15**), 40.3 (*C*H_2Ph), 32.2 (C2 and C6 of **14**), 31.0 (C2 and C6 of **15**), 30.1 (C3 and C5 of **14**), 27.8 (C3 and C5 of **15**); HRMS-CI calcd for $C_{16}H_{18}N_4O_2F$ $[M+H]^+$: 317.1414, found 317.1415.

4.1.12. *trans*-3-(4-Fluoro-benzyl)-1,3,9,11-tetraaza-dispiro[4.2.4.2]tetradecane-2,4,10,12-tetraone (16 and 17). To a mixture of **14** and **15** (100 mg, 0.316 mmol) in 4 mL of CH_2Cl_2 was added chlorosulfonyl isocyanate (48 mg, 0.34 mmol). After being stirred for 10 min at room temperature, the clear solution was concentrated in vacuo into a pale yellow form. Following addition of 3 mL of 1 N HCl the suspension was stirred for 10 min at room temperature, then heated in an oil bath at 100 °C for 1 h. After approximately 15 min of heating, the reaction mixture became homogeneous, and then a precipitate formed. The reaction mixture was cooled to room temperature; the solid was filtered, washed with water, and dried to give 0.08 g of a mixture (70%) of **16** and **17** (7/3): ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.63 (s, 1H, NH, **17**), 10.60 (s, 1H, NH, **16**), 8.98 (s, 1H, NH, **17**), 8.66 (s, 1H, NH, **16**), 8.61 (s, 1H, NH, **17**), 8.25 (s, 1H, NH, **16**), 7.28–7.24 (m, aromatic, **16** and **17**), 7.17–7.13 (m, aromatic, **16** and **17**), 4.50 (s, 2H, CH_2Ph, **17**), 4.49 (s, 2H, CH_2Ph, **16**), 2.12–2.07 (m, 4H, central ring, **16**), 2.00–1.97 (m, 4H, central ring, **17**), 1.70–1.60 (m, 8H, central ring, **16** and **17**); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 178.0 (C1′ of **16**), 177.8 (C1′ of **17**), 176.1 (C1″ of **16**), 176.0 (C1″ of **17**), 161.0 (d, C–F, J_{CF}=239 Hz, **16** and **17**), 156.4 (C3′ of **16** and **17**), 155.6 (C3″ of **16**), 155.5 (C3″ of **17**), 133.0 (aromatic, **16** and **17**), 129.2 (d, *C*–(C–C–F), $^3J_{CF}$=8 Hz, **16**), 129.1 (d, *C*–(C–C–F), $^3J_{CF}$=8 Hz, **17**), 115.3 (d, *C*–(C–F), $^2J_{CF}$=21.9 Hz, **16** and **17**), 60.8 (C1 of central ring of **17**), 60.0 (C4 of central ring of **17**), 59.6 (C1 of central ring of **16**), 58.9 (C4 of central ring of **16**), 40.2 (*C*H_2Ph of **17**), 40.1 (*C*H_2Ph of **16**), 29.1 (C2 and C6 of central ring of **16**), 29.0 (C3 and C5 of central ring of **16**), 28.6 (C2 and C6 of central ring of **17**), 28.5 (C3 and C5 of central ring of **17**); HRMS-CI calcd for $C_{17}H_{18}N_4O_4F$ $[M+H]^+$: 361.1312, found 361.1297.

