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Tetrahedron

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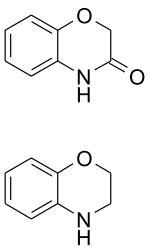
Contents

REPORT



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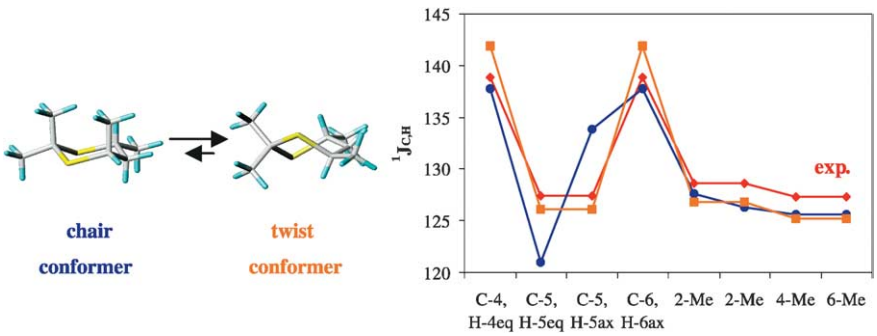
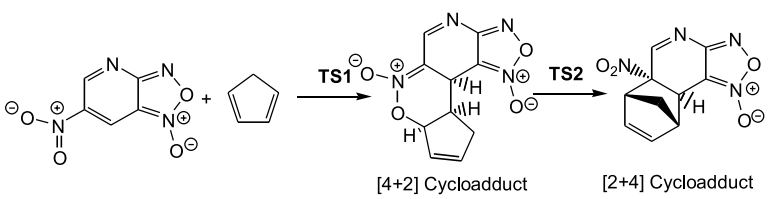
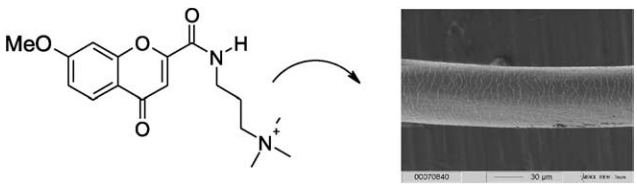
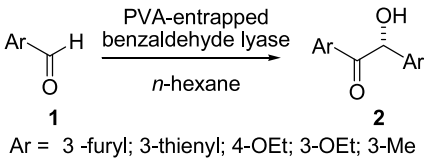
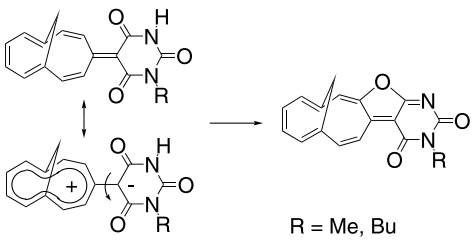


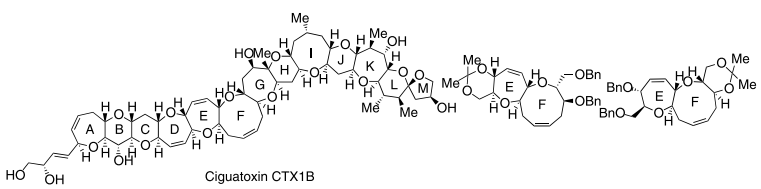


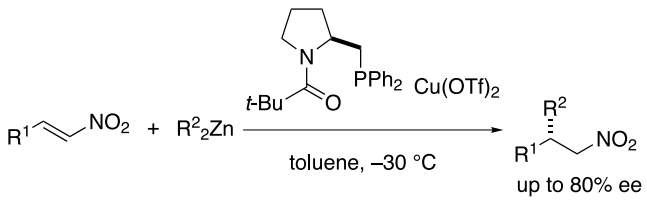


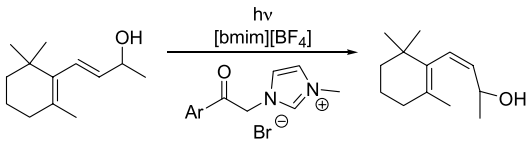
A series of chromone derivatives with a quaternary ammonium functionality providing affinity to human hair were synthesised. The UV absorption properties of the compounds depend significantly on the position of the methoxy substituent.

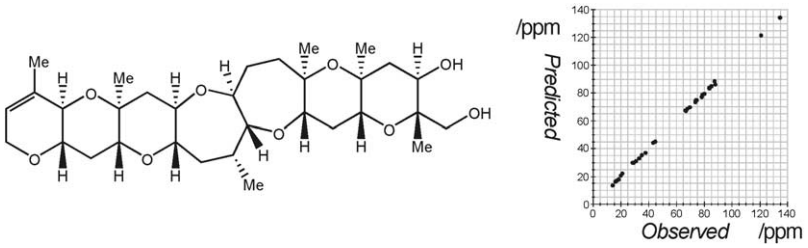


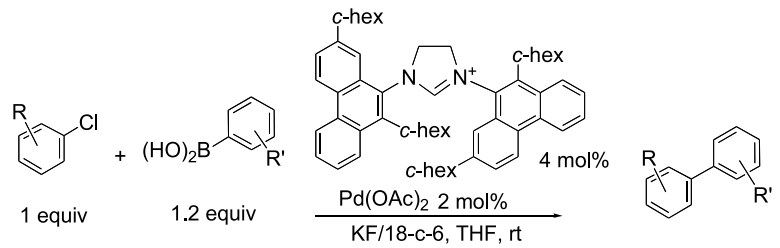


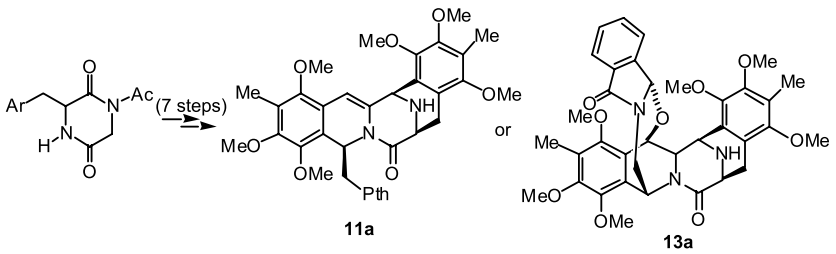


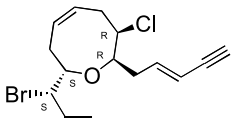






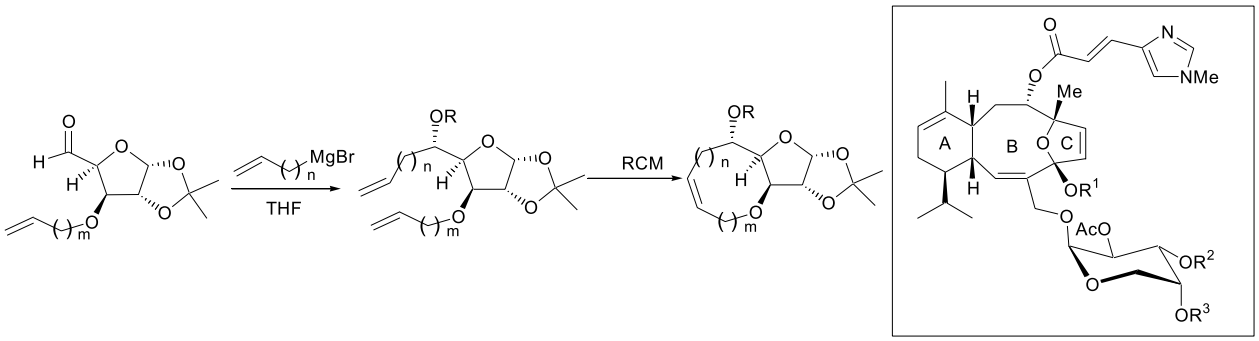


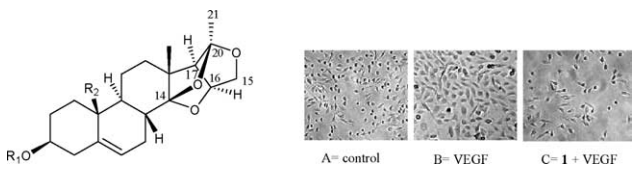


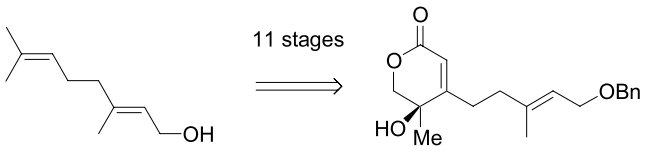


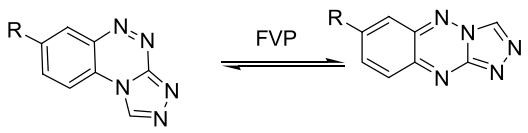
1

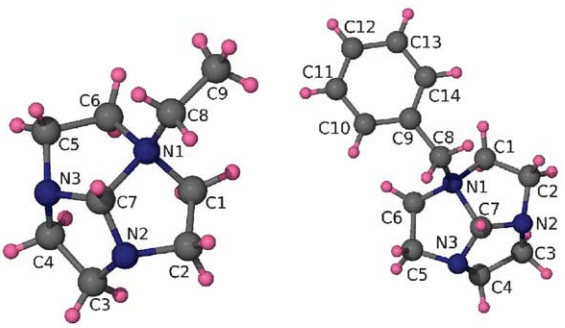
Three new halogenated C15-acetogenins, (e.g., compound **1**), have been isolated from the Chinese anaspidean mollusc *Aplysia dactylomela*. The structures have been determined by NMR analysis. The structure of **1** was further secured by a single crystal X-ray study, which also allowed to establish its absolute stereochemistry.

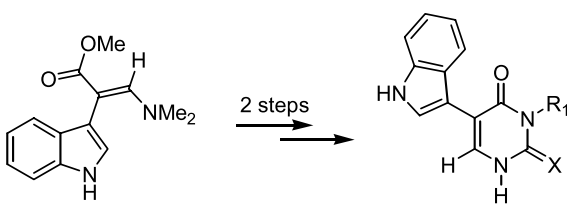




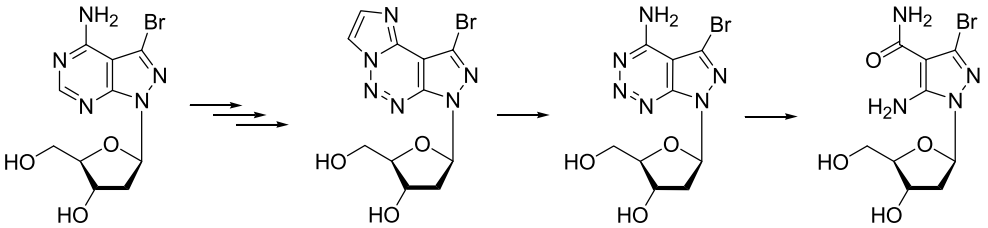


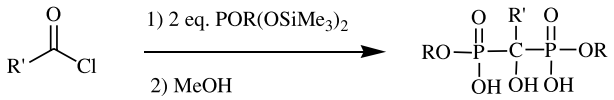


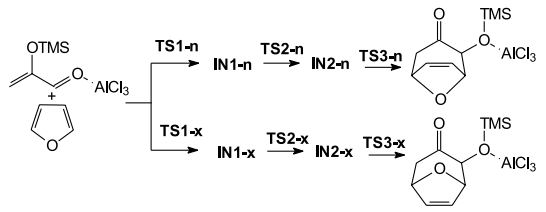


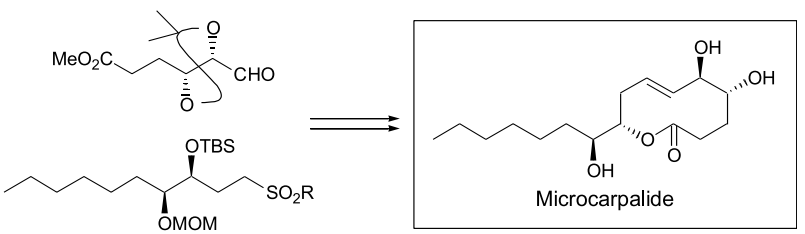


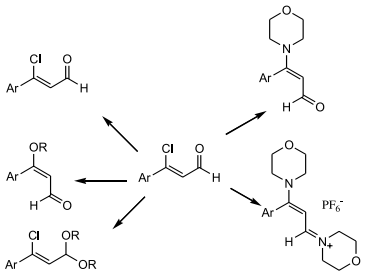
3 **10-15**

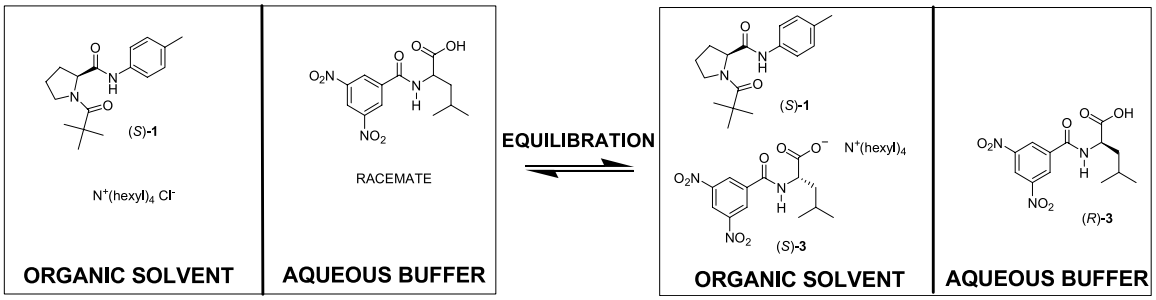












OTHER CONTENTS

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Recent advances in the synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines

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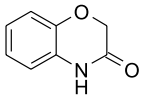
Contents

1. Introduction

The 2*H*-1,4-benzoxazin-3-(4*H*)-one (**1**) and 3,4-dihydro-2*H*-1,4-benzoxazine (**2**) scaffolds have been studied intensively as important heterocyclic systems for building natural[1,2] and designed synthetic compounds. Since the last review of 1,4-benzoxazines and 1,4-benzoxazinones in Houben Weyl in 1997,[3] the 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines have been frequently utilized as suitable skeletons for the design of biologically active compounds, ranging from herbicides and fungicides to therapeutically usable drugs.



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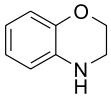

2

A literature survey provided/identified several 1,4-benzoxazine- and 1,4-benzoxazinone-based compounds (Fig. 1) in the development phase as potential new drugs. Novel antibacterial agents **3**[4] and **4**[5] are inhibitors of bacterial histidine protein kinase, and 2-oxo-1,4-benzoxazine derivative **5**[6] is potentially useful for treating infections caused by *Mycobacterium* sp. 1,4-Benzoxazine-3-one derivative **6**[7] is a potential drug for treating heart disease, myocardial necrosis or arrhythmia and 1,4-benzoxazine derivative **7**[8] possesses peroxisome proliferator-activated receptor α (PPARα) and PPARγ agonist activity and could be used in treating diabetes, hyperlipidemia and other diabetic complications. French investigators recently introduced new 8-arylalkylamino-1,4-benzoxazine neuroprotectants **8**, **9**, and **10**[9] and Schering has disclosed 1,4-benzoxazines **11**[10] and **12**[11] as inhibitors of nitric oxide synthase (NOS) which are potential drugs for treating neurodegenerative, inflammatory, autoimmune and cardiovascular disorders. 1,4-Benzoxazinone **13**[12] inhibits the coagulation serine proteases factor Xa, thrombin and factor VIIa, and 1,4-benzoxazinone **14**[13] is a potential agent for treating anxiety and depression. 1,4-Benzoxazin-3-one

Keywords: 1,4-Benzoxazine; 2*H*-1,4-Benzoxazin-3-(4*H*)-ones; 3,4-Dihydro-2*H*-1,4-benzoxazines.

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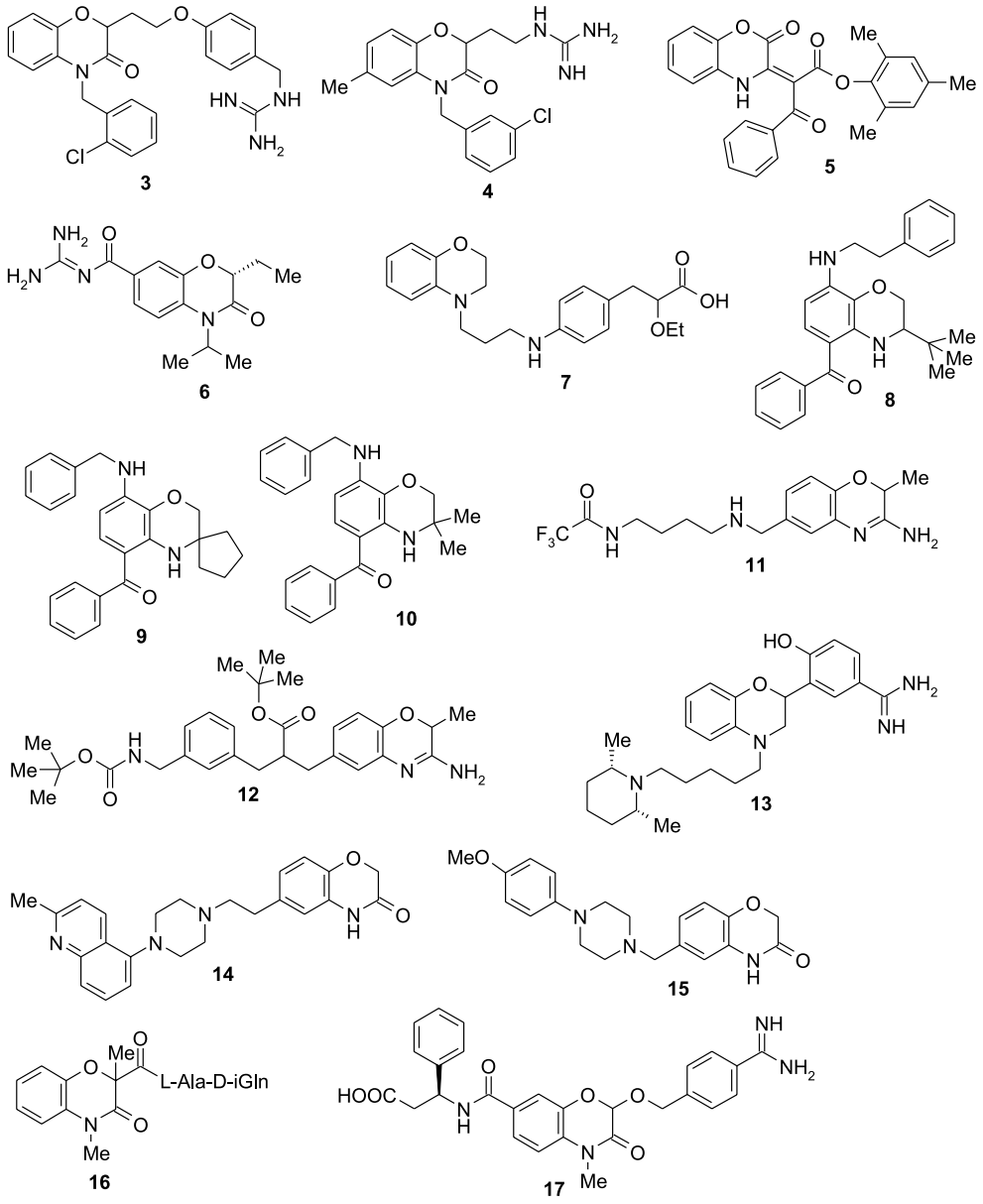


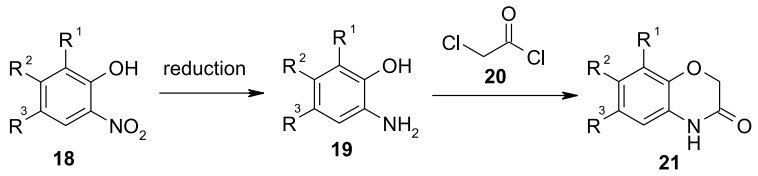
Figure 1.

derivative **15**[14] possesses D_2 receptor antagonistic activity and is a potential antipsychotic agent. The 2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acid derivative **16** was found to be a potent immunostimulant.[15] 2*H*-1,4-Benzoxazin-3(4*H*)-ones bearing a carboxylate and a benzamidine side chain are fibrinogen receptor antagonists[16] and compound **17** possesses a dual antithrombotic action, exhibiting both thrombin inhibitory and fibrinogen receptor antagonistic activities.[17] Some additional biologically active 1,4-benzoxazines were briefly described in a broader-context review.[18]

The present review provides a comprehensive coverage of the syntheses and transformations of 3,4-dihydro-2*H*-1,4-benzoxazines and 2*H*-1,4-benzoxazin-3-(4*H*)-ones published since 1996 in chemical journals and in the patent literature.

2. Synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones by ring closure

Several synthetic strategies are available to synthesize the 2*H*-1,4-benzoxazin-3-(4*H*)-one scaffold. In this section, we report novel types of ring-forming reactions yielding 2*H*-1,4-benzoxazine-3-(4*H*)-ones and recent applications of common synthetic approaches for the construction of the 2*H*-1,4-benzoxazin-3-(4*H*)-one skeleton. Synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-one derivatives **21** is achieved by reaction of chloroacetyl chloride (**20**) with substituted 2-aminophenols **19** (Scheme 1).[19–23] Usually, the synthesis of **21** is performed as a one-pot reaction which involves heating **19** and **20** in the presence of a base. Most commonly, the starting compounds are the easily available substituted 2-nitrophenols **18**, which are reduced to amines **19**, for example, by catalytic hydrogenation using palladium (Pd) on charcoal,[19] platinum



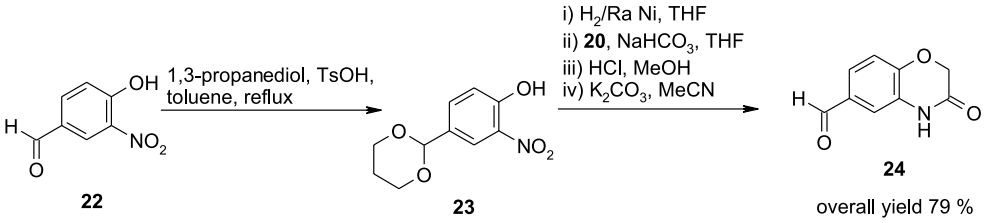
R^1	R^2	R^3	Reducing agent	Base, solvent	Yield (%) 19 → 21	Ref.
H	OMe	H	H_2, Pd/C	$NaHCO_3$	n. g.[a]	19
Br	H	H	$Na_2S_2O_3$	$NaHCO_3$, 2-butanone	45	20
F	F	COOMe	-	$BnEt_3N^+Cl^-$, $NaHCO_3$, $CHCl_3$	56	21
H	H	NO_2	-	Et_3N, CH_2Cl_2	79	22
H	F	H	-	DMF, K_2CO_3	93	23

[a]Not given.

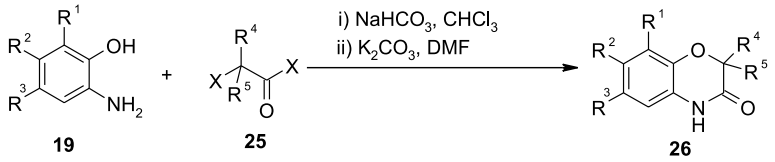
Scheme 1.

disulfide (PtS_2) on carbon[24] or sodium dithionite ($Na_2S_2O_3$).[20]

Due to the reductive conditions in the transformation of **18** to **19**, reactive groups must be protected. In the synthesis of 1,4-benzoxazinone **24**,[25,26] a key intermediate for the preparation of dopamine D_4 antagonists acting as potential atypical antipsychotic agents, the aldehyde group of **22** was

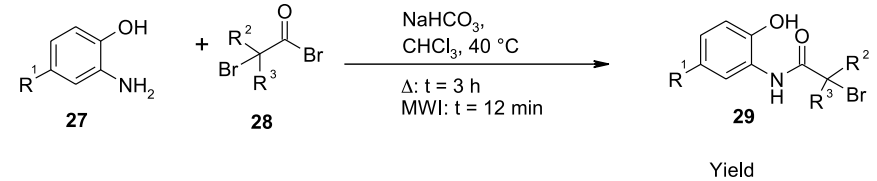


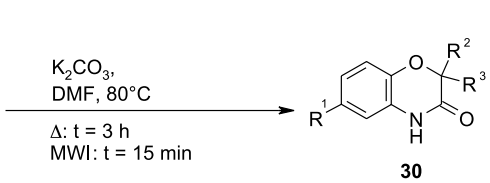
Scheme 2.



R^1	R^2	R^3	R^4	R^5	X	Yield (%)	Ref.
Br	H	H	Me	Me	Br	84	20
COOEt	H	Cl	H	Me	Cl	57	27
COOEt	H	Cl	H	Ph	Cl	38	27
H	NO_2	H	Me	Me	Br	57	28
H	NO_2	Cl	Me	Me	Br	56	29

Scheme 3.