4.1.13. (4-Hydroxymethyl-cyclohexa-1,3-dienyl)-methanol (19). Starting material **18** (1.8 g, 9.25 mmol) was dissolved in anhydrous THF (20 mL) at 0 °C, which was then subject to ultra-pure Ar flushing for 10 min. DIBAL-H (37 mL) in THF (1.0 M, 37 mmol) was added to the above solution slowly via a double-ended needle. The clear solution was stirred rigorously at 0 °C for 30 min then quenched with MeOH (3 mL), followed by adding powdered $Na_2SO_4\cdot10H_2O$ (25 g). The mixture was stirred at room temperature for 1 h and then filtered through Celite, and the filter cake was washed by copious EtOAc. The solution was evaporated in vacuo yielding a light orange oil. The oil was

dissolved in EtOAc and applied to a flash column using a CH_2Cl_2/EtOAc gradient to elute the product (1.14 g, 88%, R_f=0.24 in 3/2 CH_2Cl_2/EtOAc). Due to the instability of the product, it was used immediately for the next step: ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.89 (s, 2H, CH–CH), 4.15 (d, 4H, 2CH_2OH, J=5.7 Hz), 2.22 (s, 4H, 2CH_2), 1.93 (t, 2H, 2OH, J=5.7 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ_C 137.6 (C1 and C4), 119.0 (C2 and C3), 66.0 (CH_2OH), 23.9 (C5 and C6); HRMS-CI calcd for $C_8H_{12}O_2$ [M^+]: 140.0837, found 140.0839.

4.1.14. 1,4-Bis-hydroxymethyl-2,3-diaza-bicyclo [2.2.2]oct-5-ene-2,3-dicarboxylic acid diethyl ester (20). Diethyl azodicarboxylate (DEAD) (5.0 mL, 11.0 mmol) was added to a CH_2Cl_2 (40 mL) solution of **19** (1.40 g, 10 mmol). The solution was stirred at room temperature for 24 h. The adduct **20** was isolated by column chromatography using a CH_2Cl_2/EtOAc gradient to give a viscous, colorless oil (1.13 g, 36%, R_f=0.32 in 1/1 CH_2Cl_2/EtOAc): ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.86 (d, 1H, CH=CH, J=8.1 Hz), 6.26 (d, 1H, CH=CH, J=8.1 Hz), 4.27–4.04 (m, 8H, 2CH_2OH and 2OCH_2CH$_3$), 2.39–2.32 (m, 1H, CHHCH$_2$), 1.98–1.91 (m, 1H, CHHCH$_2$), 1.45–1.37 (m, 1H, CH$_2$CHH), 1.29–1.19 (m, 7H, CH$_2$CHH and 2CH$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 159.3 (CO$_2$Et), 158.1 (CO$_2$Et), 136.5 (C=C), 135.5 (C=C), 66.4, 65.9, 65.2, 63.2, 62.8, 62.5, 27.5 (CH$_2$CH$_2$), 26.7 (CH$_2$$CH_2$), 14.5 (CH$_3$), 14.3 (CH$_3$); HRMS-CI calcd for $C_{14}H_{23}N_2O_6$ [M+H]$^+$: 315.1556, found 315.1543.

4.1.15. 1,4-Bis-methoxymethoxymethyl-2,3-diaza-bicyclo[2.2.2]oct-5-ene-2,3-dicarboxylic acid diethyl ester (21). A solution of **20** (1.0 g, 3.1 mmol), MOM-Cl (0.5 mL, 6.25 mmol), DIPEA (1.40 mL, 7.75 mmol) in dry CH_2Cl_2 (40 mL) under N_2 was stirred at room temperature for 24 h, then quenched with 40 mL saturated $NaHCO_3$ solution. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography on silica gel (CH_2Cl_2/EtOAc, 3/2) gave **21** as a colorless oil (1.18 g, 95%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.65 (d, 1H, CH=CH, J=8.0 Hz), 6.51 (d, 1H, CH=CH, J=8.0 Hz), 4.74–4.72 (m, 4H, 2CH_2), 4.54–4.49 (m, 2H, CH_2), 4.26–4.03 (m, 6H, 3CH_2), 3.39 (s, 6H, 2CH_3), 2.34–3.30 (m, 1H, CHHCH$_2$), 1.88–1.81 (m, 2H, CH_2CH$_2$), 1.35–1.28 (m, 1H, CHHCH$_2$), 1.26 (t, 3H, CH_3, J=6.8 Hz), 1.19 (t, 3H, CH_3, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9 (CO$_2$Et), 157.7 (CO$_2$Et), 137.7 (C=C)), 134.8 (C=C), 96.6 (OCH$_2$O), 96.4 (OCH$_2$O), 69.8, 69.3, 62.8, 61.9, 61.6, 61.2, 55.2, 55.1, 28.1 (CH$_2$CH$_2$), 25.0 (CH$_2$$CH_2$), 14.3 ($CH_3CH_2$), 14.2 ($CH_3CH_2$); HRMS-CI calcd for $C_{18}H_{31}N_2O_8$ [M+H]$^+$: 403.2080, found 403.2082.

4.1.16. *cis*-(4-Ethoxycarbonylamino-1,4-bis-methoxymethoxymethyl-cyclohexyl)-carbamic acid ethyl ester (22). Under dry Ar, 10% Pd/C (0.15 g) catalyst was added slowly into a solution of **21** (0.55 g, 1.37 mmol) in CH_2Cl_2 (30 mL). The solution was degassed, placed under hydrogen atmosphere (10 psi), and stirred for 24 h. The reaction mixture was filtered through Celite on a fine fritted glass filter and the activated carbon was washed with additional EtOAc. The filtrate was evaporated in vacuo to dryness then redissolved in THF (40 mL). The THF solution was then transferred to a three-neck flask into which NH_3 was bubbled at −70 °C until approximately 150 mL were condensed. Excess Na metal was added and the solution turned dark blue. The resulting mixture was stirred at −70 °C for 1 h. The reaction was quenched by carefully adding solid NH_4Cl, and NH_3 was allowed to evaporate slowly. The residue was diluted with EtOAc (150 mL) and filtered, and the solvents were evaporated in vacuo. The resulting crude product was purified by flash chromatography over silica gel (CH_2Cl_2/EtOAc 5/1) to give **22** as a colorless paste (0.52 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.70 (s, 2H, 2NHCO), 4.58 (s, 4H, 2CH_2), 4.03 (q, 4H, 2CH_2, J=7.2 Hz), 3.66 (s, 4H, 2CH_2), 3.32 (s, 6H, 2CH_3), 2.02–1.94 (m, 4H, equatorial), 1.70–1.64 (m, 4H, axial), 1.20 (t, 6H, 2CH_3, J=7.2 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 155.1 (CO$_2$C$_2$H$_5$), 96.6 (OCH$_2$O), 69.4 (OCH$_2$C), 60.2 (OCH$_2$CH$_3$), 55.2 (OCH$_3$), 54.3 (C1 and C4 of the ring), 27.3 (C2, C3, C5, and C6 of the ring), 14.5 (CH$_3$CH$_2$); HRMS-CI calcd for $C_{18}H_{35}N_2O_8$ [M+H]$^+$: 407.2393, found 407.2388.

4.1.17. *cis*-(4-*tert*-Butoxycarbonylamino-1,4-bis-hydroxymethyl-cyclohexyl)-carbamic acid *tert*-butyl ester (23). Iodotrimethyl silane (TMSI, 0.58 mL, 4.3 mmol) was added dropwise into a solution of **22** (174 mg, 0.43 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was heated under reflux for 18 h and quenched with MeOH (1.0 mL), leading to the formation of a fine precipitates. Vacuum filtration gave a yellowish solid (presumably an iodide salt of the fully deprotected dihydroxyl diamine). This highly hygroscopic solid was used directly in the next step without further purification. MeOH (10 mL), di-*tert*-butyl-dicarbonate (0.25 g, 1.14 mmol), and triethyl amine (0.24 mL, 1.71 mmol) were added to the above solid and the reaction mixture was stirred at room temperature overnight, then quenched with 40 mL saturated $NaHCO_3$ solution. The layers were separated and the aqueous layer was extracted with EtOAc (2×40 mL). The combined organic layers were washed with water, brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatograph on silica gel (CH_2Cl_2/MeOH 9/1) gave **23** as a colorless solid (119 mg, 74%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.61 (s, 2H, 2NHCO), 3.70 (s, 4H, 2CH_2OH), 1.72 (s, 8H, equatorial and axial), 1.44 (s, 18H, 2C(CH$_3$)$_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 156.2 (CO$_2$C(CH$_3$)$_3$), 80.2 (CH$_2$OH), 68.0 (C(CH$_3$)$_3$), 55.6 (C1 and C4), 28.3 (CH$_3$), 27.8 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{18}H_{35}N_2O_6$ [M+H]$^+$: 375.2495, found 375.2498.