R^1	R^2	R^3	Yield (%) 29 → 30
Cl	H	H	62[a], 92[b]
NO_2	H	H	70[a], 96[b]
CN	H	H	80[a], 95[b]
Cl	Me	Me	65[a], 90[b]
NO_2	Me	Me	68[a], 90[b]
CN	Me	Me	70[a], 94[b]

[a]Δ: t = 3 h. [b]MWI: t = 15 min.

Scheme 4.

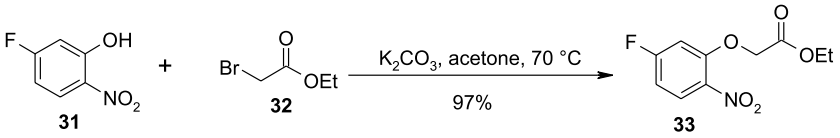
protected as an acetal; in a multistep procedure, the nitro group of acetal **23** was reduced to the corresponding 2-aminophenol which was cyclized with chloroacetyl chloride (**20**) and, finally, the acetal group was cleaved under acidic conditions providing aldehyde **24** (Scheme 2).

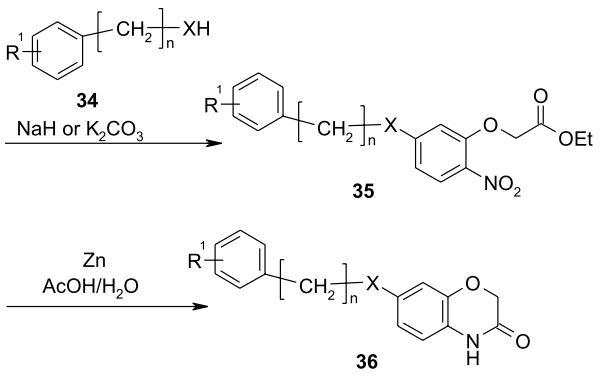
Application of α-substituted acyl halides **25** (e.g., 2-chloropropionyl chloride, 2-bromo-2-methylpropionyl bromide, etc) produces 2-aryl- or 2-alkyl-substituted 2*H*-1,4-benzoxazin-3-(4*H*)-ones **26**.[20,27–29] The yields of benzoxazinones **26** are lower when a substituent at position 2 is a phenyl ring (Scheme 3).

Caliendo and co-workers[30] have published a new approach applying microwave irradiation for the parallel synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones **30** from 2-aminophenols **27** and acyl bromides **28** in solution via **29**. Comparison of the microwave reaction with the conventional method[30,31] shows that the former technique gives better yields and that the overall reaction times are considerably reduced (Scheme 4). The generated *N*-alkyl-2*H*-1,4-benzoxazin-3-(4*H*)-one library compounds are ATP-dependent potassium channel openers, constituting potential therapeutic agents for treating hypertension, angina pectoris and asthma.

Generally, the 2*H*-1,4-benzoxazin-3-(4*H*)-one scaffold is synthesized in the first steps of a multistep synthesis and then derivatized to yield biologically active compounds. On the contrary, as seen in Schemes 5 and 6, this scaffold can also be synthesized as the very last step after the introduction of the required substituents, for example, *O*-alkylation of 5-fluoro-2-nitrophenol (**31**) with ethyl bromoacetate (**32**) produces the *O*-alkyl derivative **33** in excellent yield. At this step, **33** can be modified at position 5 with various nucleophiles **34** to afford the compounds **35**. The nitro group of **35** is reduced with zinc in acetic acid/water, yielding an amine intermediate, which undergoes spontaneous cyclization to the 7-substituted 2*H*-1,4-benzoxazin-3-(4*H*)-ones **36** possessing therapeutic potential as inhibitors of angiogenesis[32] (Scheme 5).

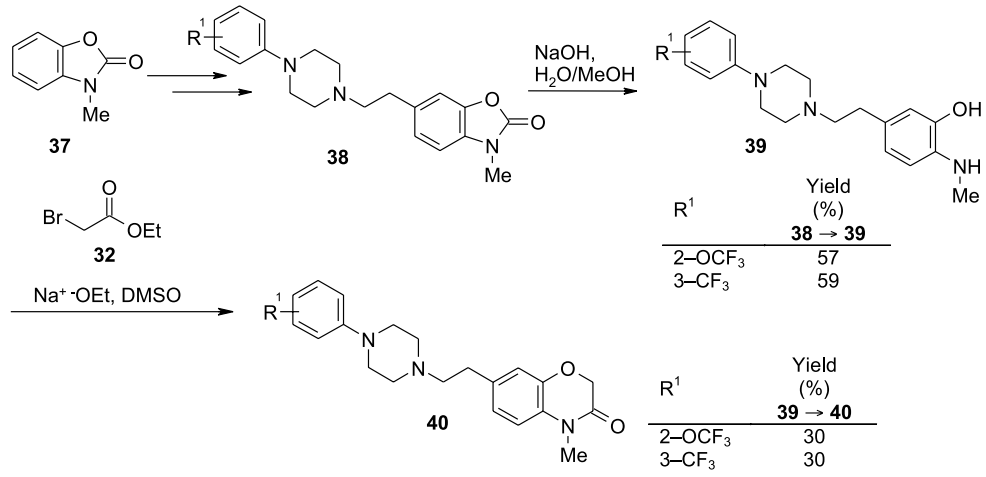
Another approach for synthesising the 2*H*-1,4-benzoxazin-3-(4*H*)-one ring in the last step, after modification of a 2-aminophenol precursor, is depicted in Scheme 6.





R^1	n	X	Yield (%) 33 → 35	Yield (%) 35 → 36
4-MeO	0	O	84	76
2-NH_2	0	S	100	39
3-NH_2	0	S	99	45
4-NH_2	0	S	60	90
4-OH	0	S	71	89
3,4-MeO	0	S	85	73
4-MeO	0	NH	30	93
2-NH_2	0	O	60	60
3-NH_2	0	O	30	39
4-AcNH	0	O	16	99
H	1	S	99	95
4-Cl	1	S	99	76
4-tBu	1	S	99	84
4-MeO	1	S	99	87

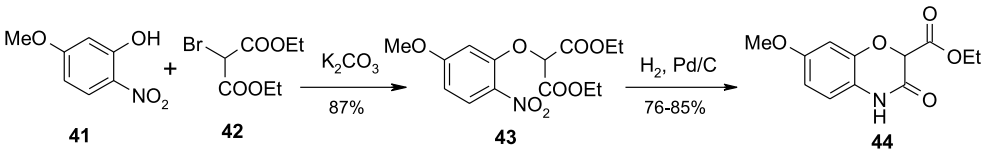
Scheme 5.



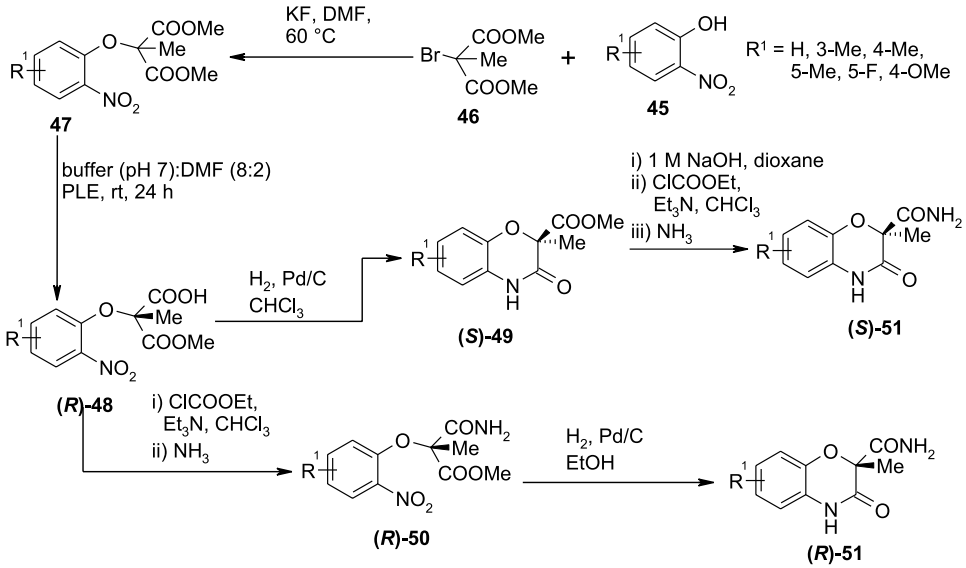
R^1	Yield (%) 38 → 39
2–OCF_3	57
3–CF_3	59

R^1	Yield (%) 39 → 40
2–OCF_3	30
3–CF_3	30

Scheme 6.



Scheme 7.

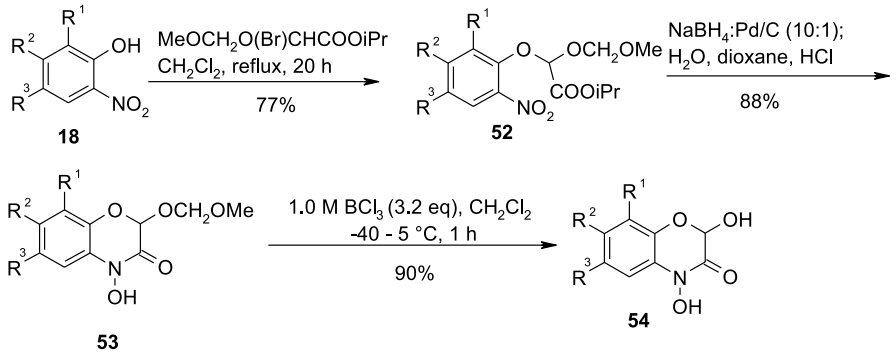


Scheme 8.

1,3-Benzoxazol-2(3*H*)-one (**37**) is used as the protected 2-aminophenol, which is substituted to give **38** and later deprotected using aqueous sodium hydroxide in methanol, yielding 5-substituted 2-methylamino phenols **39**. Reaction of **39** with ethyl bromoacetate (**32**) under strongly alkaline conditions yields 7-substituted 4-methyl-2*H*-1,4-benzoxazin-3(4*H*)-ones **40**. Taverne et al. used this approach for the synthesis of 2*H*-1,4-benzoxazin-3(4*H*)-one derivatives containing an arylpiperazine side chain, which displayed high affinity for 5-HT_{1A} and D_2 receptors.[33]

2-Bromomalonates, for example, diethyl 2-bromomalonate (**42**) (Scheme 7) and dimethyl 2-bromo-2-methylmalonate (**46**) (Scheme 8) are versatile reagents for the synthesis of 2-alkoxycarbonyl-2*H*-1,4-benzoxazin-3(4*H*)-ones. In one example, a substituted 2-nitrophenol **41** is *O*-alkylated with diethyl bromomalonate (**42**) yielding diethyl (5-methoxy-2-nitrophenoxy)malonate (**43**) which, after reduction to diethyl (5-methoxy-2-aminophenoxy)malonate, undergoes spontaneous cyclization to ethyl 7-methoxy-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylate (**44**)[24,34] (Scheme 7).

The enantioselective synthesis of (*R*)- and (*S*)-2-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxamides **51**,[35,36]



R^1	R^2	R^3	Yield (%) **52 → 53**	Yield (%) **53 → 54**
H	H	H	88	82
H	OMe	H	88	90
H	-OCH_2O-		84	83
OMe	OMe	H	99	74
OMe	OMe	H	87	87

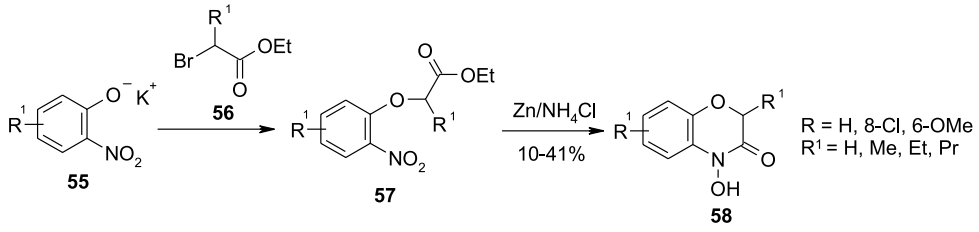
Scheme 9.

using dimethyl 2-bromo-2-methylmalonate (**46**) and the substituted *o*-nitrophenols **45**, is presented in Scheme 8. (*R*)-Monomethyl 2-methyl-2-(2-nitrophenoxy)malonates **48** are obtained by pig liver esterase (PLE)-catalyzed hydrolysis of the corresponding dimethyl malonates **47**.[35–37] (*S*)-2-Methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxamides (**51**) are obtained by reduction of the nitro group of **48** and solvent-dependent enantioselective cyclization to the methyl carboxylates (*S*)-**49**, which are transformed to the *S*-carboxamides (*S*)-**51**. Another approach is needed to produce the carboxamides (*R*)-**51**. The carboxyl group of (*R*)-**48** is de-activated, forming the carboxamide (*R*)-**50** by a mixed anhydride method. After reduction of the nitro group, spontaneous cyclization produces the carboxamides (*R*)-**51**. The enantiomers of 2-methyl- and 2,4-dimethyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acids are also obtained by resolution.[38] These compounds are important chiral peptidomimetic building blocks.

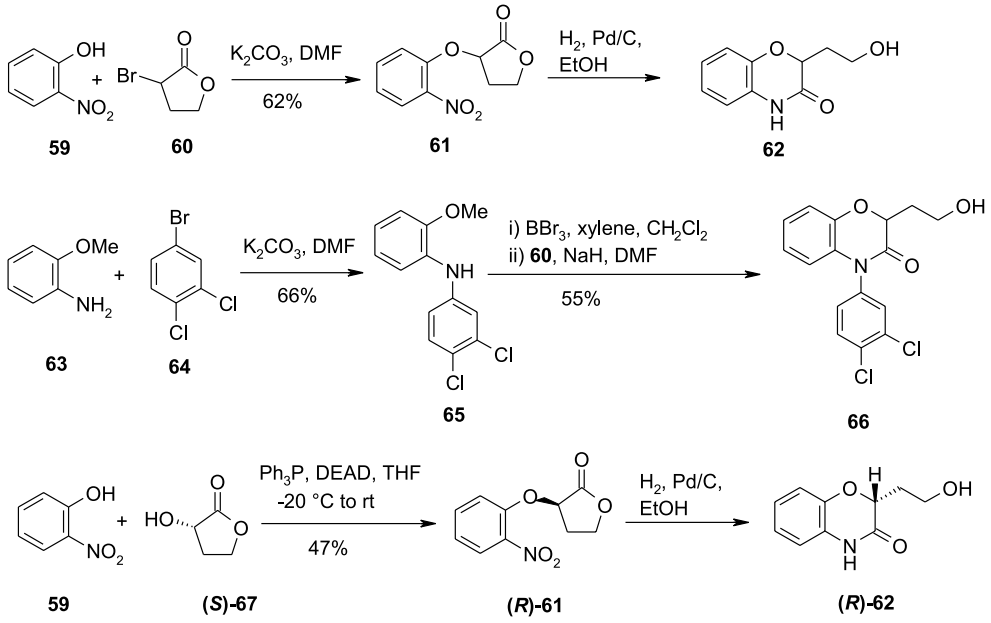
Cyclic hydroxamic acids are found in several of the major crop plants, having a role as toxins that aid in defending the plants against insect attack. An efficient synthesis of 1,4-benzoxazin-3-ones **54** containing a cyclic hydroxamic acid moiety[39] is presented in Scheme 9. 2-Nitrophenols **18** and *i*-propyl-α-bromo-*O*-methoxymethylglycolate when refluxed in dry dichloromethane afford *i*-propyl-*O*-methoxymethyl-α-(*o*-nitrophenoxy)glycolates **52**. Cyclization of **52** to cyclic hydroxamates **53** is performed reductively with a mixture of $NaBH_4$ and 10% Pd/C. Deprotection of C-2 MOM-protected alcohols **53** is accomplished with good yields using 1.0 M boron trichloride in dichloromethane (Scheme 9).

Reduction of the nitro group of ethyl 2-(*o*-nitrophenoxy)-alkanoates **57**, obtained from *o*-nitrophenol derivatives **55** and ethyl α-bromoalkanoates **56**, to the corresponding hydroxylamines using Zn/NH_4Cl and concomitant cyclization gives 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones **58** in moderate yield[40] (Scheme 10). Depending on the applied reducing agent and reaction conditions, the nitro group of **57** can be reduced to an amino (cf. Scheme 5) or hydroxylamino moiety resulting in the formation of 4-unsubstituted or 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones.

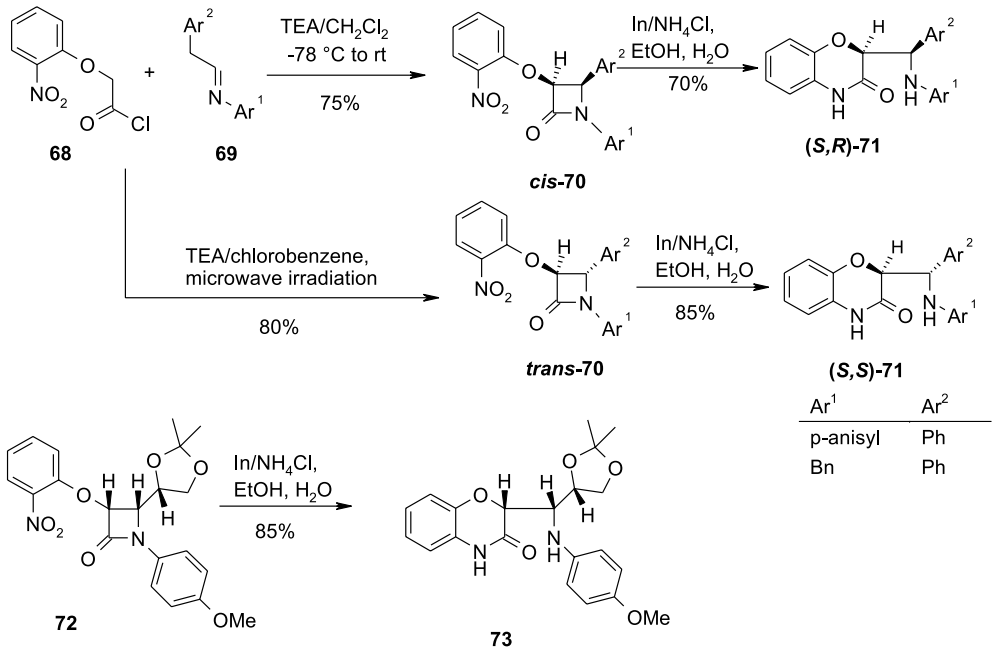
A very elegant way to prepare 2-(2-hydroxyethyl)-2*H*-1,4-benzoxazin-3-(4*H*)-ones, which have PPARγ agonist activity and possess therapeutic potential for treating type 2 diabetes, was described by Rybczynski et al.[41–44] (Scheme 11). 2-Nitrophenol (**59**) is *O*-alkylated with α-bromo-γ-butyrolactone (**60**) to provide the lactone **61**. Catalytic hydrogenation of the nitro group and concomitant cyclization yields the racemic benzoxazinone **62**. To produce the 4-substituted analogue **66**, 2-methoxyaniline (**63**) is *N*-arylated with 4-bromo-1,2-dichlorobenzene (**64**), yielding diphenylamine **65**.[44] The methoxy group of **65** is cleaved



Scheme 10.



Scheme 11.

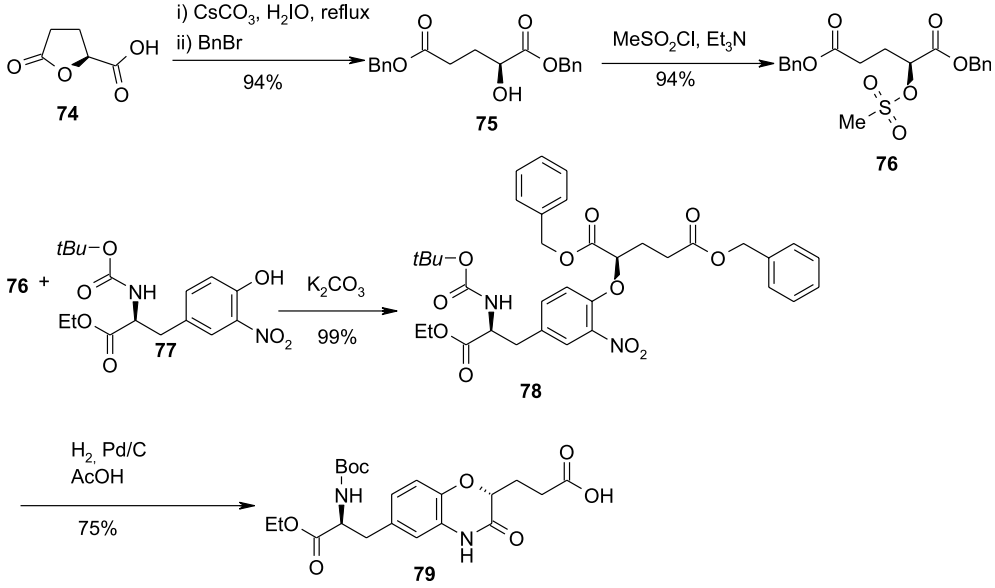


Scheme 12.

with boron tribromide in xylene/dichloromethane to produce the corresponding phenol, which is *O*-alkylated with α-bromo-γ-butyrolactone (**60**) to give the intermediate, which spontaneously cyclizes, yielding **66**. For the synthesis of (*R*)-2-(2-hydroxyethyl)-2*H*-1,4-benzoxazin-3-(4*H*)-one (*R*)-**62**, Mitsunobu reaction of 2-nitrophenol (**59**) and (*S*)-2-hydroxy-γ-butyrolactone (**67**) provides the chiral lactone (*R*)-**61**. Reduction of the nitro group by catalytic hydrogenation and concomitant cyclization yields (*R*)-**62**.[41–43]

A convenient diastereoselective synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones **71** by an indium-induced ring-forming reaction[45] is presented in Scheme 12. Annulation of 2-(2-nitrophenoxy)acetyl chloride (**68**) with imines **69** produces *cis* β-lactams **70** or *trans* β-lactams **70** depending on the reaction conditions. Slow addition of **68** to the imine solution containing triethylamine (TEA) at −78 °C affords *cis*-**70** as the only product. Reaction of the same adducts under microwave irradiation gives *trans*-**70**. Treatment of both isomers of **70** with indium/ammonium chloride in aqueous ethanol under reflux yields 2*H*-1,4-benzoxazin-3-(4*H*)-ones (*S*,*R*)-**71** and (*S*,*S*)-**71** in excellent yield. A modification of this procedure, applying a chiral auxiliary in an imine reagent, giving *cis* β-lactams **72**, can be used for the asymmetric synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones such as **73** with *R* configuration at the benzoxazinone 2-position.

In the search for new bicyclic aromatic amino acids, (*R*)-2*H*-1,4-benzoxazin-3-(4*H*)-one **79** was prepared

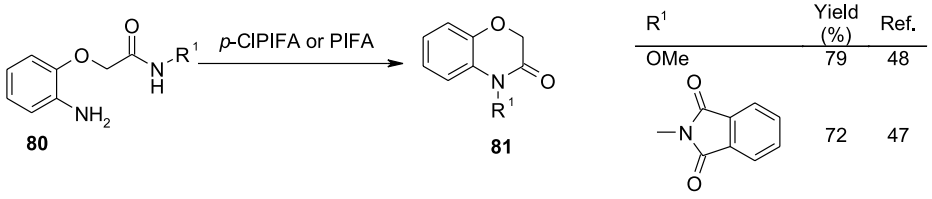


Scheme 13.