4.1.18. *cis*-1,4-Bis-*tert*-butoxycarbonylamino-cyclohexane-1,4-dicarboxylic acid (24). A solution of bis-alcohol **23** in *tert*-butyl alcohol (1 mL/0.1 mmol of alcohol) was treated at room temperature with sodium hydroxide solution (4 equiv, 0.5 M in water) and $KMnO_4$ solution (4 equiv, 10% water) and the resulting mixture was stirred overnight. After quenching the reaction with an excess of aqueous sodium thiosulfate (5%), the mixture was washed with diethyl ether and the aqueous solution was acidified to a pH of 1–2 with 1 M HCl at 0 °C. The mixture was extracted with ethyl acetate three times and the combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to yield product **24** as a colorless, sticky solid that was used for the next

step without further purification (93%); ^{1}H NMR (300 MHz, CD_3OD): δ 2.19–2.15 (m, 4H, equatorial), 1.89–1.84 (m, 4H, axial), 1.42 (s, 18H, $2C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 177.6 (CO_2H), 157.3 (CONH), 80.1 ($C(CH_3)_3$), 58.9 (C1 and C4), 30.1 (C2, C3, C5, and C6), 28.7 (CH_3); HRMS-CI calcd for $C_{18}H_{31}N_2O_8$ $[M+H]^+$: 403.2080, found 403.2075.

4.1.19. *cis*-1,4-bis-*tert*-Butoxycarbonylamino-cyclohexane-1,4-dicarboxylic acid dimethyl ester (25). Ester **25** was prepared from **24** by following the procedure described for the synthesis of **5** in 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.83 (s, 2H, 2NH), 3.73 (s, 6H, $2CH_3$), 2.26–2.21 (m, 4H, equatorial), 1.88–1.82 (m, 4H, axial), 1.43 (s, 18H, $2C(CH_3)_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 173.9 (CO_2CH_3), 154.8 (CONH), 80.1 ($C(CH_3)_3$), 57.7 (C1 and C4), 52.3 (OCH_3), 31.4 (C2, C3, C5, and C6), 28.2 (CH_3); HRMS-CI calcd for $C_{20}H_{35}N_2O_8$ $[M+H]^+$: 431.2393, found 431.2378.

4.1.20. *cis*-1,4-Bis-((*N*-benzoyl(thiocarbamoyl)amino)-1,4-dimethoxycarbonyl-cyclohexane (27). The ester **25** (0.7 g, 1.63 mmol) was treated with 35 mL CH_2Cl_2/TFA (3/7) at 0 °C. The reaction mixture was stirred at room temperature for 4 h. The volatiles were removed under vacuum and the residue was kept in a dry ice/acetone cold bath, to which a diluted TEA (5 mL) in CH_2Cl_2 (10 mL) solution was added slowly (caution: fumes formed). After 10 min of stirring, the cold bath was removed and the reaction mixture was warmed to room temperature. The volatiles were all removed in vacuo, giving **26** as a colorless oil, which was redissolved in dry CH_2Cl_2 (15 mL). Benzoyl isothiocyanate (0.48 mL, 3.58 mmol) was added by syringe. The clear solution was stirred for 12 h and then concentrated in vacuo. Flash chromatograph on silica gel (9/1 CH_2Cl_2/EtOAc) gave **27** as a yellowish oil (0.70 g, 77%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.15 (s, 2H, 2NHCO), 8.93 (s, 2H, 2NHC=S), 7.84–7.82 (m, 4H, aromatic), 7.63–7.61 (m, 2H, aromatic), 7.54–7.50 (m, 4H, aromatic), 3.79 (s, 6H, $2CH_3$), 2.70–2.65 (m, 4H, equatorial), 2.27–2.22 (m, 4H, axial); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 180.8 (C=S), 173.0 (CO_2Me), 168.0 (CONH), 134.3, 132.9, 128.8 and 128.6 (aromatic), 70.2 ($CH_2C_6H_5$), 60.2 (C1 and C4), 52.5 (CH_3), 26.1 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{26}H_{29}N_4O_6S_2$ $[M+H]^+$: 557.1529, found 557.1544.

4.1.21. *cis*-2,10-Dithioxo-1,3,9,11-tetraaza-dispiro [4.2.4.2]tetradecane-4,12-dione (28). A mixture of **27** (0.55 g, 0.99 mmol) and triethyl amine (1.5 mL) in methanol (10 mL) was heated at reflux for 18 h. The solvent was evaporated; ethanol (10 mL) was added to the residue and the solution was adjusted to a pH of 5.0 with a 5% aqueous HCl. The precipitate was collected by filtration to give **28** as white solid (0.21 g, 75%); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.7 (br s, 2H, 2NH(C=O)(C=S)), 10.4 (s, 2H, 2NHC=S), 2.09–2.05 (m, 4H, equatorial), 1.79–1.74 (m, 4H, axial); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 182.1 (C=S), 178.2 (C=O), 63.5 (C1 and C4), 27.9 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{10}H_{13}N_4O_2S_2$ $[M+H]^+$: 285.0480, found 285.0478.

4.1.22. *cis*-2,10-Dihydrazino-1,3,9,11-tetraaza-dispiro [4.2.4.2]tetradeca-1,9-diene-4,12-dione (30). Compound **30** was prepared from **28** by following the procedure described for the synthesis of **10** in 72% overall yield in two steps; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.03 (br s, 2H, 2NH), 7.29 (s, 2H, $2NH(NH_2)$), 4.46 (s, 4H, $2NH_2$), 1.97–1.93 (m, 4H, equatorial), 1.62–1.57 (m, 4H, axial); ^{13}C NMR (75.5 MHz, DMSO-d_6+TFA): δ 177.8 (C=O), 158.6 (NH_2–C=N), 64.3 (C1 and C4), 29.0 (C2, C3, C5, and C6); HRMS-CI calcd for $C_{10}H_{17}N_8O_2$ $[M+H]^+$: 281.1474, found 281.1482.

4.1.23. Bis-intercalator D1. The linker **10** (0.05 mg, 0.18 mmol) was added into a DMF solution (15 mL) containing naphthalenetetracarboxylic diimide **31** (0.26 g, 0.54 mmol),[17] PyBOP (0.279 g, 0.54 mmol), HOBT (0.082 g, 0.54 mmol), and DIPEA (0.19 mL, 1.1 mmol). The reaction mixture was stirred at room temperature for 20 h then filtered, and the crude solid product was rinsed with DMF (2×10 mL) and then with ethanol (2×10 mL). The solid was dried in vacuo, then redissolved in 1/1 TFA/CH_2Cl_2 (20 mL). The solvent was removed under vacuum after 15 min of stirring. The resulting pink solid was dissolved in 0.1% TFA/H_2O and purified by reverse-phase preparative HPLC (Amersham, AKTA Purifier, Hi-Pore RP-318 250×10 mm column) using 0.1% TFA/water as solvent A and 0.1% TFA/ACN as solvent B. The product fractions were combined and lyophilized to yield the product **D1** (0.12 g, 70%) as a light orange solid; ^{1}H NMR (500 MHz, D_2O): δ 8.60–8.56 (m, 8H), 4.68 (br s, 4H), 4.41 (t, 4H, J=6.5 Hz), 3.35 (br s, 4H), 2.75 (t, 4H, J=6.5 Hz), 1.52 (br s, 8H); UV–vis: λ_{max} ($\log_{10}\varepsilon$) 383 (4.56), 363 nm (4.46); MALDI (Applied Biosystems 4700 Proteomics Analyzer, Foster City, CA) calcd for $C_{48}H_{43}N_{14}O_{12}$ $[M+H]^+$: 1007, found 1007.