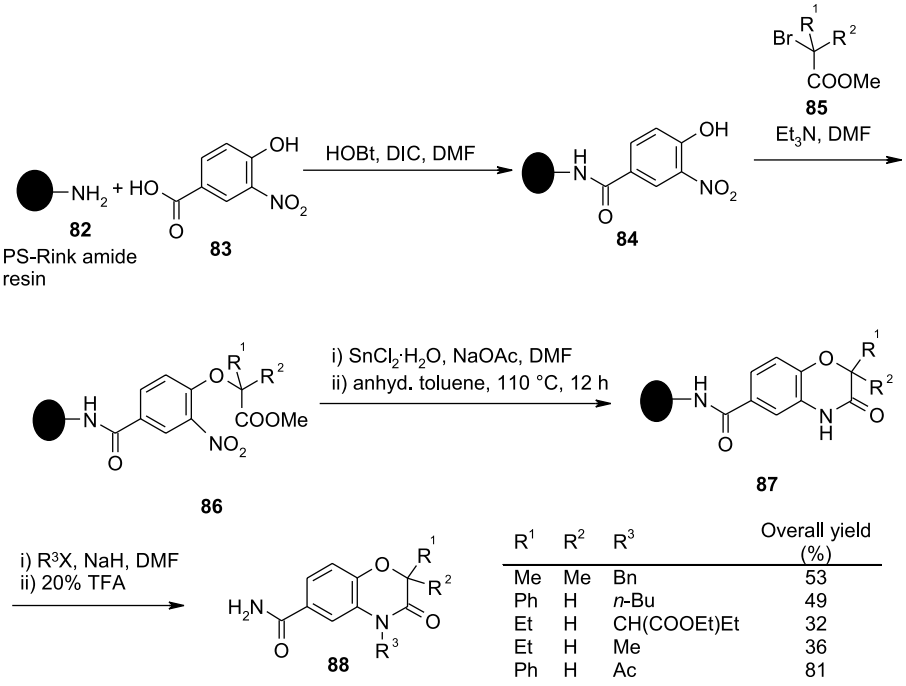
stereoselectively in a multistep synthetic sequence.[46] (2*S*)-5-Oxotetrahydrofuran-2-carboxylic acid (**74**) was hydrolyzed to (2*S*)-2-hydroxypentanedioic acid, which was protected as dibenzyl ester **75**. The 2-hydroxy group of **75** was activated by methanesulfonyl chloride, yielding **76**, which, after coupling to protected 3′-nitrotyrosine **77**, afforded compound **78**. Catalytic hydrogenation reduced the nitro group and cleaved the benzyl ester moieties of **78**, giving an intermediate which cyclized to (*R*)-2*H*-1,4-benzoxazin-3-(4*H*)-one **79** (Scheme 13).

Intramolecular cyclization of *N*-substituted 2-(2-aminophenoxy)acetamides **80**, yielding 4-substituted 2*H*-1,4-benzoxazin-3-(4*H*)-ones **81** can be achieved using 4-chlorophenyliodine(III) bis(trifluoroacetate) (*p*-ClPIFA)[47] or phenyliodine(III) bis(trifluoroacetate) (PIFA)[48] as catalyst through generation of a nitrenium ion which undergoes intramolecular electrophilic substitution (Scheme 14).

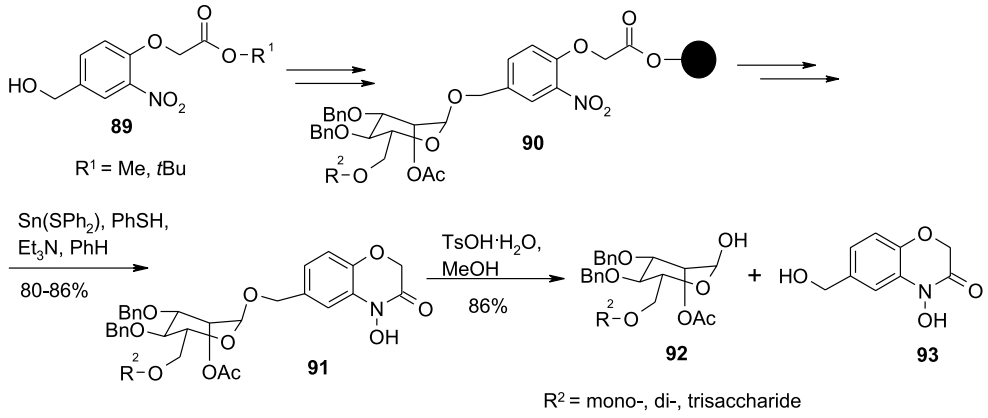
In 2001, Lee et al. published the first solid-state combinatorial synthesis of 2*H*-1,4-benzoxazin-3(4*H*)-ones.[49]



Scheme 14.



Scheme 15.



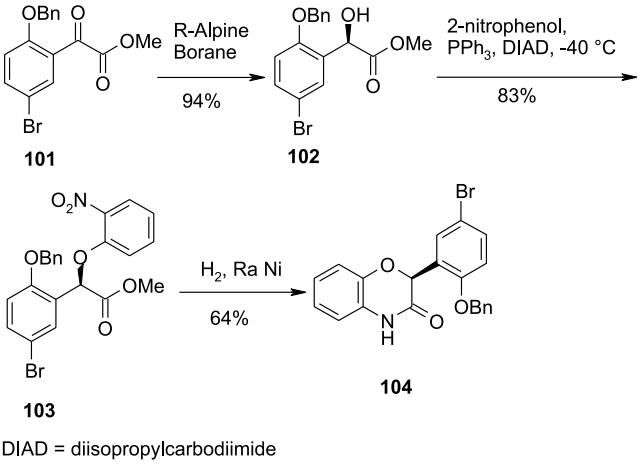
Scheme 16.

Deprotection of the Rink amide resin **82** followed by standard *N*,*N*′-diisopropylcarbodiimide/*N*-hydroxybenzotriazole promoted coupling with 4-hydroxy-3-nitrobenzoic acid (**83**), affording polymer-bound 2-nitrophenol **84**. Alkylation of **84**, with 2-alkyl/aryl-2-bromoalkanoates **85** in the presence of a base yielded compounds **86** in which the nitro group was reduced to amine by tin(II) chloride dihydrate in the presence of a sodium acetate. The latter reagent was required to act as a buffer to prevent premature cleavage of **86** from the resin. The resulting amine was cyclized to the benzoxazinones **87** in refluxing toluene. Alkylation and acylation of the amide nitrogen of **87**, followed by acidic cleavage from the resin, yielded **88** (Scheme 15).

4-Hydroxy-6-(hydroxymethyl)-2*H*-1,4-benzoxazin-3(4*H*)-one (**93**) is obtained as a side product of the solid-state synthesis of oligosaccharides **92** using [4-(hydroxymethyl)-2-nitrophenoxy]acetate **89** as linker[50] (Scheme 16). Linker **89** is attached to Wang resin and subsequently glycosilated yielding **90**, reduction of the nitro group, followed by the intermolecular cyclization releases **91**, which is hydrolyzed affording **93**.

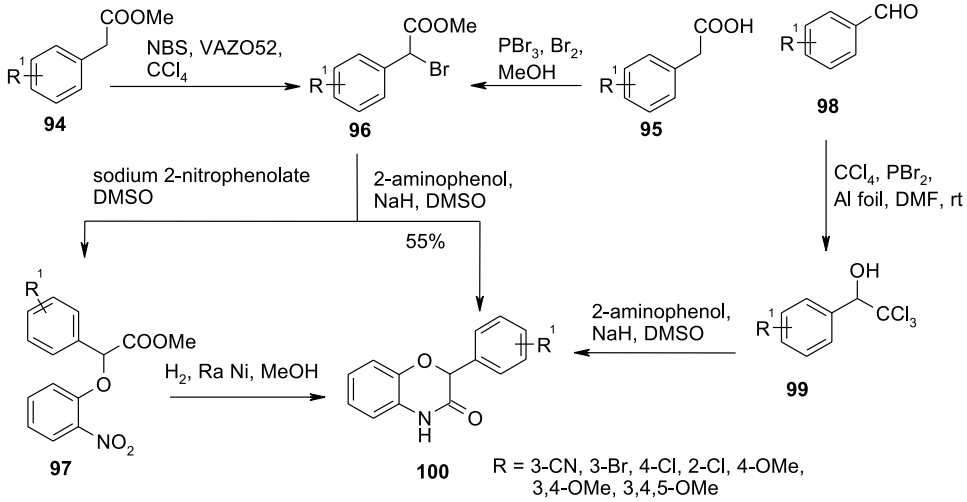
Several synthetic pathways used to prepare 2-aryl-2*H*-1,4-benzoxazin-3(4*H*)-ones **100** as novel factor Xa inhibitors[51,52] are depicted in Scheme 17. Alkylation of 2-nitrophenol with methyl α-bromo-α-arylacetates **96** (prepared from **94** or **95**) giving ether **97** as intermediate is the most favorable approach. The nitro group of **97** is reduced, providing the corresponding anilines that undergo spontaneous cyclization affording the 2-aryl-2*H*-1,4-benzoxazin-3-(4*H*)-ones **100**. In some instances, the reaction of *o*-aminophenol, instead of *o*-nitrophenol, with α-bromo-α-arylacetates **96** affords **100** directly. This pathway is particularly successful to avoid over-reduction during the transformation of **97** to **100**, which results in partial debenzylation. An alternative approach for a one-pot synthesis of 2-arylbenzoxazinones **100** is treatment of the 1-trichloromethyl carbinols **99** with 2-aminophenol in the presence of sodium hydride in dimethylsulphoxide. The advantage of this approach is that a large variety of aldehydes **98** can be easily converted into the substituted 1-trichloromethyl carbinols **99**, although the moderate yield of the benzoxazinones **100** is the main drawback.

The stereoselective synthesis of 2-aryl-2*H*-1,4-benzoxazin-3(4*H*)-ones, for example, **104** is presented in Scheme 18.[52] α-Ketoester **101** is stereoselectively reduced by *R*-alpine borane, yielding (*R*)-α-hydroxyester **102** in 94% yield and 95% enantiomeric excess. The chiral alcohol **102** is coupled with 2-nitrophenol under Mitsunobu conditions affording the ether **103**. Reduction of the nitro group of **103** yields an intermediary amine, which cyclizes to (*S*)-2-aryl-2*H*-1,4-benzoxazin-3(4*H*)-one **104**, a key intermediate in the synthesis of factor Xa inhibitors.

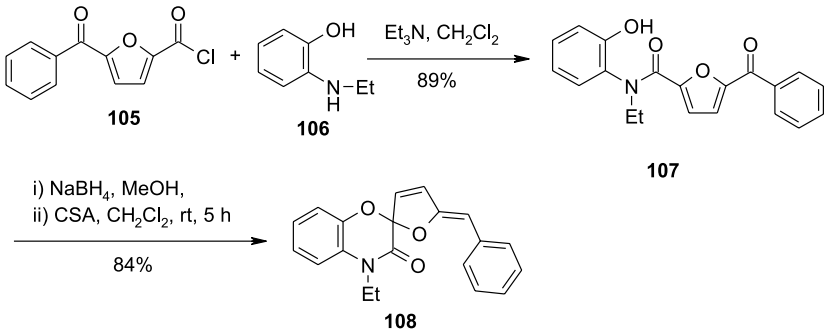


Scheme 18.

Spiro 1,4-benzoxazinone **108**, an analogue of a naturally occurring antifeeding compound tonghaosu, is prepared following the pathway[53] depicted in Scheme 19. Acid chloride **105** and *N*-ethyl-2-aminophenol (**106**) are coupled to give the carboxamide **107**. The keto group of **107** is reduced with $NaBH_4$, and cyclization of the obtained intermediate is achieved using camphorsulfonic acid (CSA) as catalyst.



Scheme 17.



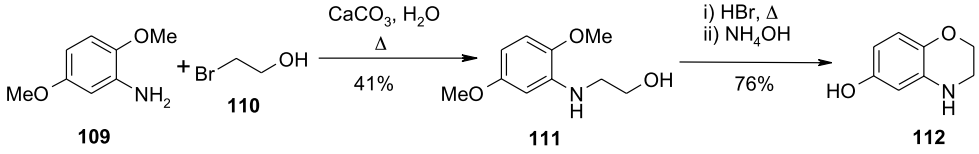
Scheme 19.

3. Synthesis of 3,4-dihydro-2*H*-1,4-benzoxazines by ring closure

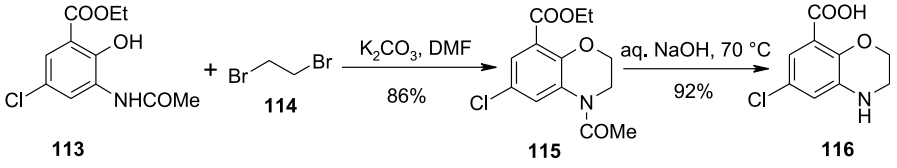
3,4-Dihydro-2*H*-1,4-benzoxazines can be synthesized by reacting 2-methoxyanilines, for example, **109**, with 2-bromoethanol (**110**), yielding [(2,5-dimethoxyphenyl)-amino]methanol (**111**), which, after cleavage of both methyl ether moieties with hydrobromic acid, undergoes cyclization to 7-hydoxy-3,4-dihydro-2*H*-1,4-benzoxazine (**112**). Compound **112** was presented as an important intermediate in the synthesis of bicyclic derivatives of oxypropanolamine, which were evaluated for inotropic, chronotropic and coronary-vasodilating activities[54] (Scheme 20).

Ethyl 3-(acetylamino)-5-chloro-2-hydroxybenzoate (**113**) reacts with 1,2-dibromoethane (**114**) producing 6-chloro-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid (**115**), which is hydrolyzed to the carboxylic acid **116**, an intermediate in the synthesis of serotonin-3 (5-HT_3) receptor antagonists[55,56] (Scheme 21).

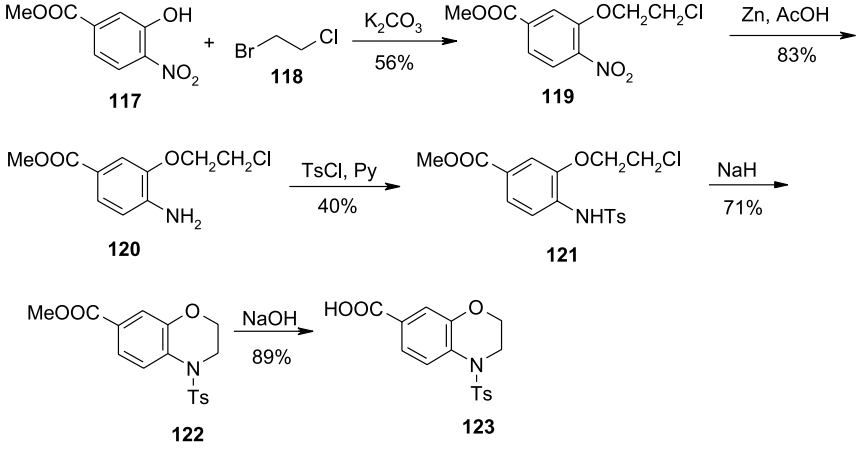
Matsuoka et al. have prepared novel methotrexate analogues incorporating the 3,4-dihydro-2*H*-1,4-benzoxazine moiety.[57] In the course of their synthesis, preparation of the benzoxazine **123** starts with *O*-alkylation of 2-nitrophenol **117** with 1-bromo-2-chloroethane (**118**) followed by zinc-mediated reduction of **119** and protection of the obtained amine **120** as the tosylate **121**. Subsequent cyclization proceeds effectively by treating **121** with sodium hydride to yield 1,4-benzoxazine **122**, which can



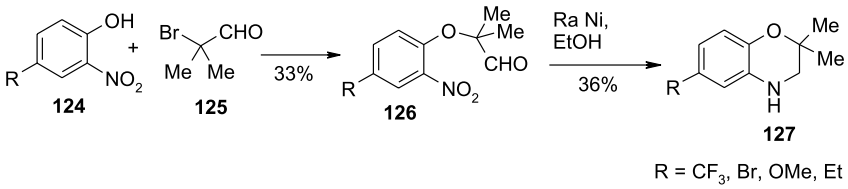
Scheme 20.



Scheme 21.



Scheme 22.



Scheme 23.

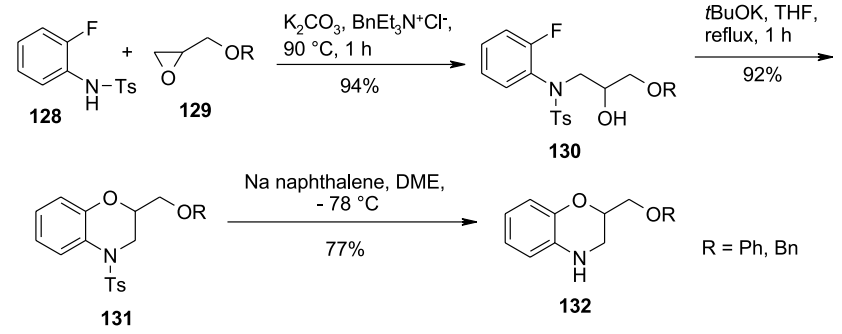
be further hydrolyzed to the carboxylic acid **123** (Scheme 22).

Ring closure to 3,4-dihydro-2*H*-1,4-benzoxazines can also be effected by a reductive amination reaction.[28] Thus, 2-bromo-2-methylpropanal (**125**) can be used for *O*-alkylation of 2-nitrophenols **124** to give the phenolic ethers **126** which are reduced, with concomitant intermolecular reductive amination, yielding 3,4-dihydro-2*H*-1,4-benzoxazines **127** (Scheme 23).

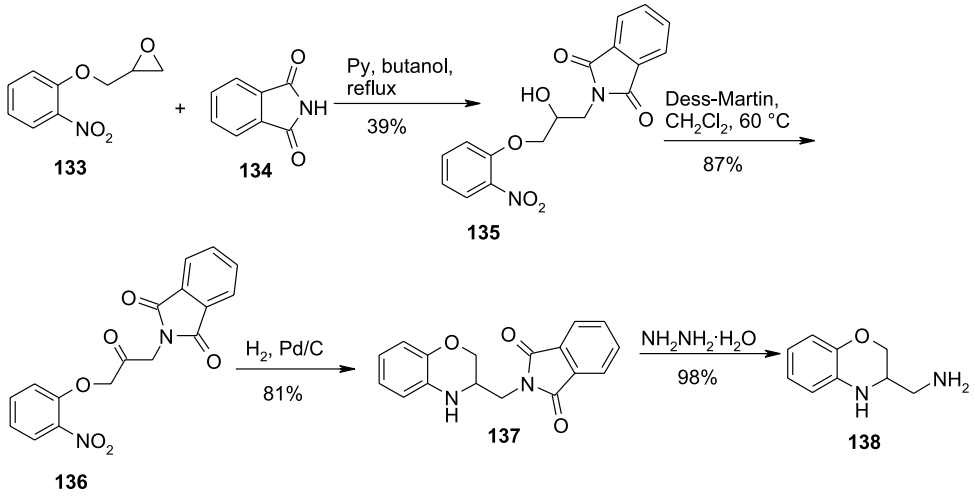
A novel synthesis of 2-substituted 3,4-dihydro-2*H*-1,4-benzoxazines, for example, 2-phenoxymethyl-3,4-dihydro-2*H*-1,4-benzoxazine (**132**; R = Ph), through formation of the C–O and C–N bonds, involves ring-opening of glycidol **129** with the *N*-nucleophile formed from 2-fluoro-*N*-tosylaniline (**128**) under phase-transfer catalysis, and subsequent intramolecular nucleophilic substitution of fluoride in **130** promoted by a non-nucleophilic strong base. Cleavage of the *N*-tosyl protecting group of **131** with sodium/naphthalene in dimethyl ether (DME) affords the 2-substituted 1,4-benzoxazines **132**[58–60] (Scheme 24). Using the (*S*)-glycidol (*S*)-**129** leads under the same conditions to enantiomerically pure 3,4-dihydro-2*H*-1,4-benzoxazine (*R*)-**132**.[58–60]

1-(3,4-Dihydro-2*H*-1,4-benzoxazin-3-yl)methanamine **138** is used as intermediate in the synthesis of pyrazino[2,1-*c*]-1,4-benzoxazines, ligands for the α_1-adrenergic receptor.[61] Epoxide **133** is opened by *N*-nucleophile **134**, giving the alcohol **135**, which is oxidized by the Dess–Martin reagent to the ketone **136**. Reduction of the nitro group of **136** and concomitant cyclization by reductive amination gives 3,4-dihydro-2*H*-1,4-benzoxazine **137**, which can be deprotected by hydrazinolysis to afford the amine **138** (Scheme 25).

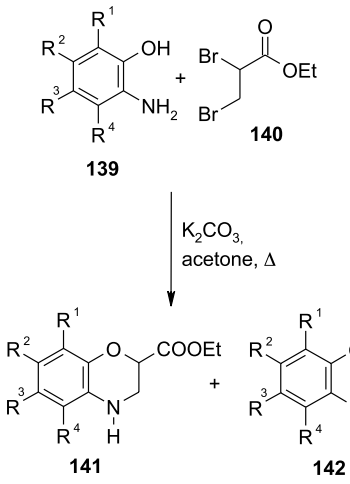
Substituted 2-aminophenols are classical starting compounds for the synthesis of 3,4-dihydro-2*H*-1,4-benzoxazines. The reaction of substituted 2-aminophenols **139** with ethyl 2,3-dibromopropionate (**140**) affords, depending on



Scheme 24.



Scheme 25.



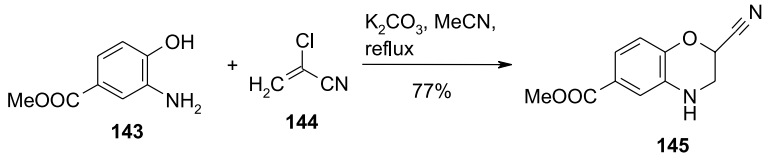
R^1	R^2	R^3	R^4	Yield (%) **141**	Yield (%) **142**	Ratio	Ref.
H	H	H	H	81	4	20:1	62
Me	H	H	H	74	13	6:1	62
MeO	H	H	H	51	9	6:1	62
Me	H	Me	H	68	13	5:1	62
H	Cl	H	H	72	n.d.[a]	-	24
Me	H	H	H	68	n.d.[a]	-	63
H	Me	H	H	90	n.d.[a]	-	63
H	H	Me	H	77	n.d.[a]	-	63
H	H	H	Me	72	n.d.[a]	-	63
H	H	OMe	H	42	n.d.[a]	-	63

[a]Not detected.

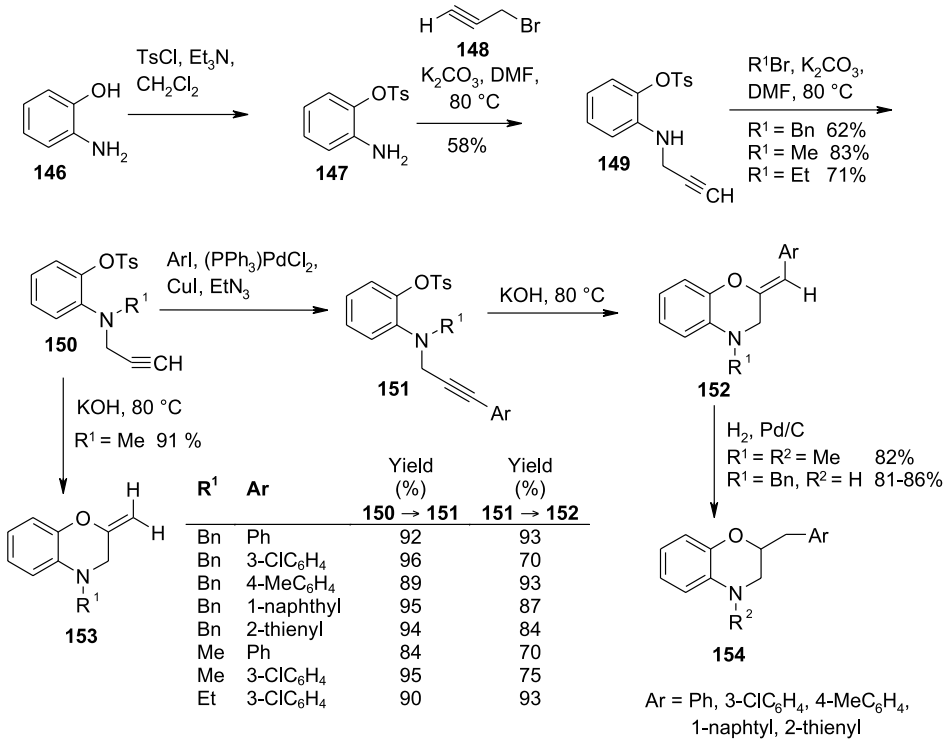
Scheme 26.

the substituents on the aromatic ring, ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates **141** as the main, and ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-3-carboxylates **142** as the minor, isomers. In the case of the unsubstituted 2-aminophenol, the ratio between the isomers is 20:1, whereas in the case of 6-substituted 2-aminophenols the ratio changes to 6:1[62] (Scheme 26).

2-Cyano-3,4-dihydro-2*H*-1,4-benzoxazines, for example, **145**,[63] can be obtained by a ring-closure reaction following the pathway presented in Scheme 27. Methyl 3-amino-4-hydroxybenzoate (**143**) and 2-chloroacrylonitrile (**144**) under reflux in acetonitrile for 18 h in the presence of K_2CO_3 provided methyl 2-cyano-3,4-dihydro-2*H*-1,4-benzoxazine-6-carboxylate (**145**) in good yield.



Scheme 27.