4.1.24. Bis-intercalator C1. The linker **30** (20.5 mg, 0.073 mmol) was added into a DMF solution containing naphthalenetetracarboxylic diimide **31** (106 mg, 0.22 mmol),[17] PyBOP (114 mg, 0.22 mmol), HOBT (34 mg, 0.22 mmol), and DIPEA (0.08 mL, 0.44 mmol). The reaction mixture was stirred for 20 h at room temperature. Diethyl ether (20 mL) was added to the above clear solution at −20 °C and a precipitate formed. Vacuum filtration gave a light orange solid that was then dissolved in 1/1 TFA/CH_2Cl_2 (20 mL). The solvents were removed under vacuum after 15 min of stirring at room temperature. The resulting solid was dissolved in 0.1% aqueous TFA and purified following the same procedure as for **D1**. The product fractions were combined and lyophilized to yield the product **C1** (44 mg, 60%) as a light orange solid; ^{1}H NMR (500 MHz, D_2O): δ 8.41 (d, 4H, aromatic Hs, J=6.0 Hz), 8.30 (d, 4H, aromatic Hs, J=6.0 Hz), 4.68 (br s, 4H), 4.43 (t, 4H, J=6.1 Hz), 3.35 (t, 4H, J=6.1 Hz), 2.65 (br s, 4H), 2.29 (m, 4H), 1.97 (m, 4H); UV–vis: λ_{max} ($\log_{10}\varepsilon$) 384 (4.42), 364 nm (4.43); MALDI (Applied Biosystems 4700 Proteomics Analyzer, Foster City, CA) calcd for $C_{48}H_{43}N_{14}O_{12}$ $[M+H]^+$: 1007, found 1007.

4.2. Dissociation kinetics

In a typical experiment, 500 μL of 0.5 mM of DNA stock solution in PIPES buffer (containing 50 mM NaCl, pH 7.0) was first incubated with 500 μL of 0.04 mM compound stock solution for 2 h. Next, 0.5 mL of the resulting

DNA/compound complex solution was mixed with 0.5 mL of 4% SDS solution in a quartz cuvette with 1 cm path length. The UV absorbance was monitored at 383–385 nm (depending on the linker structure of the dimer) after 20 s of mixing (Hewlet-Packard 8553 Multitransport UV–vis spectrophotometer).

4.3. Computer simulation

Molecular dynamics and geometry optimization computations were performed in HyperChem 7.0 (Hypercube Inc., 1115 NW 4th Street, Gainesville, FL 32601) using the Amber force field. PDB coordinates from the **G$_3$K**/d $(CGGTACC)_2$ were used and the linker segment was modified as needed.[19] During the calculation, DNA coordinates were fixed and molecular dynamics were performed on the dimer to anneal the system to obtain a lower energy minimum. Initial structures were subjected to 15 picoseconds of molecular dynamics at 1000 K to allow high degree randomization of the initial model, and then the temperature was slowly lowered to 300 K over 10 ps. After annealing a final geometry optimization was performed using the Fletcher–Reeves conjugate gradient algorithm, with a convergence cutoff value of 0.01 kcal mol^{-1}.

4.4. X-ray single crystallographic analysis

Crystallographic summary for **5** ($C_{26}H_{30}N_2O_8$): colorless prismatic crystals, monoclinic, $P2_1/c$ (No. 14), Z=2 in a cell of dimensions: a=12.7888(9), b=10.5763(7), c=9.7425(5) Å, β=105.064(4)°, V=1272.47(14) Å^3, ρ_{calc}=1.30 g cm^{-3}, μ=0.097 mm^{-1}, $F(000)$=528. A total of 4024 reflections were measured, 2200 unique (R_{int}=0.056), on a Nonus Kappa CCD using graphite monochromatized Mo Kα radiation (λ=0.71073 Å) at −120 °C. The structure was refined on F^2 to an R_w=0.254, with a conventional R=0.100 (1451 reflections with $F_o>4[\sigma(F_o)]$), and a goodness of fit=1.489 for 198 refined parameters.

Crystallographic summary for **8** ($C_{10}H_{12}N_4O_2S_2$–$2C_2H_6SO$: colorless prismatic crystals, monoclinic, $P2_1/n$ (No. 14), Z=2 in a cell of dimensions: a=8.0735(1), b=9.2155(2), c=13.6410(3) Å, β=99.819(1)°, V=1000.04(3) Å^3, ρ_{calc}=1.46 g cm^{-3}, μ=0.502 mm^{-1}, $F(000)$=464. A total of 4213 reflections were measured, 2275 unique (R_{int}=0.028), on a Nonus Kappa CCD using graphite monochromatized Mo Kα radiation (λ=0.71073 Å) at −120 °C. The structure was refined on F^2 to an R_w=0.0769, with a conventional R=0.0349 (1765 reflections with $F_o>4[\sigma(F_o)]$), and a goodness of fit=1.010 for 167 refined parameters.

Crystallographic summary for **12a** (an analogue of **12**) ($C_{17}H_{20}N_2O_4$): colorless needles, triclinic, P-1 (No. 2), Z=2 in a cell of dimensions: a=5.6474(2), b=11.2341(4), c=12.4607(4) Å, α=99.586(2), β=97.391(2), γ=91.882(2)°, V=771.84(5) Å^3, ρ_{calc}=1.36 g cm^{-3}, μ=0.098 mm^{-1}, $F(000)$=336. A total of 5249 reflections were measured, 3497 unique (R_{int}=0.026), on a Nonus Kappa CCD using graphite monochromatized Mo Kα radiation (λ=0.71073 Å) at −120 °C. The structure was refined on F^2 to an R_w=0.108, with a conventional R=0.0431 (2188 reflections with $F_o>4[\sigma(F_o)]$), and a goodness of fit=1.006 for 289 refined parameters.

Crystallographic summary for **28** ($(C_{10}H_{11}N_4S_2O_2)^{1-}$ $(C_6H_{16}N)^{1+}$): colorless lathes, monoclinic, $P2_1/c$ (No. 14), Z=8 in a cell of dimensions: a=12.2470(3), b=11.9535(3), c=27.0891(7) Å, β=96.674(1)°, V=3938.82(17) Å^3, ρ_{calc}=1.30 g cm^{-3}, μ=0.290 mm^{-1}, $F(000)$=1648. A total of 11,100 reflections were measured, 6658 unique (R_{int}=0.070), on a Nonus Kappa CCD using graphite monochromatized Mo Kα radiation (λ=0.71073 Å) at −120 °C. The structure was refined on F^2 to an R_w=0.140, with a conventional R=0.0617 (3580 reflections with $F_o>4[\sigma(F_o)]$), and a goodness of fit=1.168 for 460 refined parameters.

Crystallographic data (excluding structure factors) for **5**, **8**, **12a**, and **28** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 293250, 293251, 293248, and 293249, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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Corrigendum

Corrigendum to "Asymmetric transfer hydrogenation of α,β-unsaturated, α-tosyloxy and α-substituted ketones" [Tetrahedron 62 (2006) 1864]

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In the original submission of this paper, an author, Jerome Hannedouche was omitted. Dr Hannedouche carried out the work on the cyclic and acyclic enone reductions in the paper. The correct author list reads as stated above.

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