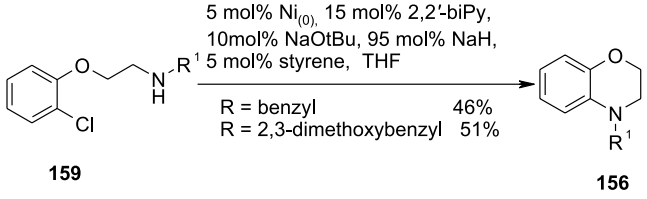
R^1	Ar	Yield (%) 150 → 151	Yield (%) 151 → 152
Bn	Ph	92	93
Bn	3-ClC_6H_4	96	70
Bn	4-MeC_6H_4	89	93
Bn	1-naphthyl	95	87
Bn	2-thienyl	94	84
Me	Ph	84	70
Me	3-ClC_6H_4	95	75
Et	3-ClC_6H_4	90	93

Scheme 28.

2-Arylidene/methylene-3,4-dihydro-2*H*-1,4-benzoxazines **152** and **153** are prepared by a ring-closure reaction from 2-aminophenols and propargyl bromide (**148**). The starting 2-aminophenol (**146**) is *O*-tosylated with *p*-toluenesulfonyl chloride in the presence of triethylamine in dichloromethane (tosylation in the presence of pyridine leads to *N*-tosylation) to give the *O*-tosyl derivative **147**. Propargyl bromide (**148**) is coupled to **147** in the presence of potassium carbonate in dimethylformamide to afford 2-(prop-2-ynylamino)phenyl *p*-toluenesulfonate **149**, which is *N*-alkylated to give the compounds **150**. Removal of the *O*-tosyl group by alkaline hydrolysis leads to spontaneous cyclization to the 2-methylene-3,4-dihydro-2*H*-1,4-benzoxazines (**153**). The palladium-catalyzed reaction of **150** with aryl iodides, carried out in the presence of bis(triphenylphosphine)palladium(II) dichloride [$PdCl_2(PPh_3)_2$] as catalyst and cuprous iodide as co-catalyst, gives the 3-arylpropargylamines **151**. These are readily cyclized in the presence of potassium hydroxide to unstable 1,4-benzoxazines **152** bearing an exocyclic double bond at position 2. The synthesis is completely regio- and stereoselective and no seven-membered ring compounds or any compounds of *E*-stereochemistry are isolated. 4-Alkyl(benzyl)-2-alkyl(aryl)idene-3,4-dihydro-2*H*-1,4-benzoxazines **152** and **153** are highly unstable in halogenated solvents and on silica gel. Hydrogenation with Pd/C as catalyst gives stable 2-arylmethyl-3,4-dihydro-2*H*-1,4-benzoxazines **154**.[64] The 4-benzyl group is removed during the hydrogenation procedure (Scheme 28).

3,4-Dihydro-2*H*-1,4-benzoxazines **156** and **158**[65] are synthesized by intramolecular C–O bond formation using palladium-catalyzed intramolecular etherification of aryl halides employing di-*tert*-butylphosphonobiaryl ligands (L) and palladium(II) acetate (Scheme 29). The ligands L facilitate reductive elimination to release the steric strain of the palladium(II) aryloxy intermediate. Primary (**155**), as well as secondary (**157**), alcohol substrates **157** are efficiently cyclized in the presence of a mild base (e.g., caesium carbonate or potassium phosphate), which enables the use of an ester group-containing aryl bromide. The presence of an unprotected arylamine does not interfere with the C–O bond formation and no amination side products are observed. It is also possible to cyclize enantiomerically pure alcohols **157** with conservation of enantiomeric purity in the products **158**.

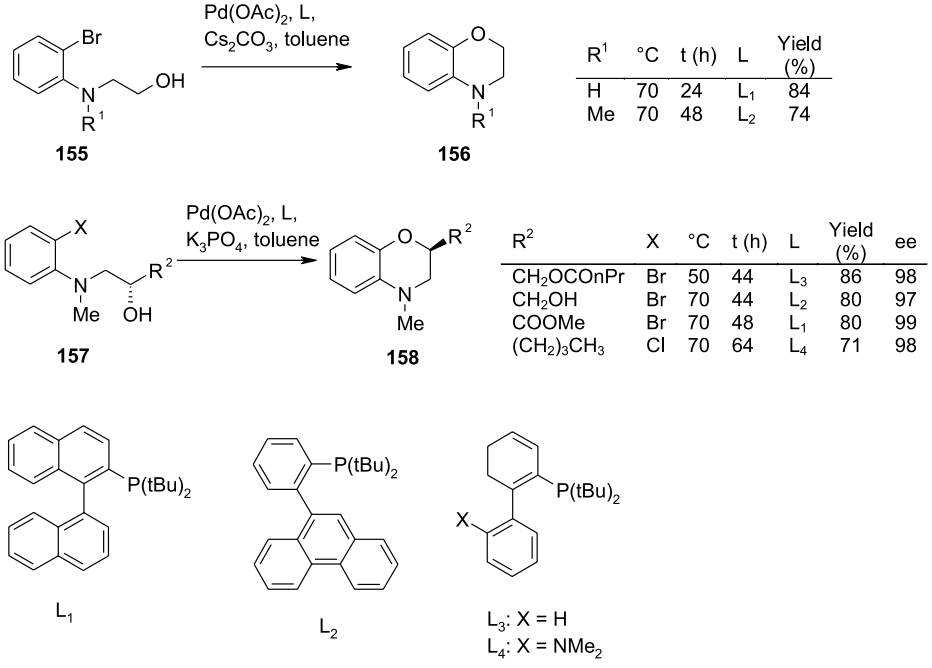
Nickel-mediated intramolecular amination of aryl chlorides **159** yields 3,4-dihydro-2*H*-1,4-benzoxazines **156** by the in situ-generated Ni(0) catalyst associated with 2,2′-bipyridine[66] (Scheme 30). The same procedure has also been applied also to the formation of five- and seven-membered rings.



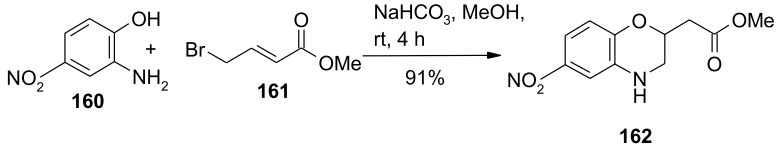
Scheme 30.

In the course of synthesis of integrin receptor antagonists, the key intermediate **162** was prepared in a reaction of methyl (2*E*)-4-bromobut-2-enoate (**161**) with 2-aminophenol **160** via an intramolecular Michael addition[67] (Scheme 31).

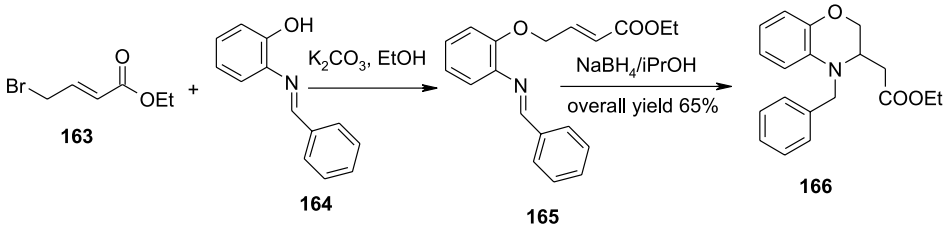
Another example using intramolecular Michael addition for the synthesis of 3,4-dihydro-2*H*-1,4-benzoxazines is presented in Scheme 32. *O*-Alkylation of imine **164**, using ethyl 4-bromocrotonate (**163**), affords the imine **165**. In situ reduction of **165** gives the corresponding amine, which



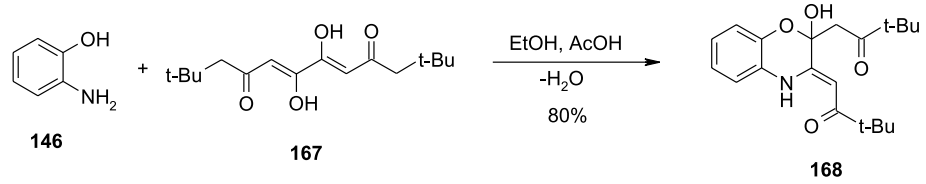
Scheme 29.



Scheme 31.



Scheme 32.



Scheme 33.

spontaneously undergoes intramolecular Michael addition to afford the benzoxazine **166** in good overall yield.[62]

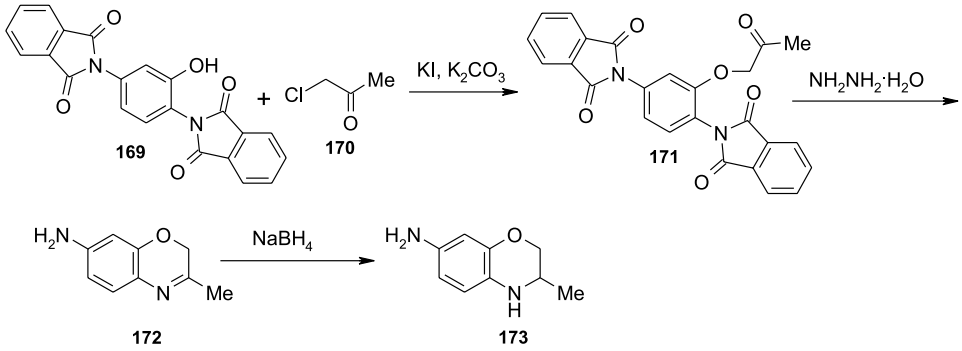
The action of 2-aminophenol (**146**) on 5,6-dihydroxy-2,2,9,9-tetramethyldeca-4,6-diene-3,8-dione (**167**) leads to the formation of a stable cyclic hemiacetal **168**[68] (Scheme 33).

O-Alkylation of the 2,5-diaminophenol derivative **169** with 1-chloroacetone (**170**) affords the phenoxyacetate **171**. The phthalimide protecting groups are removed by hydrazinolysis and the obtained phenylenediamine intermediate undergoes cyclization, yielding the cyclic imine **172**, which is reduced to 3-methyl-3,4-dihydro-2*H*-1,4-benzoxazin-7-amine (**173**), an intermediate in the synthesis of antibacterial agents[69] (Scheme 34).

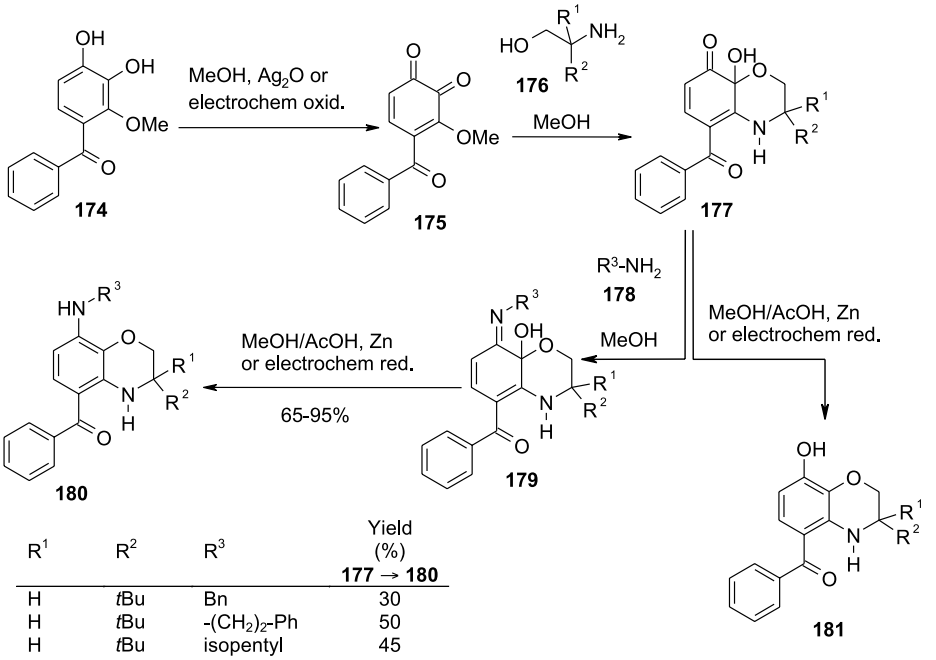
8-Amino-1,4-benzoxazine derivatives **180** were synthesized as potential neuroprotective agents for treating cerebral ischaemia leading to neurodegenerative disease.[70] A new two-step one-pot electrochemical procedure for preparing **180** involves the initial oxidation of diaryl ketone **174** to 1,2-quinone **175** followed by substitution of the methoxy group with aminoalcohols **176** and, after intramolecular ring closure, the formation of 8a-hydroxy-3,4-dihydro-2*H*-1,4-benzoxazin-8(8a*H*)-one derivatives **177** in 60–95% yield. The imines **179** are formed in the reaction of **177** with an excess of amine **178**, and subsequently reduced through two-electron transfer to the 8-amino derivatives **190**. 8-Hydroxy derivatives **181** are prepared by the reduction of the C-8 carbonyl, using zinc in methanol/acetic acid followed by elimination of water (Scheme 35).

4. Synthesis of 3,4-dihydro-2*H*-1,4-benzoxazines by reduction of 2*H*-1,4-benzoxazin-3-(4*H*)-ones

2*H*-1,4-Benzoxazin-3-(4*H*)-ones **26** are readily reduced to 3,4-dihydro-2*H*-1,4-benzoxazines **182** by strong reducing agents such as lithium aluminum hydride[71] or borane[28] (Scheme 36). It should be noted that the use of boron trifluoride diethyl etherate/sodium borohydride[56] enables



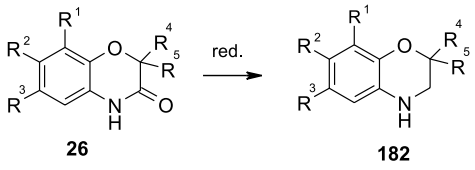
Scheme 34.



R^1	R^2	R^3	Yield (%) 177 → 180
H	*t*Bu	Bn	30
H	*t*Bu	-$(CH_2)_2$-Ph	50
H	*t*Bu	isopentyl	45
Me	Me	Bn	15
Me	Me	isopentyl	50
Me	Me	CH_2CH_2OH	60
Me	Me	$CH(CH_2OH)_2$	45
	-$(CH_2)_4$-	Bn	35
	-$(CH_2)_4$-	*i*Bu	50
Ph	Ph	Bn	50
Ph	Ph	isopentyl	50
H	CH_2OH	$CH(CH_2OH)_2$	15
Me	CH_2OH	CH_2CH_2OH	50
Me	CH_2OH	$CH(CH_2OH)_2$	25
CH_2OH	CH_2OH	CH_2CH_2OH	60

R^1	R^2	Yield (%) 174 → 177	Yield (%) 177 → 181
H	tBu	95	85
	-$(CH_2)_4$-	60	80
H	CH_2OH	65	75

Scheme 35.



R^1	R^2	R^3	R^4	R^5	Reducing agent	Yield (%)	Ref.
F	H	H	H	H	$LiAlH_4$	65	71
H	NO_2	H	Me	Me	BH_3	93	28
COOEt	H	F	H	H	$BF_3{\cdot}Et_2O$, $NaBH_4$	83	56
COOEt	H	Br	H	H	$BF_3{\cdot}Et_2O$, $NaBH_4$	74	56

Scheme 36.

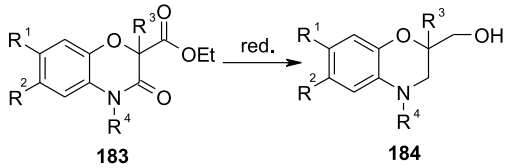
selective reduction of the benzoxazinone lactam moiety in the presence of the carboxylic ester moiety.

The use of a strong reducing agent, however causes concomitant reduction of some functional groups present in the molecule, which allows one-pot multiple functionalization. Ethyl 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates **183** are reduced to (3,4-dihydro-2*H*-1,4-benzoxazin-2-yl)methanols **184** with lithium borohydride,[24] borane[72] or lithium aluminum hydride[73] (Scheme 37).

5. Modification of substituents in 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 2*H*-1,4-benzoxazines

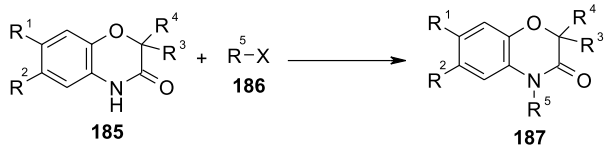
5.1. Modification of substituents in the 1,4-oxazine ring of 1,4-benzoxazin-3-(4*H*)-ones

Although a substituent at position 4 of 2*H*-1,4-benzoxazin-3-(4*H*)-ones can be introduced in a precursor which is cyclized to 2*H*-1,4-benzoxazin-3-(4*H*)-one, a more frequently used approach is *N*-alkylation of the 2*H*-1,4-benzoxazin-3-(4*H*)-one scaffold **185**. Classically, sodium hydride,[30,51] potassium fluoride,[74] or potassium carbonate[75]



R^1	R^2	R^3	R^4	Reducing agent	Yield (%)	Ref.
H	H	H	H	$LiBH_4$	62	24
H	H	Me	H	BH_3	76	72
H	H	H	Pr	$LiAlH_4$	78	73
Cl	H	H	Me	$LiAlH_4$	98	73

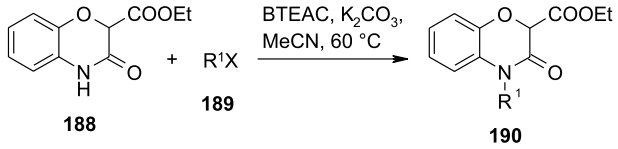
Scheme 37.



R^1	R^2	R^3	R^4	R^5	X	Method	Yield (%)	Ref.
NO_2	H	Me	COOEt	Me	I	KF	96	74
NO_2	H	H	H	CH_2COOEt	Br	KF	72	74
H	H	H	C_6H_4-3-CN	$(CH_2)_5Br$	Br	NaH	56	51
H	H	H	H	$(CH_2)_2O$-C_6H_4-4-CHO	Br	K_2CO_3	91	75
H	Cl	H	H	CH_2COOEt	Br	NaH	40[a],87[b]	30
H	NO_2	Me	Me	CH_2COOEt	Br	NaH	55[a],92[b]	30
H	NO_2	H	H	$(CH_2)_2COOEt$	Br	NaH	65[a],94[b]	30
H	CN	Me	Me	$(CH_2)_2COOEt$	Br	NaH	41[a],86[b]	30
H	Cl	H	H	$(CH_2)_3COOEt$	Br	NaH	55[a],85[b]	30
H	CN	Me	Me	$(CH_2)_3COOEt$	Br	NaH	60[a],92[b]	30

[a]Δ: t = 3 h; [b]MWI: t = 18 min

Scheme 38.



R^1	X	Yield (%)
Me	I	72
Et	Br	45
Bn	Br	57

Scheme 39.

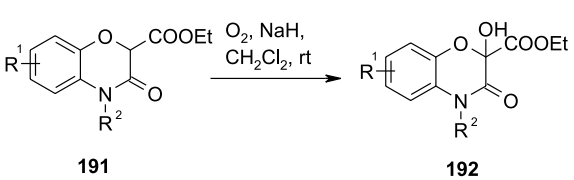
are used to activate the amide nitrogen of **185**. Microwave irradiation (MWI)[30] of the reaction mixture of **185** and **186** reduces the reaction time and enhances the yield of **187** (Scheme 38).

In the *N*-alkylation of 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates, for example, **188**, with alkyl halides **189**, phase-transfer conditions are usually employed to produce **190**,[76] since the use of strong bases leads also to alkylation in position 2[76] (Scheme 39).

In alkylation of alkyl 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates **191** at position 2,[76] however carbanion oxidation is a competing reaction which can prevail under oxidative reaction conditions. Thus, 2-hydroxy derivatives **192** can be synthesized from alkyl 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates **191** through carbanion oxidation[77,78] (Scheme 40).

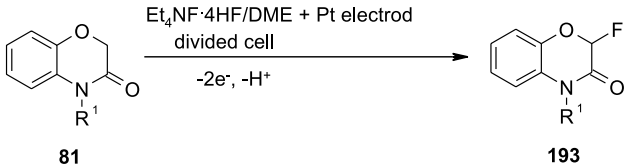
Introduction of fluorine in position 2 of *N*-substituted 2*H*-1,4-benzoxazin-4(3*H*)-one derivatives **81**, to give **193**, can be performed by anodic monofluorination using a divided cell containing $Et_4NF{\cdot}4HF$ and platinum plate electrodes[79] (Scheme 41).

2-Bromo-2*H*-1,4-benzoxazin-3-(4*H*)-one (**194**) is transformed to 2-amino-2*H*-1,4-benzoxazin-3-(4*H*)-one (**195**) by treating with gaseous ammonia. 2-Mercapto-2*H*-1,4-benzoxazin-3-(4*H*)-one (**196**) is obtained from **194** in a two-step procedure which involves the formation and alkaline



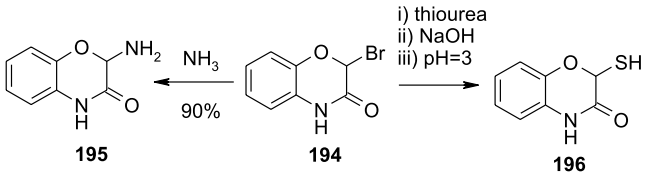
R^1	R^2	Yield (%)
H	Me	82
H	Et	75
H	Pr	76
H	Bn	90
H	CH_2COOEt	86
H	$(CH_2)_3COOEt$	56
H	$CH_2COOtBu$	71
6-COOEt	Me	85
7-COOEt	Me	79

Scheme 40.



R^1	Yield (%)
Me	51
CH_2COOEt	42
Bn	49

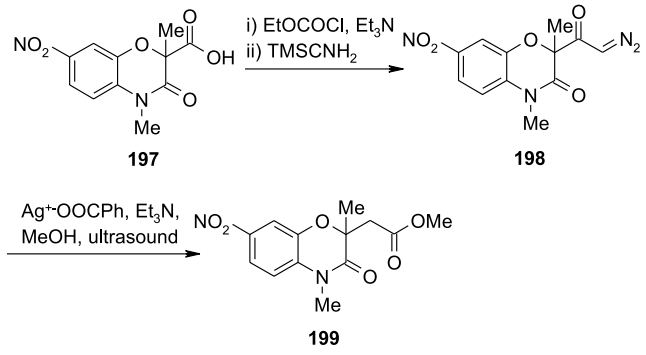
Scheme 41.



Scheme 42.

cleavage of the corresponding istothiouronium bromide[80] (Scheme 42).

Homologation of 3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates, for example, transformation of carboxylic ester **197** to homologous ester **199**, a useful peptidomimetic template, can be performed by a modified Arnsdt–Eisert synthesis. The mixed anhydride prepared from compound **197** with ethyl chloroformate reacts with trimethylsilyl diazomethane affording the diazoacetyl derivative **198**. Ultrasound-promoted Ag^{+}/base catalyzed Wolff rearrangement of diazoketone **198** gives the homologated methyl ester **199**[81] (Scheme 43).

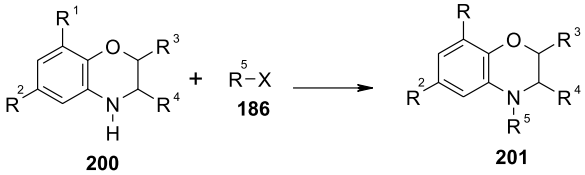


Scheme 43.

5.2. Modification of substituents in the 1,4-oxazine ring of 3,4-dihydro-2*H*-1,4-benzoxazines

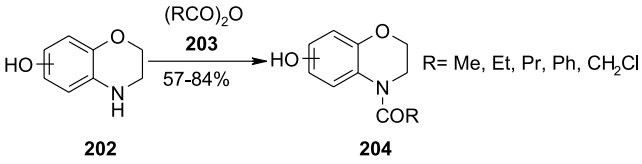
N-Alkylation of 3,4-dihydro-2*H*-1,4-benzoxazines **200** to form **201** is achieved with dialkylsulfates[55] or with alkyl halides in the presence of a base.[82] In the case of 3-substituted 3,4-dihydro-2*H*-1,4-benzoxazines **200** the yields can be lower[63] (Scheme 44).

Anhydrides **203** are employed for the selective *N*-acylation



R^1	R^2	R^3	R^4	R^5	X	Base	Yield (%)	Ref.
H	H	COOEt	H	Me	I	K_2CO_3	86	82
H	H	COOEt	H	Bn	Cl	K_2CO_3	76	82
H	MeO	H	COOEt	Me	I	K_2CO_3	38	63
Me	H	H	COOEt	Me	I	K_2CO_3	45	63
H	COOMe	CN	H	Me	I	K_2CO_3	45	63
CN	H	Me	Me	2-oxotetrahydro-furan-3-yl	Br	NaH	8	28

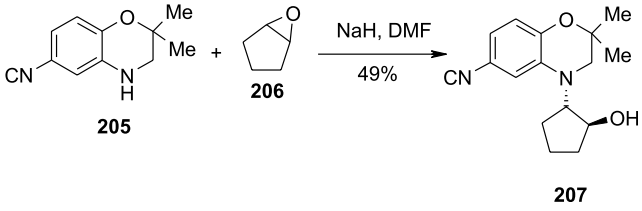
Scheme 44.



Scheme 45.

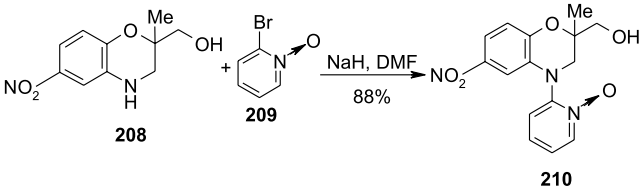
of 3,4-dihydro-2*H*-1,4-benzoxazinols **202** to produce 4-acyl-3,4-dihydro-2*H*-1,4-benzoxazinols **204**[54] (Scheme 45).

N-Alkylation of 2,2-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazine-6-carbonitrile (**205**) by ring opening of cyclopentene oxide (**206**) in the presence of sodium hydride yields the *trans* alcohol **207**[28] (Scheme 46).



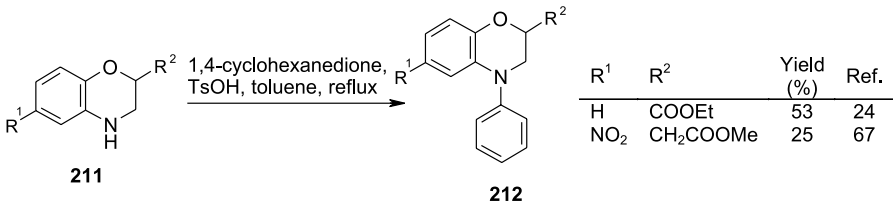
Scheme 46.

Two exceptional examples of the *N*-arylation of 3,4-dihydro-2*H*-1,4-benzoxazines were reported recently. In the course of synthesizing potassium channel activators, several pyridine *N*-oxide derivatives, for example, **210**, were obtained by nucleophilic substitution of 2-bromopyridine *N*-oxide (**209**) with the 3,4-dihydro-2*H*-1,4-benzoxazine derivative **208** in the presence of sodium hydride[28,29,72] (Scheme 47).



Scheme 47.

Treating 3,4-dihydro-2*H*-1,4-benzoxazine **211** with 1,4-cyclohexanedione in the presence of *p*-toluenesulfonic acid

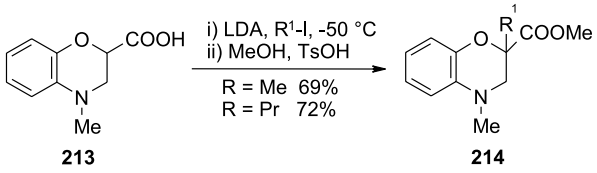


R^1	R^2	Yield (%)	Ref.
H	COOEt	53	24
NO_2	CH_2COOMe	25	67

Scheme 48.

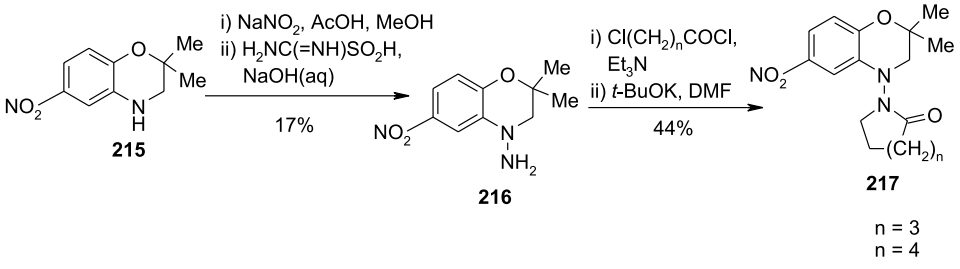
in toluene afforded 4-phenyl-3,4-dihydro-2*H*-1,4-benzoxazine **212** (Scheme 48).

N-Substituted 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylic acids, for example, **213**, are alkylated at position 2 by treatment with lithium diisopropylamide (LDA) in tetrahydrofuran at −50 °C under an argon atmosphere, followed by quenching of the dianion with methyl iodide or propyl iodide. Refluxing the crude product in methanol for 18 h in the presence of *p*-toluenesulfonic acid finally provides the methyl esters **214**[63] (Scheme 49).

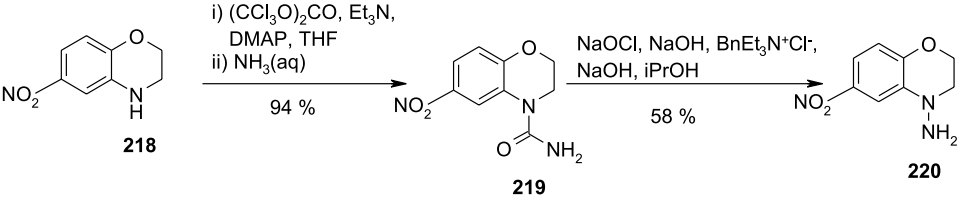


Scheme 49.

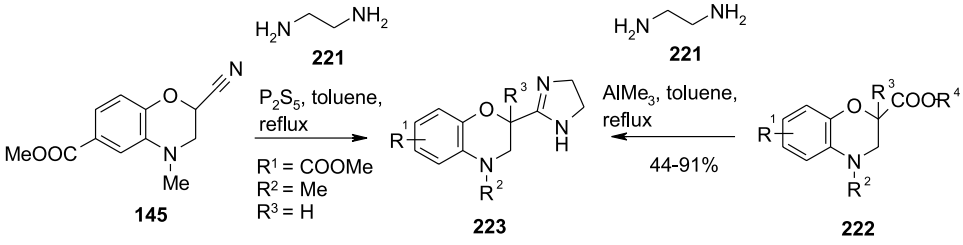
Introduction of the lactam ring into the 4-position of 1,4-benzoxazine **215** to give cromakalim analogues **217**[28] is carried out as shown in Scheme 50. Nitrosation of **215**, followed by reduction of the nitroso intermediate with formamidinesulfinic acid, gives the 4-amino-3,4-dihydro-2*H*-1,4-benzoxazine derivative **216** which, on treatment with 4-chlorobutyryl chloride or 5-chlorovaleryl chloride,



Scheme 50.



Scheme 51.



R^1	R^2	R^3	R^4	Yield (%) 222 → 223
H	Me	Me	Me	81
H	Me	Pr	Me	44
6-CH_2OH	Me	H	Et	62
H	H	H	Et	66
5-Me	Bn	H	Et	85
6-Me	Pr	H	Et	78

Scheme 52.

followed by ring closure with potassium *tert*-butoxide, gives the lactams **217** (Scheme 50).

Treating 6-nitro-3,4-dihydro-2*H*-1,4-benzoxazine (**218**) with triphosgene and subsequently with ammonium hydroxide yields the carboxamide **219** which, after Hofmann rearrangement, affords the hydrazine **220**[83] (Scheme 51).

Carbonitrile **145** is transformed to imidazoline **223** (R^1=CO_2Me; R^2=Me; R^3=H) with retention of the 6-methoxycarbonyl group by reaction with ethylenediamine (**221**) and phosphorous pentasulfide in refluxing toluene. In contrast, different 3,4-dihydro-2*H*-1,4-benzoxazine-2-carboxylates **222** can be transformed to imidazolines **223** using ethylenediamine and trimethylaluminum as catalyst in boiling toluene[63] (Scheme 52).

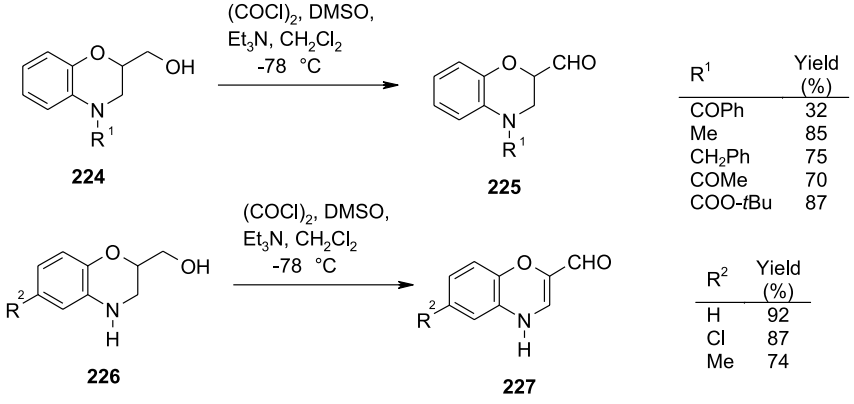
N-Substituted 3,4-dihydro-2*H*-1,4-benzoxazin-2-yl methanols **224** are oxidized under Swern conditions to the *N*-substituted 3,4-dihydro-2*H*-1,4-benzoxazine-2-carbaldehydes **225**. Interestingly, under the same reaction conditions, *N*-unsubstituted 3,4-dihydro-2*H*-1,4-benzoxazin-2-yl methanols **226** are oxidized to the 4*H*-1,4-benzoxazine-2-carbaldehydes **227**[84] (Scheme 53). Oxidation of 3,4-dihydro-2*H*-1,4-benzoxazin-3-yl methanols using Swern and Dess–Martin reagents is not successful and leads only to degradation products.[62]

In a synthesis of high-affinity selective dopamine D_4 receptor antagonists **231**, 3,4-dihydro-2*H*-1,4-benzoxazine-3-one (**1**) is converted into 3-methoxy-2*H*-1,4-benzoxazine (**228**), using Meerwein's salt (trimethyloxonium tetrafluoroborate). Compound **228** is condensed with amine **229**, affording intermediate **230**, which is then selectively methylated with sodium hydride/methyl iodide to give **231**[85] (Scheme 54).

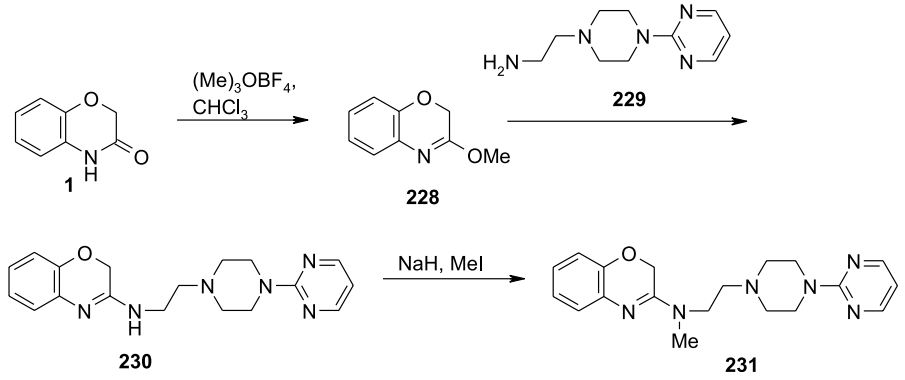
5.3. Modification of substituents in aromatic ring of 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 2*H*-1,4-benzoxazines

Classical reactions for introducing substituents into aromatic rings are applicable to 3,4-dihydro-2*H*-1,4-benzoxazines and 2*H*-1,4-benzoxazin-3-(4*H*)-ones. The influence of ring heteroatoms, however defines the substitution pattern. Regioselective bromination of the aromatic ring of 3,4-dihydro-2*H*-1,4-benzoxazine **232**, with no formation of the 6-bromomethyl derivative, is achieved by refluxing a mixture of **232** and *N*-bromosuccinimide (NBS), with [2,2′-azobis(2-methylpropionitrile)] (AIBN) as catalyst. 3,4-Dihydro-2*H*-1,4-benzoxazine **233**, when submitted to a Stille reaction with tetramethyltin in the presence of tetrakis(triphenylphosphine)palladium(0) as catalyst, affords 6,7-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazine **234**[63] (Scheme 55).

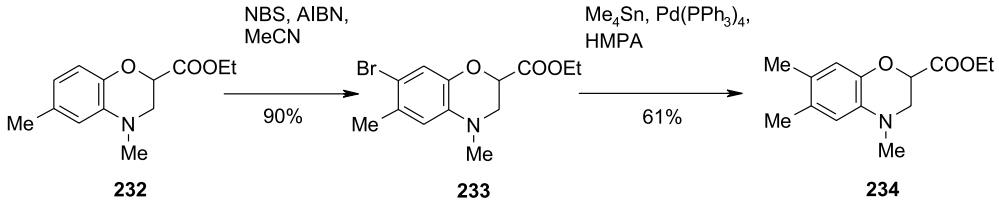
Generally, bromination of 2*H*-1,4-benzoxazin-3(4*H*)-ones with bromine in tetrachloromethane leads to bromination at position 2, yielding 2-bromo-2*H*-1,4-benzoxazin-3(4*H*)-ones.[86] Bromination of 6-acetyl-4-methyl-2*H*-1,4-benzoxazin-3(4*H*)-one (**235**) with bromine in acetic acid,



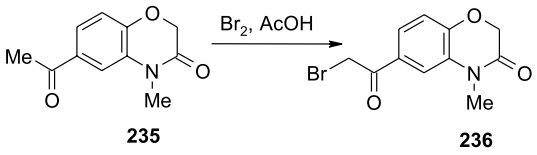
Scheme 53.



Scheme 54.



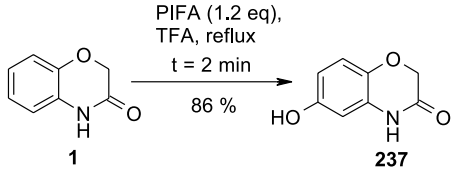
Scheme 55.



Scheme 56.

however, occurs selectively at the 7-acyl group yielding product **236**[87] (Scheme 56).

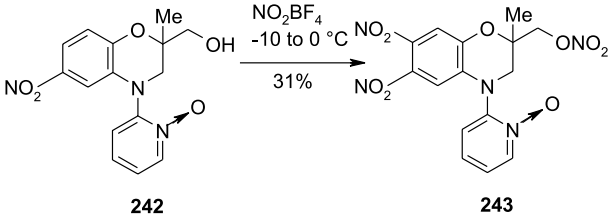
In a reaction of 2*H*-1,4-benzoxazin-3-(4*H*)-one (**1**) with phenyliodine(III) bis(trifluoroacetate) (PIFA), a hydroxy group is introduced into position 6, affording the 6-hydroxy derivative **237**[88] (Scheme 57). This procedure is usually used for *para* hydroxylation of substituted anilides and benz-annulated lactams. However, in the case of 2*H*-1,4-benzoxazin-3-(4*H*)-ones, the participation of the ring oxygen is strong enough for the hydroxyl group to be incorporated into the meta position relative to the ring nitrogen atom.



Scheme 57.

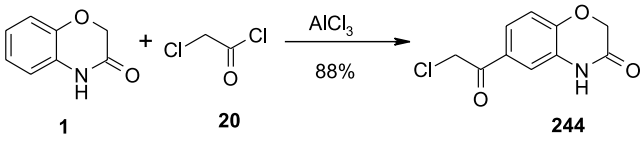
In the synthesis of compounds showing affinity for dopamine D_2, as well as serotonin 5-HT_{1A} and 5-HT_2 receptors, chlorine in position 6 of **238** is removed by catalytic hydrogenation in alkaline solution, affording the 3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid **239**, which is sulfonated at position 6 with chlorosulfonic acid, yielding a 6-chlorosulfonyl intermediate. Addition of ammonium hydroxide yields 6-(aminosulfonyl)-4-methyl-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid (**240**). The chlorosulfonyl intermediate can be reduced with Zn/H_2SO_4 and, after reaction with methyl iodide, 4-methyl-6-(methylthio)-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxylic acid (**241**) is obtained[55] (Scheme 58).

A nitro group is introduced into 1,4-benzoxazine **242** on treatment with nitronium tetrafluoroborate with concomitant nitration of the side chain hydroxyl group, yielding a nitratomethyl-6,7-dinitro-3,4-dihydro-2*H*-1,4-benzoxazine derivative **243**[72] (Scheme 59).

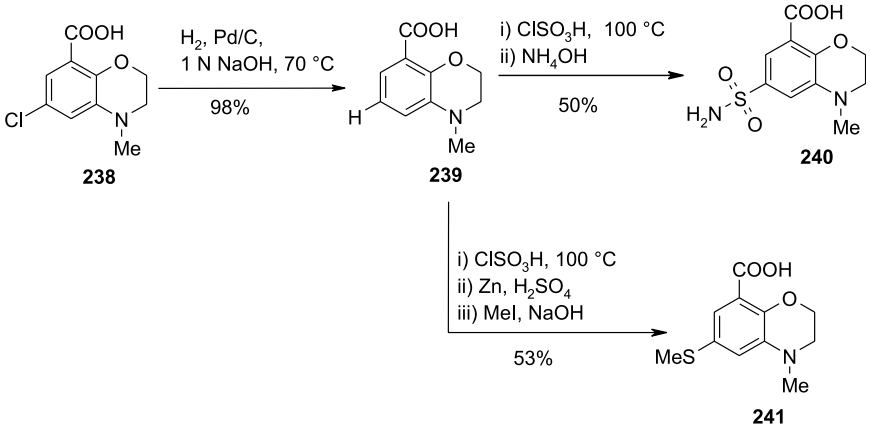


Scheme 59.

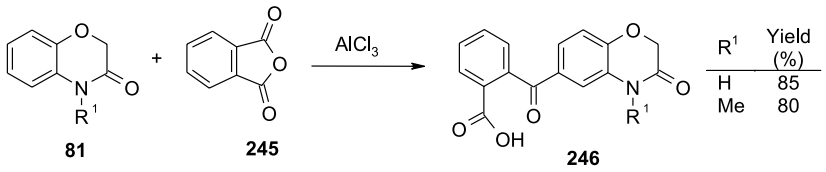
Freidel–Crafts acylation is a very convenient method for the introduction of alkyl or acyl substituents into the aromatic ring of 1,4-benzoxazin-3(4*H*)-ones. Thus, treating 2*H*-1,4-benzoxazin-3-(4*H*)-one (**1**) with chloroacetyl chloride (**20**) and anhydrous aluminum chloride yields



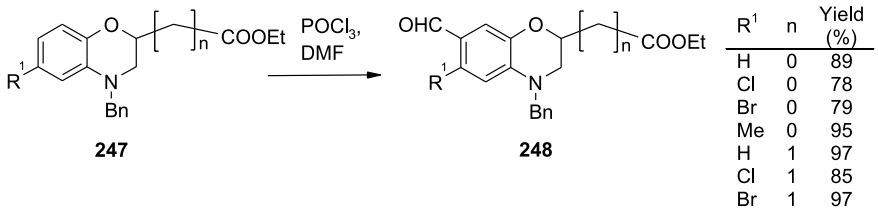
Scheme 60.



Scheme 58.



Scheme 61.



Scheme 62.

6-chloroacetyl-2*H*-1,4-benzoxazin-3-(4*H*)-one (**244**), an intermediate in the synthesis of a potential selective COX-2 inhibitor[89] (Scheme 60).

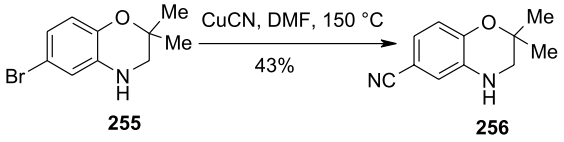
2*H*-1,4-Benzoxazin-3-(4*H*)-ones **81** are acylated at position 6 when treated with phthalic anhydride (**245**) and anhydrous aluminum chloride as a catalyst, yielding the diaryl ketones **246**[90] (Scheme 61).

Regioselective formylation of ethyl 3,4-dihydro-2*H*-1,4-benzoxazine-2-propionate or 2-acetate derivatives **247** is achieved by treating **247** with phosphorous oxychloride, yielding 7-formyl-3,4-dihydro-2*H*-1,4-benzoxazines **248**[91] (Scheme 62).

A 4-(*t*-butyloxycarbonyl)-piperazine moiety is introduced into position 8 of 8-bromo-3,4-dihydro-2*H*-1,4-benzoxazine **249** by aromatic nucleophilic substitution of bromine in **249** with 1-Boc-piperazine (**250**) using tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) and 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) as catalysts to produce **251** (Scheme 63).[22]

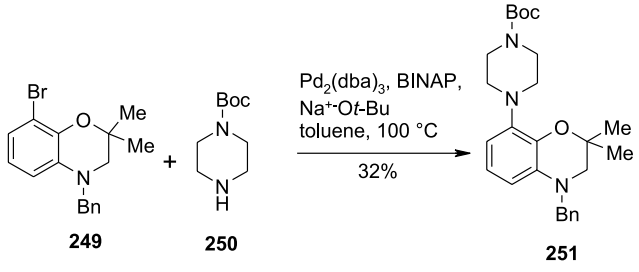
A pyridin-4-yl substituent is introduced into position 6 of 6-bromo-2*H*-1,4-benzoxazin-3-(4*H*)-one (**252**) by Suzuki coupling with pyridin-4-ylboronic acid (**253**) using tetrakis(triphenylphosphine)palladium(0) as catalyst, affording 8-pyridin-4-yl-2*H*-1,4-benzoxazin-3(4*H*)-one (**254**)[21] (Scheme 64).

6-Bromo-2,2,-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazine (**255**) is transformed to carbonitrile derivative **256** by refluxing **255** with copper(I) cyanide in *N*,*N*-dimethylformamide[28] (Scheme 65).

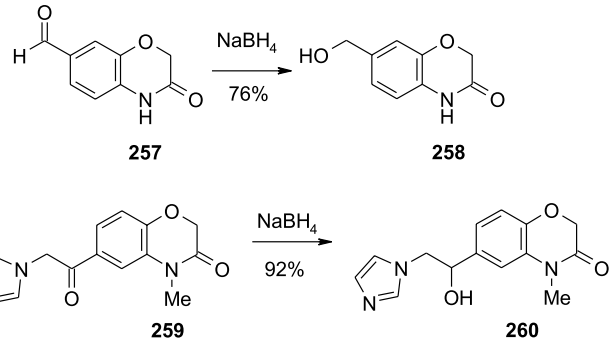


Scheme 65.

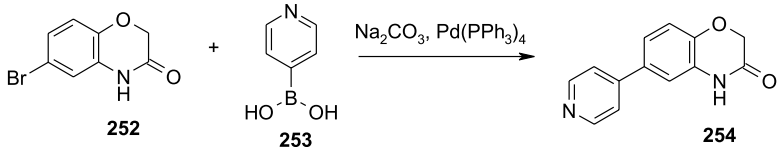
In 1,4-benzoxazinones, selective reductions of ester, ketone and aldehyde substituents can be performed without concomitant reduction of the lactam moiety. Thus, aldehyde **257** is efficiently reduced to the corresponding benzylic alcohol **258**[25] and ketone **259** is effectively reduced to the



Scheme 63.



Scheme 66.



Scheme 64.

secondary alcohol **260**[79] with sodium borohydride in methanol (Scheme 66).

6. Conclusions

This review covers the achievement in the synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines in the last 9 years. New palladium-catalyzed intramolecular etherification and nickel-mediated intramolecular amination made possible application of other starting compounds than classically used 2-aminophenols allowing different functionalization. There was a substantial progress made in the stereo controlled synthesis of 2-substituted 1,4-benzoxazines and 1,4-benzoxazinons. New synthetic techniques, for example, solid state synthesis and use of microwave irradiation also proved to be useful in the synthesis of 2*H*-1,4-benzoxazin-3-(4*H*)-ones and 3,4-dihydro-2*H*-1,4-benzoxazines. Main progress was also made in the application of 1,4-benzoxazines and 1,4-benzoxazinons as central scaffolds for designed biologically active compounds. 1,4-Benzoxazines and 1,4-benzoxazinones are easily accessible and functionalized heterocycles whose application in the synthesis of biologically active compounds will rise with the increasing demand for peptide mimetics.

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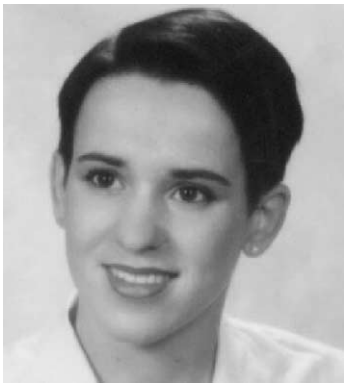
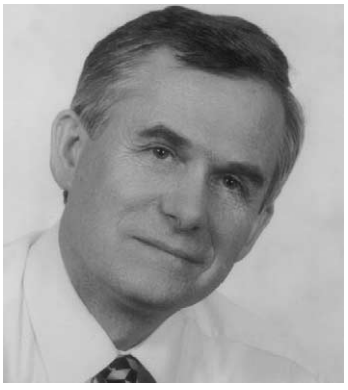
Biographical sketch

Janez Ilaš was born in 1980 in Novo Mesto, Slovenia. He graduated at the University of Ljubljana, Faculty of Pharmacy. In 2003 he joined the group of Professor D. Kikelj where he is currently working on his PhD thesis in the field of design and synthesis of new antithrombotic compounds. His research interests include medicinal chemistry, combinatorial chemistry, peptidomimetics and molecular modeling.

Assoc. Prof. Dr. Marija Sollner Dolenc completed her doctoral studies at the University of Ljubljana. Since 1987, she is a member of the Medicinal Chemistry Department at the Faculty of Pharmacy, University of Ljubljana. She has been conducting research on design and synthesis of compounds with influence on immune system and integrins. The emphasis of her work is on the development of new synthetic methods for preparation peptidomimetics with the same activity as endogenous bioactive peptides.



Petra Štefanič Anderluh was born in 1975 in Ljubljana, Slovenia. She studied at the Faculty of Chemistry and Chemical Technology and at the Faculty of Pharmacy at the University of Ljubljana. She received the BSc degree in Chemistry in 1998 and the Master of Pharmacy degree in 2000 followed by the PhD degree at the Faculty of Pharmacy in 2003 under the mentorship of Professor D. Kikelj in the field of design and synthesis of novel antithrombotics. In 2003, she moved to Drug Discovery Unit in Lek Pharmaceuticals, Ljubljana, Slovenia, where she works on development of new antibacterial agents. Her research interests include medicinal chemistry, heterocyclic chemistry, peptidomimetics, parallel chemistry and microwave-assisted chemistry.



Danijel Kikelj received his Master of Pharmacy Degree in 1978 and a Master of Science Degree in 1983 from the University of Ljubljana, Slovenia. He received his PhD in 1988 from the University of Heidelberg (Germany) under supervision of Professor R. Neidlein in the field of heterocyclic chemistry. After returning to the Faculty of Pharmacy, University of Ljubljana he took the position of an Assistant Professor of pharmaceutical chemistry in 1990. He was promoted to Associate Professor in 1995 and to Full Professor of pharmaceutical chemistry in 2000. From 1991 till 1992, he was a Professor of pharmaceutical chemistry at the Faculty of Pharmacy of the Heidelberg University. He is author of a chapter on quinazolines and co-author of a chapter on thiazoles in *Houben Weyl—Science of Synthesis*. His current research interests focus on the design and synthesis of peptidomimetics, stereoselective synthesis and heterocyclic chemistry.

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Application of ^1J(C,H) coupling constants in conformational analysis

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Abstract—Conformational equilibria for a number of methyl substituted 1,3-dioxanes **1**, 1,3-oxathianes **2** and 1,3-dithianes **3** were calculated at the HF and DFT levels of theory. In addition to the chair conformers also the energetically adjacent twist conformers were considered and the positions of the corresponding conformational equilibria estimated. On the basis of the global energy minima of conformers, participating in the conformational equilibria, the $^1J_{C,Hax,equ}$ coupling constants were calculated using the GIAO method and compared with the experimental values obtained from $^{13}C,^1H$ coupled ^{13}C NMR spectra. The Perlin effect, the influence of the solvent and the suitability of this NMR parameter for assigning the conformational equilibria present are critically discussed.

1. Introduction

The C,H coupling constant $^1J_{C,H}$ is not very widely applied in structural chemistry. In textbooks, the dependence of $^1J_{C,H}$ on hybridization of the carbon atom and the inductive as well as the heavy atom effects of substituents attached to the carbon atom are mentioned.[1] In addition, the linear dependence of $^1J_{C,H}$ on increasing heteroatom substitution[2] was observed and the dependence of this NMR parameter on the size of alicyclic and heterocyclic rings was employed successfully to assign CH fragments of 5/6-membered acetals[3] and epoxides.[4] The conformational dependence of $^1J_{C,H}$ coupling constants was used also to study the structure of 4,6-dioxo-1,3-dioxanes,[5] to indicate overall structural change of heparin derivatives due to O/N sulfuration/acetylation,[6] to employ them as a function of ring geometry to provide another tool in conformational analysis of furanose ring systems[7] and to indicate the stereochemistry at the anomeric center of 2,3-*O*-anhydro-furanosides.[8]

On the other hand, the different values of the $^1J_{C,H}$ coupling constants of axial and equatorial protons to the same adjacent carbon atom in cyclohexane and its hetero analogs (Perlin effect) have been the topic of a large number of publications and the source of the difference has been strongly and controversely discussed since the seventies of last century. Perlin and Casu[9] were the first to discuss this effect: the value of the coupling to the equatorial proton proved to be 8–10 Hz larger and the difference was interpreted as the effect of an $n\rightarrow\sigma^*$ stereoelectronic interaction of the oxygen lone pairs and the axial antiperiplanar C–H bond.[10] Since that time, the magnitude of $^1J_{C,H}$ in pyranosyl rings was employed as a reliable indicator of the stereochemistry at the anomeric centre[7] until Bailey et al.[11] in 1,3-dithiane and Juaristi and Cuevas[12,13] in a number of 1,3-dithianes observed the opposite sequence of $^1J_{C,H}$ coupling and concluded that factors other than $n\rightarrow\sigma^*$ stereoelectronic interaction are responsible for the Perlin effect. Beside the inductive effects of substituents and ring heteroatoms[13] the balance of a number of stereoelectronic interactions was assigned to be responsible for the Perlin/reversed Perlin effect.[13–15] Finally, the corresponding C–H bond length, depending on various stereoelectronic influences [$n\rightarrow\sigma_{C-H}^*$, $\sigma_{C-H}\rightarrow\sigma_{C-H}^*$, $\sigma_{C-C}\rightarrow\sigma_{C-H}^*$, $\sigma_{C-X}\rightarrow\sigma_{C-H}^*$ (X=O,N,S)][16,17] was postulated to be responsible for the size of the $^1J_{C,H}$ coupling constants (consistent with the Fermi contact term in $^1J_{C,H}$ being inversely dependent on the distance between the coupled nuclei).[18–20]

The $^1J_{C,H}$ coupling constant was also found to depend (in addition to changes in stereochemical interactions and molecular geometry) on the torsional angle of the methoxy group in methyl-α- and β-D-xylopyranoside[21,22] or the corresponding twist in oligosaccharides and on the solvent

Keywords: $^1J_{C,Hax,equ}$ Coupling constants; Conformational analysis; NMR; 1,3-Dioxanes; 1,3-Oxathianes; 1,3-Dithianes; Theoretical calculations; GIAO; Perlin effect.
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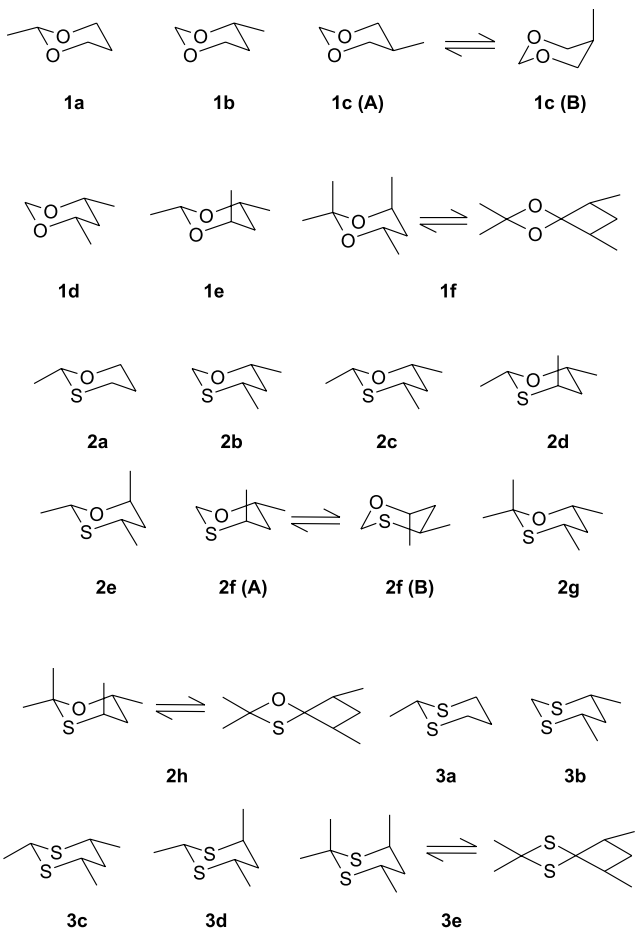
dielectric constant;[23a,b] the dihedral angle dependence was corroborated by FPT INDO semiempirical quantum chemical calculations[23a,b] and, later, by ab initio DFT calculations:[21,23] calculated and experimentally determined values being in fair agreement.

The $^{1}J_{C,C}$ coupling constant, on the other hand, proved to be dependent on the conformation/configuration of epimeric piperidine N-oxides and of their structural analogs,[24] but do not provide any experimental evidence for hyperconjugation in the ground state.[25]

The NMR parameters, chemical shift δ and coupling constant J, can be accurately calculated nowadays; DFT methods were especially successful in that respect and especially in case of chemical shifts of light nuclei like ^{1}H, ^{13}C or ^{15}N such calculations can attain 'chemical accuracy'. For instance, one of us and his co-workers used calculated vicinal H,H coupling constants, $^{3}J_{H,H}$, of conformers for determining conformational equilibria being still fast on the NMR time scale.[26] Except the $^{3}J_{H,H}$ in Ref. 26, calculated coupling constants have not yet been applied to conformational analysis. It is the major objective of this paper to test the corresponding possibilities of the $^{1}J_{C,H}$ coupling. Therefore, a collection of differently methyl substituted 1,3-dioxanes **1**, 1,3-oxathianes, **2** and 1,3-dithianes **3**, shown in Scheme 1, have been selected for this study.



Scheme 1. Anancomeric conformers and conformational equilibria of the compounds studied.

2. Results and discussion

2.1. Conformational analysis of 1–3

Proton coupled ^{13}C NMR spectra of the saturated six-membered heterocyclic compounds **1–3** were taken and the $^{1}J_{C,H}$ coupling constants extracted (Table 1). All of the conformational equilibria of the compounds studied have already been investigated earlier. In the cases of the methyl substituted 1,3-dioxanes **1a–d**,[27–29] 1,3-oxa-thianes **2a–g**[30] and 1,3-dithianes **3a–d**[31–36] chair conformers having the methyl substituents in the sterically favoured equatorial position (**1a–d**, **2a–c**, **3a–c**) proved to be strongly preferred; in case of **1c** both chair conformers contribute to the conformational equilibrium.[27–29] In the case of *trans*−4,6-dimethyl-1,3-oxathiane (**2f**) the two non-identical chair conformations 4eq6ax (88%) and 4ax6eg (12%) also interconvert fast on the NMR time scale (at room temperature where ^{13}C NMR spectra were obtained) as shown earlier by vicinal H,H coupling constants and chemical equilibration.[30,32] Finally, in the case of 2,2,4,6-tetramethyl substituted 1,3-dioxane **1f**, 1,3-oxathiane **2h** and 1,3-dithiane **3e**, 2,5-twist conformers with isoclinal methyls at C-2 and pseudoequatorial methyls at C-4,6 have been shown experimentally to clearly predominate in comparison with the chair forms with *syn*-diaxial substitution at C-2 and C-4 or C-6.[1c,27–30]

First, the structures shown in Scheme 1 were calculated employing HF and DFT ab initio quantum mechanical methods. Usually, the conformational equilibria of the heterocyclic compounds studied favour greatly one chair conformation, in other words they are anancomeric;[1c,27–36] therefore, the inverted chair conformers with majority of their methyl substituents in axial positions (with the exception of **1c** and **2f**) were not included in the calculations. In three cases, namely **1f**, **2h** and **3e**, however, the calculations showed that twist conformers are more or at least equally stable than the corresponding chair forms in moderate agreement with the experimental findings which showed that the former clearly predominate.[1c,27–36]

The results of the calculations are given in Table 2. The free energy differences of the conformers are of manifold interest: (i) The calculated free energy differences for the two chair conformers of **1c** and **2f** are close to the experimental findings. (ii) In case of **1a–e**, **2a–c** and **3a–d** the twist conformers proved to be over 4 kcal mol^{-1} less stable than the corresponding chair forms—confirming the chair stereochemistry of these compounds. (iii) In the case of *trans*-4,6-diMe (**2f**), *r*-2,*t*-4,*c*-6- (**2d**) and *r*-2,*c*-4,*t*-6-triMe (**2e**), and 2,2-*cis*-4,6-tetraMe (**2g**) 1,3-oxathianes the chair-twist energy difference was found to be smaller (<3 kcal/mol), however, still high enough to make these compounds to attain >99% of the chair forms in solution. (iv) Finally, for the 2,2,4,6-tetraMe-substituted compounds **1f**, **2h** and **3e**, the 2,5-twist conformation was calculated to be more or at least equally stable than the chair in agreement with the experimental findings which, however, show that in fact the former clearly dominate in these cases.[1c,27–30,34]

Table 1. Experimental $^{13}C,^{1}H$-coupling constants $^{1}J_{C,H}$/Hz

Compound	C-2, H-2equ	C-2, H-2ax	C-4, H-4equ	C-4, H-4ax	C-5, H-5equ	C-5, H-5ax	C-6, H-6equ	C-6, H-6ax	Methyl
1a	—	159.3	146.4	140.8	126.8	129.7	146.4	140.8	126.9
1b	167.5	157.2	—	139.2	125.5	129.3	146.4	140.9	126.2
1c	166.7	159.1	145.0	140.9	—	130.5	145.0	140.9	126.0
1d	167.7	157.5	—	140.5	124.8	129.2	—	140.5	126.3
1e	—	159.5	—	140.4	125.5	129.0	149.2	—	125.6 (6), 126.2 (4)
1f	—	—		143.3	126.3	126.3		143.3	126.2 (2), 126.0 (4), 126.0 (6)
2a	—	157.5	139.8	139.8	125.4	131.2	147.3	138.3	128.0
2b	156.6	156.6	—	141.9	124.4	129.6	—	140.6	127.3 (4), 127.0 (6)
2c	—	157.6	—	139.5	124.5	129.2	—	139.8	128.0 (2), 126.2 (6), 127.5 (4)
2d	—	157.9	139.6	—	124.7	129.3	—	139.2	128.2 (2), 126.3 (6), 126.7 (4)
2e	—	158.3	—	139.2	124.9	129.2	146.9	—	120.0 (2), 125.6 (6), 127.4 (4)
2f	157.2	157.2	139.9	—	125.2	129.2	—	140.3	126.3 (6), 127.0 (4)
2g	—	—	—	138.5	124.6	129.1	—	139.2	126.1 (6), 127.0 (2a), 127.8 (2e), 127.4 (4)
2h	—	—		138.5	126.0	127.5		141.4	125.4 (6), 127.0 (2a), 127.0 (4), 127.8 (2e)
3a	—	154.3	137.9	137.9	126.4	131.9	137.9	137.9	129.3
3b	145.0	154.1	—	138.7	123.6	131.2	—	138.7	127.7
3c	—	154.6	—	138.5	124.2	130.2	—	138.5	127.7 (4), 127.7 (6), 129.7 (2)
3d	—	154.6	—	139.4	124.9	130.7	136.7	—	129.6 (2), 127.0 (6), 128.0 (4)
3e	—	—		138.9	127.4	127.4		138.9	127.3 (4), 127.3 (6), 128.6 (2)

The solvent effect ($CDCl_3$) was not considered in the energy calculations. Although the calculated energy differences (Table 2) in favour of the preferred conformers are large enough to allow the statements given above as to the position of the conformational equilibria of **1–3**, the solvation energies for both the chair and twist conformers of compounds **1f**, **2h** and **3e** were calculated as an example employing the SCIPCM method;[37] the results are collected in Table 3. Even if the solvation energy of the chair conformer is somewhat higher (from 0.18–0.46 kcal/mol) dramatical changes could not be observed and the conclusions based on the previous calculations in the gas state remain the same.

Since the calculations support nicely experimentally verified conformational equilibria of **1–3**, we can proceed and estimate the values of the $^{1}J_{C,H}$ coupling constants in order to compare them with the experimental ones and discuss the applicability of this NMR parameter as a tool in conformational analysis of substituted saturated six-membered rings.

2.2. Ab initio DFT calculation of the $^{1}J_{C,H}$ coupling constants of 1–3

The $^{1}J_{C,H}$ coupling constants were calculated paying particular attention to the various contributions to the $^{1}J_{C,H}$ spin–spin couplings studied. According to the theory, spin–spin coupling arises from several electron–nuclear interactions: the Fermi-contact term (FC), the paramagnetic (PSO) and the diamagnetic spin–orbit terms (DSO) and, finally, the spin-dipole term (SD);[17] more details and the calculation of these terms have been reviewed.[38,39] Various correlative methods for the calculation of spin–spin couplings have been developed; in the case of **1–3** we applied the ADF computer program.[40] As an example, different contributions on the calculated $^{1}J_{C,H}$ the 2,5-twist conformer of the 1,3-dithiane **3a** are given in Table 4. For light nuclei like carbon and hydrogen, the Fermi contact term is often the most important electron–nuclear interaction; the paramagnetic and the diamagnetic spin–orbit terms tend to cancel each other and the spin-dipole term is often negligible.[26] This general behaviour was found also for the compounds studied (cf. Table 4): the C,H coupling is

Table 2. Energies of preferred conformers of substituted 1,3-diheterocyclohexanes (cf. Scheme 1) as calculated ab initio at the HF/6-311G** and B3LYP/6-311G** level of theory (absolute energies cf. Supplementary data)

Compound	Hetero atoms in position		Methyls on chair conformers			Chair–twist, HF/6-311G** in kcal/mol	Chair–twist, B3LYP/6-311G** in kcal/mol
	1	3					
1a	O	O	2e			5.42	5.75
1b	O	O		4e		4.87	5.42
1c (**A**)	O	O		5e		4.90[a]	5.41[b]
1c (**B**)	O	O		5a		4.21	4.79
1d	O	O		4e	6e	7.18	7.53
1e	O	O	2e	4e	6a	5.47	5.52
1f	O	O	2a,e	4a	6e	−2.60	−2.20
2a	O	S	2e			4.75	4.52
2b	O	S		4e	6e	6.31	6.22
2c	O	S	2e	4e	6e	6.63	6.20
2d	O	S	2e	4a	6e	2.84	2.59
2e	O	S	2e	4e	6a	2.42	2.45
2f (**A**)	O	S		4a	6e	2.67[c]	2.81[d]
2f (**B**)	O	S		4e	6a	3.01	2.98
2g	O	S	2a,e	4e	6e	5.20	4.69
2h	O	S	2a,e	4a	6e	−0.53	−0.32
3a	S	S	2e			4.54	4.24
3b	S	S		4e	6e	6.76	6.43
3c	S	S	2e	4e	6e	7.69	7.12
3d	S	S	2e	4a	6e	8.50	7.69
3e	S	S	2a,e	4a	6e	−0.56	−0.32

[a] $\Delta G°(\mathbf{A}\text{-}\mathbf{B}) = -0.69$ kcal mol^{-1}.
[b] $\Delta G°(\mathbf{A}\text{-}\mathbf{B}) = -0.62$ kcal mol^{-1}.
[c] $\Delta G°(\mathbf{A}\text{-}\mathbf{B}) = -1.59$ kcal mol^{-1}.
[d] $\Delta G°(\mathbf{A}\text{-}\mathbf{B}) = -1.35$ kcal mol^{-1}.

Table 3. Energies of **1f**, **2h** and **3e** when considering the solvent effect on the SCRF/SCIPCM//B3LYP/6-311G** level of theory [dielectric constant 4.8 (chloroform)]

Compound	SCIPCM—difference chair–twist in kcal/mol	Solvation energy	
		Chair in kcal/mol	Twist in kcal/mol
1f	−1.95	1.68	1.44
2h	−0.14	1.50	1.32
3e	0.14	1.58	1.12

dominated by the Fermi contact contribution (ca. 98%); both the diamagnetic and paramagnetic orbital contributions proved to be positive and increased the calculated coupling constants by about 1.5–2%. The spin dipole term was found to be negligible. Thus, when calculating of $^{1}J_{C,H}$ coupling constants for this kind of organic compounds, the Fermi contact term can describe sufficiently the structural (configuration, conformation) and electronic influences on this NMR parameter.

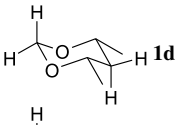
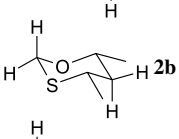
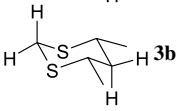
On the other hand, the inverse dependence of the Fermi contact term in $^{1}J_{C,H}$ on the distance between the two coupling nuclei was not adequately realized. Actually, the

Table 4. Calculated C,H-coupling constants of **3a** ($^{1}J_{C,H}$/Hz) separated into the various contributing electron–nuclear interactions

Partition in $^{1}J_{C,H}$	C-2, H-2ax	C-4, H-4ax	C-4, H-4equ	C-5, H-5ax	C-5, H-5equ	C-6, H-6ax	C-6, H-6equ	Methyl[a]
Diamagnetic orbital contribution	1.3	1.0	1.1	1.0	1.0	1.0	1.1	0.8
Paramagnetic orbital contribution	0.5	1.1	1.0	1.4	1.4	1.1	1.0	1.6
Fermi-contact + spin-dipolar contribution	150.7	132.0	141.9	122.4	122.9	131.7	141.8	124.0
Fermi-contact contribution	150.4	131.7	141.6	122.3	122.7	131.4	141.6	123.8
Total calculated spin–spin coupling	152.5	134.1	143.9	124.8	125.2	133.8	143.9	126.4
C–H distance[b]	1.080	1.079	1.078	1.083	1.083	1.079	1.078	1.080

[a] Average of three C,H-coupling constants in the calculated conformation.
[b] Distances in Angstroms Å.

Table 5. Difference in the C,H-coupling constants $^{1}J_{C,H}$/Hz of C-2 and C-5 in **1d**, **2b** and **3b** (Perlin effect)

Compound		C-2			C-5		
		$^{1}J_{C\text{-}2,Hax}$	$^{1}J_{C\text{-}2,Heq}$	Δ^{a}	$^{1}J_{C\text{-}5,Hax}$	$^{1}J_{C\text{-}5,Heq}$	Δ^{a}
 **1d**	Obsd	157.5	167.7	10.2	129.2	124.8	−4.4
	Calcd	151.6	162.7	11.1	129.3	122.8	−6.4
 **2b**	Obsd	156.6	156.6	0.0	129.6	124.4	−5.2
	Calcd	153.6	158.3	4.7	129.6	122.8	−6.8
 **3b**	Obsd	154.1	145.0	−9.1	131.2	123.6	−7.6
	Calcd	157.7	143.8	−15.9	133.8	121.0	−12.8

[a] Perlin effect: $^{1}J_{C,Hequ} - {}^{1}J_{C,Hax}$.

bond length variation as calculated with respect to $^{1}J_{C,H}$ variations is too small to be correlated. However, there is a qualitative agreement since the smallest values for $^{1}J_{C,H}$ obtained experimentally or calculated theoretically, belong to the largest C,H bond distances (1.083 Å—124–125 Hz). No further differentiation was possible when the couplings get larger (1.078–1.080 Å—134–153 Hz).

2.3. The Perlin/reversed Perlin effect.[9–17]

The Perlin effect $^{1}J_{C,Hequ} > {}^{1}J_{C,Hax}$ has been used for a long time as a reliable indication of the stereochemistry at the anomeric centre of carbohydrates. This situation is correctly reproduced in the present calculations: in 4,6-diMe-1,3-dioxane **1d** the experimental result for C-2 is excellently in

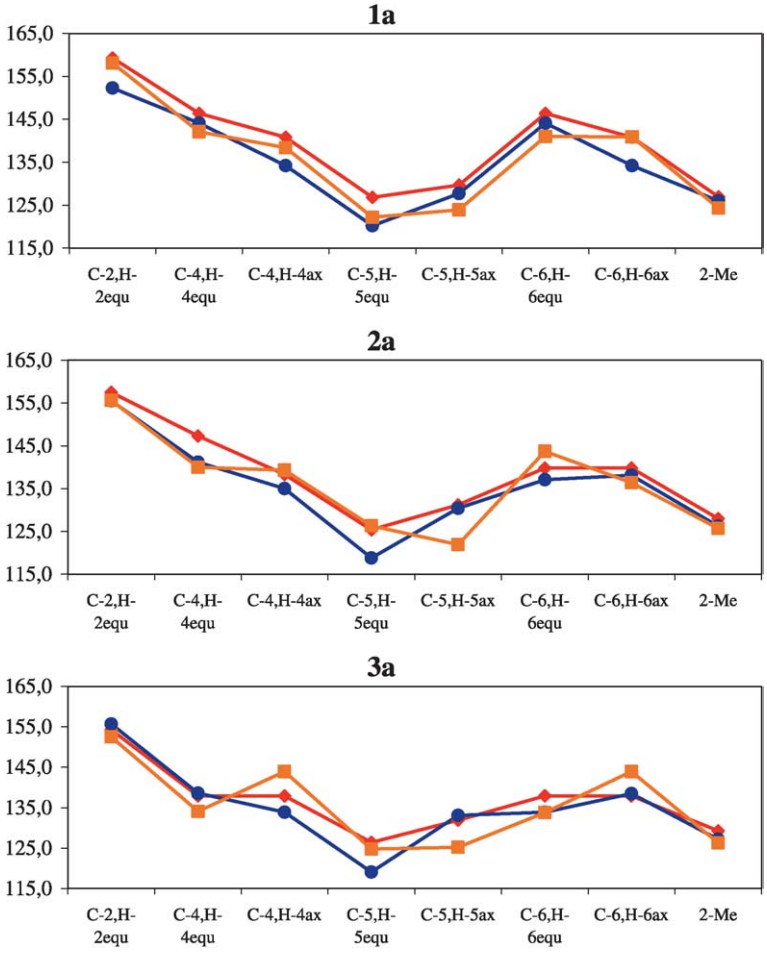


Figure 1. Comparison of experimental (◇) and theoretically calculated $^{1}J_{C,H}$/Hz values of chair (●) and twist (□) conformer in compounds **1a**, **2a** and **3a**.

Table 6. Experimental and calculated C,H-coupling constants $^1J_{C,H}$/Hz and their correlation coefficients $^1J_{C,H}$(exp.) versus $^1J_{C,H}$(calcd)

1a			**1b**			**1c**					**1d**		
$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair (80%)[a]	Chair (20%)[b]	Chair (80/20)	$^1J_{C,H}$	Exp.	Chair
C-2, H-2ax	159.3	152.3	C-2, H-2equ	167.5	163.1	C-2, H-2equ	166.7	163.5	163.4	163.5	C-2, H-2equ	167.7	162.7
C-4, H-4equ	146.4	144.1	C-2, H-2ax	157.2	151.7	C-2, H-2ax	159.1	151.7	151.0	151.6	C-2, H-2ax	157.5	151.6
C-4, H-4ax	140.8	134.2	C-4, H-4ax	139.2	133.9	C-4, H-4equ	145.0	145.2	146.2	145.4	C-4, H-4ax	140.5	134.6
C-5, H-5equ	126.8	120.2	C-5, H-5equ	125.5	121.7	C-4, H-4ax	140.9	135.3	133.2	134.9	C-5, H-5equ	124.8	122.9
C-5, H-5ax	129.7	127.7	C-5, H-5ax	129.3	128.6	C-5, H-5ax	130.5	126.6	118.7	125.0	C-5, H-5ax	129.2	129.3
C-6, H-6equ	146.4	144.1	C-6, H-6equ	146.4	144.0	C-6, H-6equ	145.0	145.2	146.2	145.4	C-6, H-6ax	140.5	134.6
C-6, H-6ax	140.8	134.2	C-6, H-6ax	140.9	135.2	C-6, H-6ax	140.9	135.3	133.2	134.9	Methyl (4)	126.3	124.6
Methyl (2)	126.9	126.0	Methyl (4)	126.2	124.6	Methyl (5)	126.0	123.1	124.1	123.3	Methyl (6)	126.3	124.6
		0.97344[c]			0.99332			0.97938	0.94779	0.97532			0.99317

1e			**1f**				**2a**			**2b**		
$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	Twist	$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair
C-2, H-2ax	159.7	153.6	C-4, H-4equ	143.3	135.1	138.7	C-2, H-2ax	157.5	155.5	C-2, H-2equ	156.6	158.3
C-4, H-4ax	140.4	134.6	C-5, H-5equ	126.3	126.5	124.6	C-4, H-4equ	147.3	141.2	C-2, H-2ax	156.6	153.6
C-5, H-5equ	125.0	124.8	C-5, H-5ax	126.3	125.3	124.6	C-4, H-4ax	138.3	135.0	C-4, H-4ax	141.9	139.1
C-5, H-5ax	129.0	126.7	C-6, H-6ax	143.3	139.4	138.8	C-5, H-5equ	125.4	118.8	C-5, H-5equ	124.4	122.8
C-6, H-6equ	149.2	140.4	Methyl (2eq)	126.2	125.8	124.4	C-5, H-5ax	131.2	130.4	C-5, H-5ax	129.6	129.5
Methyl (2)	126.2	125.5	Methyl (2ax)	126.2	123.7	124.4	C-6, H-6equ	139.8	137.1	C-6, H-6ax	140.6	137.5
Methyl (6)	125.6	124.5	Methyl (6)	126.0	124.5	124.1	C-6, H-6ax	139.8	138.1	Methyl (4)	127.3	125.3
Methyl (4)	126.2	122.7	Methyl (4)	126.0	122.6	124.1	Methyl (2)	128.0	126.2	Methyl (6)	127.0	123.0
		0.98808			0.96294	0.99989			0.98097			0.99144

2c			**2d**			**2f**				
$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair (88%)[a]	Chair (12%)[b]	Chair (88/12)
C-2, H-2ax	157.6	155.5	C-2, H-2ax	157.9	155.5	C-2, H-2equ	157.2	154.4	153.6	153.7
C-4, H-4ax	139.5	135.1	C-4, H-4ax	139.6	135.1	2-2, H-2ax	157.2	156.6	158.3	158.1
C-5, H-5equ	124.5	121.4	C-5, H-5equ	124.7	123.4	C-4, H-4equ	139.9	135.2	139.1	138.6
C-5, H-5ax	129.2	131.7	C-5, H-5ax	129.3	129.3	C-5, H-5equ	125.2	123.2	122.8	122.8
C-6, H-6ax	139.8	137.6	C-6, H-6equ	139.2	134.7	C-5, H-5ax	129.2	129.3	129.5	129.5
Methyl (2)	128.0	125.9	Methyl (2)	128.2	126.2	C-6, H-6ax	140.3	135.5	137.5	137.3
Methyl (6)	126.2	124.5	Methyl (6)	126.3	124.2	Methyl (4)	127.0	124.5	123.0	123.2
Methyl (4)	127.5	125.3	Methyl (4)	126.7	124.3	Methyl (6)	126.3	124.1	125.3	125.2
		0.98422			0.99165			0.99160	0.99116	0.99203

2e			**2g**			**2h**				**3a**		
$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	Twist	$^1J_{C,H}$	Exp.	Chair
2-2, H-2ax	158.3	157.3	C-4, H-4ax	138.5	137.2	C-4, H-4ax	138.5	136.6	140.6	C-2, H-2ax	154.3	155.7
C-4, H-4equ	139.2	137.4	C-5, H-5equ	124.6	121.5	C-5, H-5equ	126.0	124.1	124.7	C-4, H-4equ	137.9	138.6
C-5, H-5equ	124.9	122.9	C-5, H-5ax	129.1	131.7	C-5, H-5ax	127.5	128.7	125.6	C-4, H-4ax	137.9	133.9
C-5, H-5ax	129.2	129.4	C-6, H-6ax	139.2	136.2	C-6, H-6equ	141.4	134.1	140.3	C-5, H-5equ	126.4	119.1
C-6, H-6ax	146.9	138.7	Methyl (2eq)	127.8	126.5	Methyl (2eq)	127.8	126.6	125.9	C-5, H-5ax	131.9	133.1

Table 6 (*continued*)

2e			**2g**			**2h**				**3a**		
$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	Twist	$^1J_{C,H}$	Exp.	Chair
Methyl (2)	128.0	126.2	Methyl (2ax)	127.0	125.1	Methyl (2ax)	127.0	125.1	125.1	C-6, H-6equ	137.9	133.9
Methyl (6)	125.6	123.0	Methyl (4)	127.4	125.4	Methyl (6)	125.4	124.4	124.2	C-6, H-6ax	137.9	138.5
Methyl (4)	127.4	125.3	Methyl (6)	126.1	124.5	Methyl (4)	127.0	123.9	125.1	Methyl (2)	129.3	127.2
		0.97889			0.95212			0.93133	0.98907			0.96650
3b			**3c**			**3d**				**3e**			
$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair	$^1J_{C,H}$	Exp.	Chair		$^1J_{C,H}$	Exp.	Chair	Twist
C-2, H-2equ	145.0	143.8	C-2, H-2ax	154.6	156.1	C-2, H-2ax	154.6	156.0		C-4, H-4equ	138.9	137.8	141.9
C-2, H-2ax	154.1	157.7	C-4, H-4ax	138.5	137.89	C-4, H-4ax	139.4	137.8		C-5, H-5equ	127.4	121.0	126.1
C-4, H-4ax	138.7	137.8	C-5, H-5equ	124.2	121.1	C-5, H-5equ	124.9	122.6		C-5, H-5ax	127.4	133.9	126.1
C-5, H-5equ	123.6	121.0	C-5, H-5ax	130.2	133.9	C-5, H-5ax	130.7	132.1		C-6, H-6ax	138.9	137.8	141.9
C-5, H-5ax	131.2	133.8	C-6, H-6ax	138.5	137.8	C-6, H-6equ	136.7	132.1		Methyl (2eq)	128.6	127.6	126.8
C-6, H-6ax	138.7	137.8	Methyl (2)	129.7	127.3	Methyl (2)	129.6	127.3		Methyl (2ax)	128.6	126.3	126.8
Methyl (4)	127.7	125.4	Methyl (6)	127.7	125.4	Methyl (4)	128.0	125.4		Methyl (6)	127.3	125.6	125.2
Methyl (6)	127.7	125.4	Methyl (4)	127.7	125.4	Methyl (6)	127.0	124.3		Methyl (4)	127.3	125.6	125.2
		0.98848			0.98092			0.98609				0.82363	0.99855

[a] Major conformer **A** (cf. Schemes 1 and 2).
[b] Minor conformer **B** (cf. Schemes 1 and 2).
[c] Correlation coefficient.

agreement with the calculations ($\Delta^1J_{C,H}=10.2$ Hz; 11.1 Hz calculated)—cf. Table 5. In the corresponding 1,3-oxathiane **2b** the two $^1J_{C,H}$ coupling constants approach each other and reverse the sequence in the corresponding 1,3-dithiane **3b** ($^1J_{C,Hequ}<{}^1J_{C,Hax}$)—reversed Perlin effect. This result is also well reproduced by the present calculations—hereby, the difference between the two coupling constants is calculated to be a few Hz larger (4.7 and 6.8 Hz); due to this reason the calculated difference between the two $^1J_{C,H}$ coupling constants in **2b** is still positive ($\Delta=4.7$ Hz) while the two couplings were already experimentally identical. However, the experimental tendency in $^1J_{C,H}$, when comparing the three compounds in Table 5, is also well corroborated.

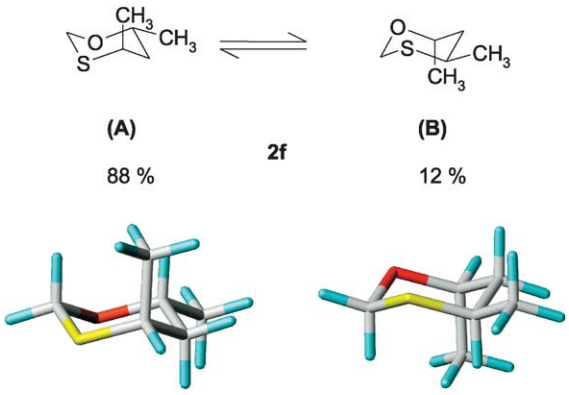
In case of C-5 the sequence $^1J_{C,Hequ}<{}^1J_{C,Hax}$ proved to be common, again well supported by the present calculations; also in this case the differences calculated theoretically for $^1J_{C,H}$ are larger than those of the experimental couplings. This is even enhanced in **3b** in comparison with **1d** and **2b**, obviously overestimating but correctly corroborating the experimental $^1J_{C,H}$ differences between axial and equatorial positions.

2.4. Conformational equilibria of 1–3 in the light of $^1J_{C,H}$ coupling constants calculated at ab initio DFT level of theory

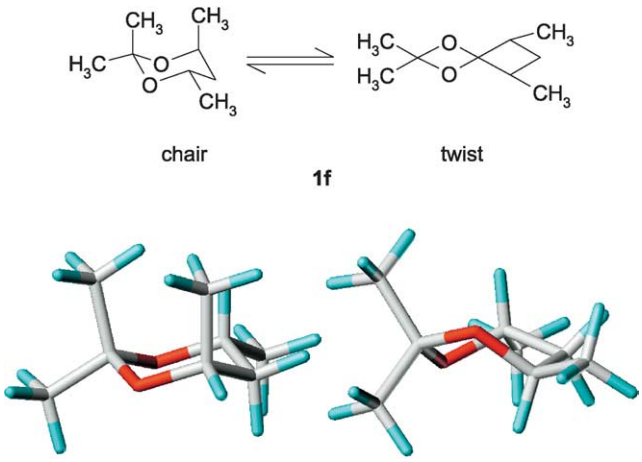
Experimental and theoretically calculated $^1J_{C,H}$ coupling constants are compared in Table 6. The results are of manifold interest:

(i) The calculated values are mostly somewhat lower than the experimental couplings in case of 1,3-dioxanes **1** and 1,3-oxathianes **2**; in case of 1,3-dithianes **3** the calculated couplings are more often larger than the experimental ones. In addition, the agreement between the experimental and theoretically calculated couplings is somewhat less in case of **3**. When considering the correlation coefficients $^1J_{C,H}$(exp.) versus $^1J_{C,H}$(calcd), the agreement appears to be excellent (esp. when differences between two related couplings are considered) to apply the calculated $^1J_{C,H}$ coupling constants to explain the relative, up to the absolute size of these NMR parameters at least in 6-membered saturated heterocyclic ring systems.

(ii) Even if the conformational status as to the preference of the chair conformers (**1a–e**, **2a–g**, **3a–d**) is already completely clear from the large energy differences as compared to the energetically nearest twist conformer, the comparison of the $^1J_{C,H}$ values as calculated for the twist conformers proved to be really discriminating: the agreement with the C,H couplings calculated for the chairs proved to be much better (cf. Table 6). The diagrams in Figure 1 for **1a**, **2a** and **3a** illustrate graphically the above observation: the relative sizes of $^1J_{C,Hax,equ}$ of C-6 (in **1a**), of C-4 and C-6 (in **2a**) and of C-4 and C-6 in **3a**, respectively, do not fit at all with the values calculated for the twist conformers. Thus, even if the energy difference between the chair and the energetically nearest twist conformer is not very large (cf. **2d–g**–vide supra), the calculated $^1J_{C,Hax,equ}$ prove to be an unequivocal indicator of the preferred chair conformation.



Scheme 2. Conformational equilibrium of **2f** still fast on the NMR time scale.



Scheme 3. Conformational equilibrium of **1f** still fast on the NMR time scale.

(iii) In case of the *trans*-4,6-dimethyl-1,3-oxathiane **2f** two chair conformers (cf. Scheme 2) participate in the conformational equilibrium (fast at ambient temperature on the NMR time scale); the energy difference calculated (cf. Table 2) prefers **2f**(**A**) as found previously.[33] The calculated C,H couplings in the two alternative chair conformers are not much different, but the better correlation coefficient exp. versus calcd $^{1}J_{C,Hax,equ}$ couplings (cf. Table 6) corroborate the experimental findings and hence the usefulness of the application of $^{1}J_{C,H}$ in a conformational sense. The same situation prevails for **1c**.

(iv) Finally the twist conformers of **1f**, **2h** and **3e** which have been proved earlier experimentally.[27–30,33,34] Based on the energy calculations (cf. Table 2) the 2,5-twist conformer should be >99% in **1f** (cf. Scheme 3) and also slightly

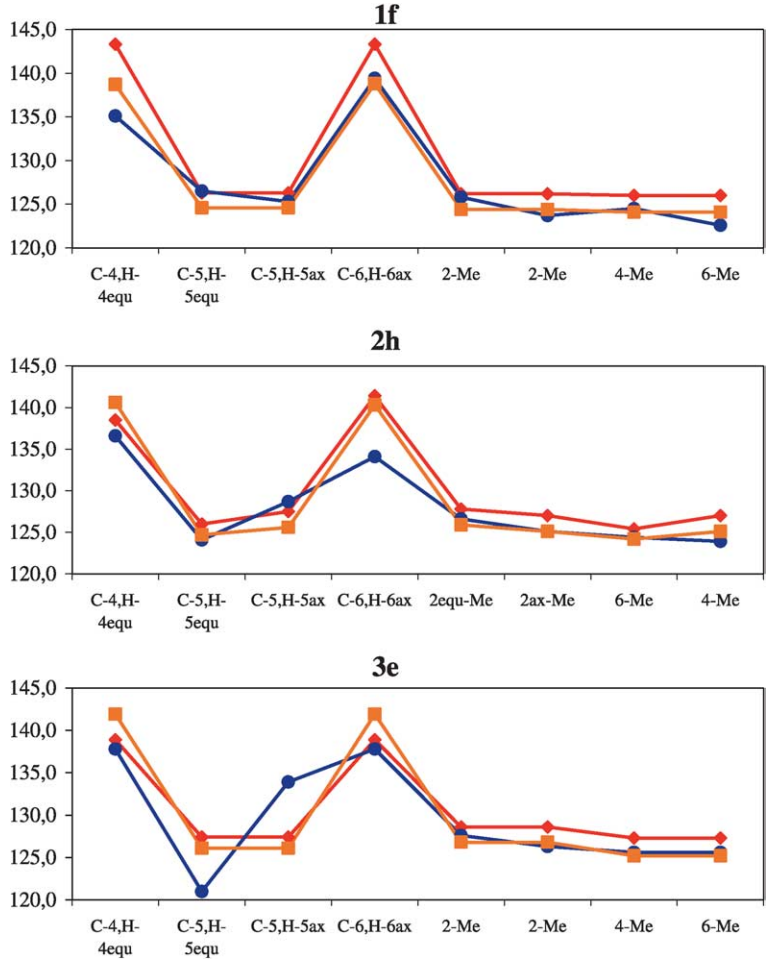


Figure 2. Comparison of experimental (◇) and theoretically calculated $^{1}J_{C,H}$/Hz values of both the chair (●) and twist (□) conformer in compounds **1f**, **2h** and **3e**.

favoured in respect of the corresponding chair conformers of **2h** and **3e**. These results are corroborated by the $^{1}J_{C,H}$ calculations: the correlation coefficient exp. versus calcd $^{1}J_{C,Hax,equ}$ couplings (cf. Table 6) being by far better when comparing to those calculated for the twist conformers, which in fact supports the experimental findings about the relatively high predominance of all three twist forms.[28,33,34] The diagrams in Figure 2 visualize the characteristic differences between the $^{1}J_{C,H}$ couplings in the chair and 2,5-twist conformers of **1f**, **2h** and **3e**. The two plots in each diagram are much closer to each other in case of the twist conformers; the $^{1}J_{C,Hax,equ}$ couplings for C-5 are most illustrative for the conformational status. While they are identical or almost identical for the twist conformers (both the calculated and experimental values), the two couplings calculated for the corresponding chair conformers are clearly different. The encouraging results on the presence of twist conformers in some of the conformational equilibria in question shows that the theoretical method employed fits very well in describing the behaviour of the $^{1}J_{C,H}$ couplings in the 2,5-twist conformers in the studied compounds.[27–30,33,34]

3. Conclusions

GIAO calculations, based on global energy minima of the preferred conformers of a number of methyl substituted 1,3-dioxanes **1**, 1,3-oxathianes **2** and 1,3-dithianes **3**, are very useful when estimating the C,H coupling constants in this kind of compounds. The agreement between calculated (dominated by the Fermi contact term) and experimental values of preferred conformer(s) proved to be in general very good; solvent effects are of minor significance only. The presence of the Perlin effect could be proved theoretically and the preferred conformers in the conformational equilibria identified by applying this NMR parameter and by comparing calculated values with the experimental ones even in the case of conformational equilibria being still fast on the NMR time scale. GIAO calculations appear to be useful in determining the $^{1}J_{C,H}$ coupling behaviour of the 2,5-twist conformers of the title compounds and thus in helping to identify these conformers in solution so confirming the conclusions based on ^{13}C NMR chemical shift correlations and thermochemical observations. Theoretically calculated $^{1}J_{C,Hax,equ}$ coupling constants thus seem to be very useful in conformational analysis of small organic compounds as it was reported previously for the $^{3}J_{H,H}$[26] and $^{3}J_{C,H}$ coupling analogs.[41]

4. Experimental

The samples studied were available from earlier work[28–30,32–34] or were prepared conventionally.[34c] The proton-coupled ^{13}C NMR spectra were recorded on a JEOL GX-400 NMR instrument for 0.2 M solutions in $CDCl_3$ (used as a field-frequency lock signal) at ambient temperature at 100.54 MHz. The measured $^{1}J_{C,H}$ values were independent of concentration of NMR solutions.

Ab-initio calculations were carried out with the GAUSSIAN 98 program[42] using the 6-311G** basis set[43] at the Hartree–Fock and the B3LYP[44] level of theory. Geometry optimization of all configurations were performed without constraints (x, y, z coordinates and absolute energies of the compounds studied are given in Supplementary data).

The SCRF/SCIPCM (self-consistent reaction field/self-consistent isodensity polarized continuum model)[37] method were used to consider the solvent effect; the dielectric constant of chloroform ($\varepsilon=4.8$) was applied.

The values of the coupling constants were calculated with the Amsterdam Density Functional (ADF)[40] program. The VWN+BLYP (Vosko–Wilk–Nusiar+Becke–Lee–Yang–Parr)[45] generalized gradient approximation (GGA) were used to determine the unperturbed molecular orbitals. All calculations were performed using a core double zeta, valence triple zeta, and double polarized basis (TZ2P) implemented in the ADF program.[40] All compounds were optimized without restrictions; the key word converge grad=0.0001 was used. All calculations were adopted without symmetry options.

The spin–spin coupling constants were calculated using Fermi-contact interactions including the spin-dipolar (FC-SD), the paramagnetic spin–orbit (PSO), and the diamagnetic spin–orbit (DSO) contributions. Calculations of only the Fermi-contact interactions were also performed.[46]

The quantum chemical calculation were processed on SGI Octane (R 12000) computers and a Linux cluster computer at Potsdam University.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.05.083

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A DFT study of the polar Diels–Alder reaction between 4-aza-6-nitrobenzofuroxan and cyclopentadiene

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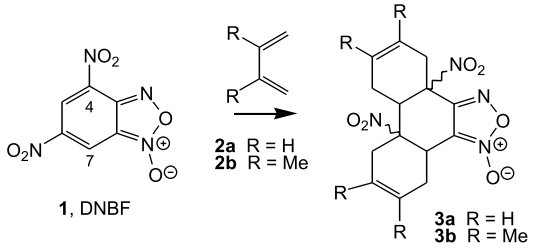
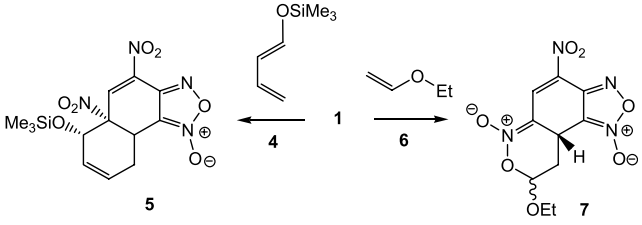
Abstract—The polar Diels–Alder reaction between 4-aza-6-nitrobenzofuroxan (ANBF) and cyclopentadiene has been studied using DFT procedures at the B3LYP/6-31G* level. Only one highly asynchronous transition state structure associated to the formation of the [4+2] adduct **13** is found. A further [3,3] sigmatropic shift on the [4+2] cycloadduct **13** allows its conversion into the thermodynamically more stable [2+4] cycloadduct **14**. The analysis of the global and local electrophilicities of the reagents correctly explain the behaviour of ANBF as a strong electrophile in polar cycloadditions.

1. Introduction

Nitrobenzofuroxans are compounds with a high susceptibility to undergo covalent nucleophilic addition or substitution processes with very weak nucleophiles, leading to numerous synthetic, biological, and analytical applications.[1–4] In 1973, Kresze and Bathelt[5] reported that the treatment of 4,6-dinitrobenzofuroxan **1**, DNBF, with 1,3-butadiene, **2a**, and 2,3-dimethylbutadiene, **2b**, affords the adducts **3a** and **3b**, respectively, (see Scheme 1). Formation of these compounds was accounted for in terms of normal-electron-demand (NED) Diels–Alder (DA) type reactions. The potential field of reactivity of nitrobenzofuroxans toward DA reactions has been explored by Terrier et al.[6,7] Thus, the reaction of **1** with 1-trimethylsilyloxybutadiene **4** proceeds with a high endostereoselectivity as well as a high regioselectivity to give nearly quantitatively the adduct **5** with no evidence for the subsequent formation of a diadduct of type **3** (see Scheme 2).[6] On the other hand, the reaction of **1** with ethyl vinyl ether **6** (2 equiv) produces a 4:1 mixture of the two epimeric adducts of **7** (see Scheme 2).[7]



Scheme 1.



Scheme 2.

Recently, these authors studied the reaction of DNBF, **1**, with an excess of cyclopentadiene **8**, Cp, to give the domino adduct **11** (see Scheme 3).[8] The reaction of DNBF with **8** leads to the initial formation of the adducts **9** and **10**, which is followed by the stereoselective formation of the highly functionalized compound **11**.[8] Experiments carried out under different temperature and concentration conditions indicated that the adducts **9** and **10** have a similar thermodynamic stability but the formation of **10** is kinetically more favored than that of **9**. Formation of the compound **11** implies that the addition of the second molecule of **8** is kinetically more favored at the remaining nitroalkene moiety of **9**.

Keywords: Polar Diels–Alder reactions; Benzofuroxans; Electrophilicity; Reaction mechanisms; DFT calculations.
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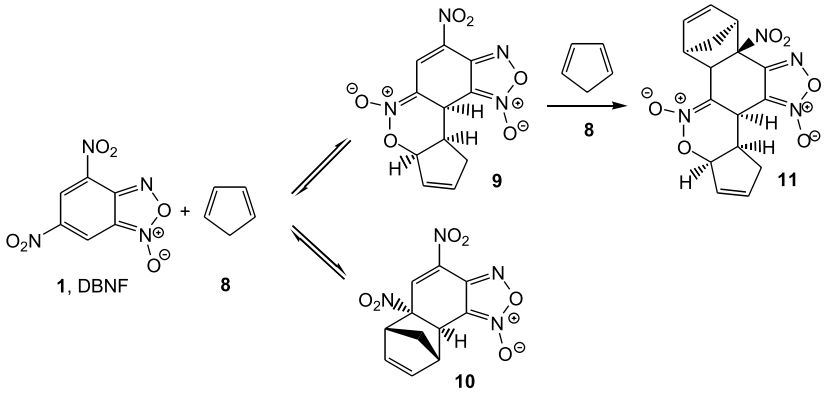


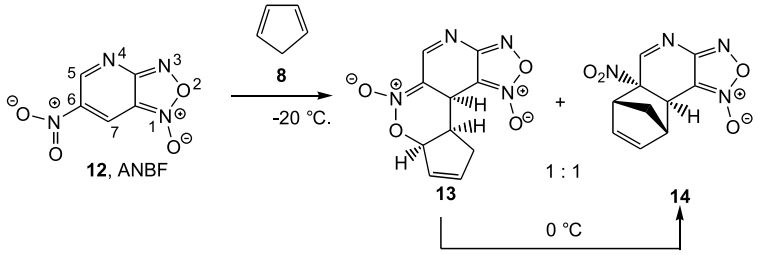
Scheme 3.

More recently, Terrier et al. studied the reactivity of 4-aza-6-nitrobenzofuroxan **12**, ANBF, as an aza analogue of DNBF.[9] The treatment of ANBF with a large excess of **8** in dichloromethane overnight at room temperature led to the formation of the cycloadduct **14** (see Scheme 4). Formation of the cycloadduct **14** takes place with a complete *endo* selectivity through the approach of the π-system of **8** over the nitro substituent of ANBF. When the reaction was initially carried out at −20 °C, the NMR spectra revealed the formation of the cycloadduct **13** together with that of **14**. Raising the temperature to 0 °C, the complete conversion of **13** into **14** was observed.[9] While **13** is the formal [4+2] cycloadduct of an inverse-electron-demand (IED) DA reaction of ANBF with **8**, **14** is the formal [2+4] cycloadduct of a NED DA reaction. The [*m*+*n*] notation indicates only the number of π electrons of ANBF, *m*, and **8**, *n*, that are involved in the formation of these formal cycloadducts.

Several experimental reports have appeared describing the use of simple or substituted alkenes in hetero-DA reactions with nitroalkenes as the heterodiene contributors.[10–15] The molecular mechanism of the DA reaction of nitroethylene toward substituted electron-rich ethylenes (propene, methyl vinyl ether, MVE, and dimethylvinylamine) has been studied computationally.[16] These reactions are highly asynchronous concerted processes, which can formally be viewed as induced by a nucleophilic attack of the electron-releasing substituted ethylene to the conjugated β-position of nitroethylene acting as the electrophilic center. In the transition state structure (TS), which exhibits a strong zwitterionic character, the related C–C bond formation process proceeds ahead of the second sigma bond process, that is the O–C bond, involved in the IED-DA cycloaddition. The feasibility of the reaction was related to the polar character of the TS; for instance, while for dimethylvinylamine, the more nucleophilic species of the series, the cycloaddition presents a very low activation energy, the reaction with MVE requires the presence of a Lewis acid coordinated to nitroalkene to make it feasible.[16]

The use of the global electrophilicity index,[17] ω, defined within the density functional theory (DFT)[18,19] was reported[20] to classify the dienes and dienophiles currently used in DA reactions on a unique scale of electrophilicity. A good correlation between the difference in electrophilicity for the diene and dienophile pair, $\Delta\omega$, and the feasibility of the cycloaddition was found.[20] For instance, the nitroethylene/propene, nitroethylene/MVE and nitroethylene/dimethylvinylamine cycloadditions present a $\Delta\omega$ of 2.01, 2.19 and 2.34 eV, respectively. The increase of the $\Delta\omega$ value with the large nucleophilic character of the substituted ethylene is in agreement with the decrease of the activation energy. Coordination of a Lewis acid to nitroalkene increases the $\Delta\omega$ for the nitroethylene–BH_3/MVE reaction to 3.91 eV, allowing the cycloaddition to take place along a more polar process.[21] In addition, the proposed local electrophilicity index, ω_k, has been also found to be a useful tool that correctly explains the regioselectivity of polar DA reactions.[22]

Recently, the reaction of DNBF with an excess of **8** reported by Terrier et al.[8] to give the domino adduct **11** has been theoretically studied (see Scheme 3).[23] The electrophilicity of DNBF is $\omega = 5.46$ eV.[23] This large value justifies its participation in a polar cycloaddition as a strong electrophile.[20] In addition, the large electrophilicity of the [4+2] cycloadduct **9**, 4.06 eV, accounts for its participation in the cycloaddition with a second molecule of **8** to give the adduct **11**. Interestingly, each of these cycloadditions takes place in a stepwise process characterized by the initial formation of a zwitterionic intermediate resulting from the nucleophilic



Scheme 4.

attack of **8** to the more electrophilic position of DNBF, that is the C7 position, or of the cycloadduct **9**, that is the C5 one.[23]

In this paper a theoretical study for the DA reactions between ANBF and Cp reported by Terrier et al.[9] has been carried out (see Scheme 4). The aim of this work is to understand the mechanism for the formation of the cycloadducts **13** and **14**, and in particular to determine whether ANBF behaves as a strong electrophile toward Cp in polar DA reactions. Firstly, the polar nature of this cycloaddition will be assessed using the reactivity indexes defined within the DFT theory. An extensive exploration of the potential energy surface (PES) for this cycloaddition will be performed in order to understand the mechanism for the formation of the cycloadducts **13** and **14**. Finally, the different behavior of the ANBF system compared with that of the DNBF one will be discussed.

2. Computational methods

DFT calculations have been carried out using the B3LYP[24,25] exchange-correlation functionals, together with the standard 6-31G* basis set.[26] The optimizations were carried out using the Berny analytical gradient optimization method.[27,28] The stationary points were characterized by frequency calculations in order to verify that the TSs have one and only one imaginary frequency. The intrinsic reaction coordinate (IRC)[29] path was traced in order to check the energy profiles connecting each TS to the two associated minima of the proposed mechanism by using the second order González–Schlegel integration method.[30,31] The electronic structures of stationary points were analyzed by the natural bond orbital (NBO) method.[32,33] All calculations were carried out with the Gaussian 98 suite of programs.[34]

Solvent effects have been considered by B3LYP/6-31G* optimization of the gas-phase structures using a self-consistent reaction field (SCRF)[35] based on the polarizable continuum model (PCM) of the Tomasi's group.[36–38] Since these cycloadditions are carried out in dichloromethane, we have selected its dielectric constant at 298.0 K, $\varepsilon = 8.93$.

3. The global and local electrophilicity indices

The global electrophilicity index ω, which measures the stabilization in energy when the system acquires an additional electronic charge ΔN from the environment, has been given by the following simple expression:[17]

$$\omega = \frac{\mu^2}{2\eta} \tag{1}$$

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 $\eta \approx \varepsilon_L - \varepsilon_H$, respectively.[18,19]

Besides the global electrophilicity index, it is possible to define its local (or regional) counterpart condensed to atoms.[22] The local electrophilicity index ω_k condensed to atom k is easily obtained by projecting the global quantity onto any atomic center k in the molecule by using the electrophilic Fukui function (i.e., the Fukui function for nucleophilic attack, f_k^+, resulting in:[22]

$$\omega_k = f_k^+ \omega \tag{2}$$

4. Results and conclusions

4.1. DFT analysis based on the reactivity indices

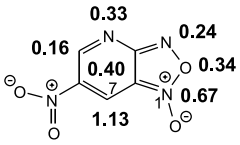
Recent studies devoted to DA reactions have shown that the global indices defined in the context of DFT are a powerful tool to understand the behavior of polar cycloadditions.[39–41] In Table 1 the static global properties: electronic chemical potential μ, chemical hardness η, and global electrophilicity ω, of 4-aza-6-nitrobenzofuroxan (**12**) and cyclopentadiene (**8**) are presented.

Table 1. Electronic chemical potential (μ, in a.u.), chemical hardness (η, in a.u.) and global electrophilicity (ω, in eV) 4-aza-6-nitrobenzofuroxan (**12**), the cycloadducts **13** and **14**, and cyclopentadiene (**8**)

	μ	η	ω
12	−0.2084	0.1229	4.81
Nitroethylene + BH_3	−0.2047	0.1317	4.33
13	−0.1761	0.1408	3.00
14	−0.1777	0.1542	2.79
Nitroethylene	−0.1958	0.2001	2.61
8	−0.1107	0.2016	0.83

The electronic chemical potential of Cp (**8**), $\mu = -0.1107$ a.u., is less than that of ANBF (**12**), $\mu = -0.2084$ a.u., thereby indicating that the net charge transfer will take place from Cp towards ANBF, in clear agreement with the charge transfer analysis performed at the corresponding TS (vide infra). The electrophilicity of ANBF is 4.81 eV, a value that falls in the range of strong electrophiles within the ω scale.[20] This value is lower than that for DNBF, 5.46 eV.[23] The presence of a second electron-withdrawing 4-NO_2 group in the latter substrate is responsible for its larger electrophilicity as compared to ANBF. It remains, however, that the electrophilicity value of ANBF is larger than that for nitroethylene, $\omega = 2.60$ eV, or for the nitroethylene:BH_3 complex, $\omega = 4.33$ eV (see Table 1), accounting for the super-electrophile reactivity of this nitrobenzofuroxan,[9] and its capacity to be part of polar cycloaddition reactions. The $\Delta\omega$ for the reaction between ANBF and Cp, 3.98 eV, is slightly larger than that for the reaction between the nitroethylene:BH_3 complex and MVE, 3.91 eV. Therefore, in spite of Cp being a weaker nucleophile than MVE, the large electrophilicity of ANBF accounts for its facile participation in this DA reaction.

Since this DA reaction has a large polar character, the analysis of the local electrophilicity,[22] ω_k, allows to explain the regiochemistry of these cycloadditions. The local electrophilicity values for ANBF are summarized in Scheme 5. The C7 carbon of ANBF presents the largest local electrophilicity value, $\omega_7 = 1.13$ eV. Therefore, this



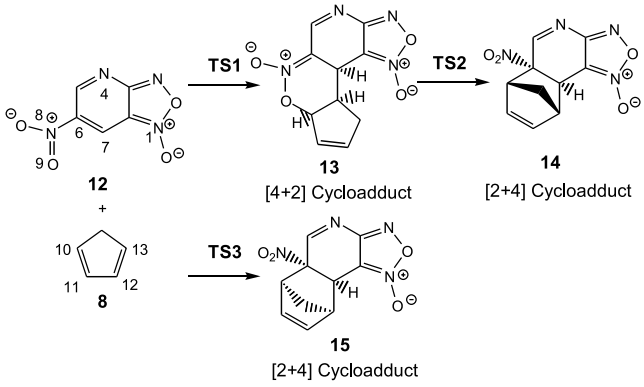
12, ANBF

Scheme 5. Local electrophilicity values, ω_k in eV, of 4-aza-6-nitrobenzofuroxan (**12**).

center will be the most electrophilic center of ANBF. Note that the local electrophilicity at the C7 carbon is twice the value of ω_k at the N1 nitrogen atom, which corresponds to the second more electrophile site of the molecule. This analysis is in complete agreement with the total regioselectivity observed experimentally through covalent hydration of ANBF at the C7 carbon,[9] and with the asynchronicity found at the bond formation process (see later).

4.2. Analysis of the PES of the DA reaction between ANBF and Cp

An exhaustive exploration of the PES for the DA reactions between ANBF and Cp allows to find only one TS, **TS1**, associated to a highly asynchronous bond formation process. From this TS, IRC calculations allow to characterize the [4+2] cycloadduct **13**. The different stationary points of this DA reaction have been depicted in Scheme 6 together with the atom numbering, while the energetic results are



Scheme 6.

Table 2. B3LYP/6-31G* total (E, in a.u.) and relative energies[a] (ΔE, in kcal/mol) of the stationary points of the Diels–Alder reaction between 4-aza-6-nitrobenzofuroxan (**12**) and cyclopentadiene (**8**), in vacuo and in dichloromethane

	In vacuo		In dichloromethane	
	E	ΔE	E	ΔE
8	−194.101064		−194.102506	
12	−711.364363		−711.370418	
TS1	−905.445640	12.4	−905.459717	8.3
13[b]	−905.483954	−11.6	−905.495346	−14.1
TS2	−905.444950	12.8	−905.459804	8.2
14[b]	−905.475763	−6.5	−905.484810	−7.5
TS3	−905.437725	17.4	−905.453487	12.2
15	−905.475341	−6.2	−905.484225	−7.1

[a] Relative to **8** and **12**.
[b] The MP2/6-31G* total energies of **13** and **14** are −902.9117787 and −902.9245776 a.u., respectively.

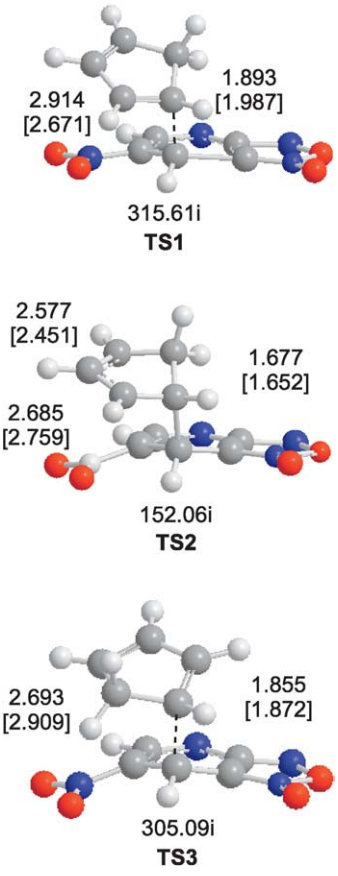


Figure 1. B3LYP/6-31G* geometries of the transition structures involved in the Diels–Alder reaction between 4-aza-6-nitrobenzofuroxan (**12**) cyclopentadiene (**8**). The bond lengths directly involved in the reaction are given in Ångströms. The values in dichloromethane are given in square brackets. The unique imaginary frequency is also given.

listed in Table 2. The geometries of the TSs are presented in Figure 1.

In gas-phase, the B3LYP/6-31G* activation barrier associated to the nucleophilic attack of Cp to ANBF with formation of the formal [4+2] cycloadduct **13** is 12.4 kcal/mol. This barrier is slightly larger than that associated with the nucleophilic attack of Cp to DBNF, 11.0 kcal/mol,[23] in agreement with the larger electrophilic character of DBNF, and in consequence, with the large polar character of the cycloaddition of Cp with DBNF (see later). All attempts to find a TS connecting the reagents, that is ANBF+Cp, with the [2+4] cycloadduct **14** have failed. The exploration of the PES allows to find a TS, **TS2**, that permits the conversion of the formal [4+2] cycloadduct **13** into the formal [2+4] cycloadduct **14**. This conversion is associated to a Claisen rearrangement, a [3,3] sigmatropic shift.[42] The energy level of **TS2**, 12.8 kcal/mol, is close to that associated to **TS1**. Formation of the [4+2] cycloadduct **13** is exothermic by −11.6 kcal/mol. At this level of calculations **13** is lower in energy than **14**, while HF/6-31G* calculations give **14** 1.8 kcal/mol more stable than **13**. Further MP2/6-31G* geometrical optimization for the two cycloadducts gives the cycloadduct **14** 8.0 kcal/mol more stable than **13**, in clear agreement with the experimental outcome.[9]

This DA reaction takes place experimentally with a total *endo* selectivity.[9] To test our computational model the channel associated to the *exo* approach of Cp relative to the NO_2 substituent of ANBF was also studied (see Scheme 6). The DFT calculations give **TS3** 5.0 kcal/mol higher in energy than **TS1**, in clear agreement with the absence of formation of the adduct **15** and therefore with the experimental outcome (see Table 2).

The geometries of the TSs are given in Figure 1. The length of the C7–C10 and O9–C11 forming-bonds at the TS associated to the nucleophilic attack of Cp to ANBF, that is, **TS1** are 1.893 and 2.914 Å, respectively. This is in accord with the highly imbalanced character of this TS. A similar situation prevails in **TS3**. Also noteworthy in **TS1** is the fact that the O9–C11 distance is appreciably shorter than that between the C6 and C13 atoms (3.219 Å). This feature, which is a consequence of the coulombic attraction between the ends of the zwitterionic **TS1** favors the O9–C11 bond-formation and therefore the formation of the [4+2] cycloadduct **13** rather than that of the related NED-DA adduct **14**.[16] In addition, these favorable coulombic interactions are responsible for the large *endo* selectivity observed in this polar cycloaddition.[16] At the TS associated to the [3,3] sigmatropic shift, **TS2**, the lengths of the O9–C11 breaking and C6–C3 forming-bonds are 2.685 and 2.577 Å, respectively.

The extent of bond-formation along a reaction pathway is provided by the concept of Wiberg bond order (BO).[43] At **TS1** the BO value of the C7–C10 forming bond is 0.57, while the values of the BOs between the O9 and C11 and the C6 and C13 atoms are 0.09 and 0.07, respectively. At **TS3** the BO value of the C7–C10 forming bond is 0.62, while the value of the BO between the C6 and C13 atoms is 0.17. On this basis, the more unfavorable *exo*-**TS3** will be slightly more advanced than the *endo* one. It remains that the data confirm that along the nucleophilic attack of Cp to ANBF only the C7–C10 bond process has progressed to a large extent at **TS1**, in agreement with other arguments (vide supra). At the TS associated to the [3,3] sigmatropic shift, **TS2**, the BO values of the O9–C11 breaking and C6–C13 forming bonds are 0.05 and 0.23, respectively, suggesting that the O9–C11 breaking-bond is ahead of the C6–C13 forming-bond process in this TS. The BO value of the C7–C10 bond at this TS is 0.80.

The natural population analysis[32,33] (NPA) allows the evaluation of the charge transfer along this polar cycloaddition. The B3LYP/6-31G* atomic charges at the TSs associated to the nucleophilic attack have been partitioned between the Cp and the ANBF frameworks. The negative charge transferred from the donor Cp to the acceptor ANBF is 0.40 e at **TS1** and 0.39 e at **TS3**, thereby confirming the zwitterionic nature of these TSs. Along this polar addition there is a large charge transfer as a consequence of the large electrophilicity of ANBF (see section 4.1). These values are slightly smaller than that evaluated at the TS associated to the *endo* attack of Cp to DNBF, 0.41 e,[23] in agreement with the larger electrophilic character of DNBF than that of ANBF.

The analysis of the atomic motion at the unique imaginary frequency of **TS1** (315.61i cm^{-1}) and **TS3** (305.09i cm^{-1}) indicates that these TSs are mainly associated to the movement of the C7 and C10 atoms along the C7–C10 bond-formation; the movement of the C6, O9, C11 and C13 atoms being negligible. This analysis reinforces the two-center interaction at this polar DA reaction that is anticipated by the analysis of the global and local electrophilicity indices. At **TS2** the analysis of the atomic motion at the unique imaginary frequency, 152.06i cm^{-1}, indicates that the O9–C11 breaking and C6–C13 forming-bond processes are coupled.

Solvent effects of dichloromethane have been modeled using the PCM method by means of geometrical optimizations of the stationary points. Table 2 reports the relative energies, while their corresponding geometries are given in Figure 1. Solvent effects stabilize all stationary points between 5 and 11 kcal/mol. The more stabilized species are the TS associated to the nucleophilic attack due to its zwitterionic character. In consequence, the activation barrier for the cycloaddition decreases 4.1 kcal/mol. In dichloromethane, **TS1** is 3.9 kcal/mol lower in energy than **TS3**, in clear agreement with the regioselectivity experimentally observed.[9]

Inclusion of solvent by the PCM approach produces minor changes in the geometries of the TSs. All attempts to locate a zwitterionic intermediate and a second TS associated to a stepwise mechanism were unsuccessful.[44] In consequence, the one-step mechanism also prevails in dichloromethane. The length of the C7–C10 forming bond at the TSs associated to the nucleophilic attack of Cp to ANBF increases by ca. 0.1 Å; 1.987 Å at **TS1** and 1.872 Å at **TS3**.

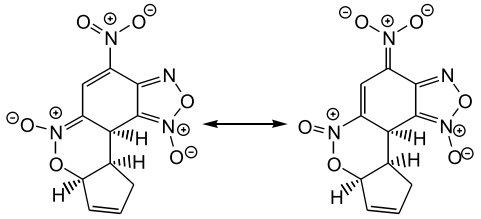
This DFT analysis indicates that while the formal [4+2] cycloadduct **13** is formed through one-step mechanism associated to the nucleophilic attack of Cp to ANBF, the formal [2+4] cycloadduct **14** is formed through a domino process that comprises formation of the cycloadduct **13** and a [3,3] sigmatropic shift on **13** to yield the **14**. A similar [3,3] sigmatropic shift for the conversion of a [2+4] cycloadduct into a thermodynamically more stable [4+2] one has been recently reported.[42] In the present study no channel connecting the reagents, ANBF and Cp, with the [2+4] cycloadduct **14** could be found. In addition, it is noteworthy that along the *exo* channel the [2+4] cycloadduct **15** is directly formed through **TS3**. In consequence, formation of both the formal [4+2] or [2+4] cycloadducts will take place through TS associated to the nucleophilic attack of Cp to ANBF and, in the absence of steric hindrance, formation of the [4+2] cycloadduct is controlled by the attracting coulombic interactions between the more positively and negatively centers at the corresponding zwitterionic TS.[45]

4.3. The ANBF versus DNBF behaviour

At this stage, some additional comments can be made regarding the finding that the most stable [2+4] adduct **14** derives from the initially formed [4+2] cycloadduct **13** via a [3+3] sigmatropic shift. This is a reactivity pattern, which strongly contrasts with the situation observed in the DNBF/Cp system. In this instance, both experimental[8] and

theoretical studies[23] agreed that the interaction proceeds as shown in Scheme 3 with a competitive initial formation of the adducts **9** and **10**. Subsequent formation of the adduct **11** follows, reflecting the greater susceptibility of the remaining nitroalkene moiety of the [4+2] adduct **9** than of the [2+4] adduct **10** to suffer a second addition process. In as much as the diadduct **11** is the thermodynamically more stable product of the overall interaction, its formation has the effect to drive the complete equilibrium system of Scheme 3 toward completion of the second condensation process.

On the basis of Schemes 3 and 6, it appears that the reactivities of DNBF and ANBF differ by the different reactivity of the [4+2] cycloadducts **9** and **13**, respectively. This situation can perhaps be understood in terms of the different electronic effects exerted by the 4-NO_2 and 4-aza substituents on these cycloadducts. Acting through its strong −M effect, the 4-NO_2 group can contribute efficiently to the large electrophilicity of the [4+2] adduct **9** of DNBF (structures **9a** and **9b** in Scheme 7). Such a situation does not operate in the ANBF system where the 4-aza group acts mainly through a −I effect. This analysis is in agreement with the larger electrophilicity of the cycloadduct **9**, 4.06 eV,[23] relative to that of the cycloadduct **13**, 3.00 eV, accounting well for the participation of the former in a subsequent polar DA reaction towards a second Cp molecule. On the other hand, the less reactive cycloadduct **13** undergoes a [3,3] sigmatropic shift leading to the thermodynamically more stable [2+4] adduct **14**.



Scheme 7.

5. Conclusions

The polar DA reaction between 4-aza-6-nitrobenzofuroxan and cyclopentadiene has been studied using quantum chemical procedures at the B3LYP/6-31G* level of theory. An exhaustive exploration of the PES for this DA reaction allows to find only one highly asynchronous TS associated to the nucleophilic attack of the end of the conjugated π-system of Cp (C10) to the electrophilically activated C7 carbon atom of ANBF. At this TS the formation of the second sigma bond between the oxygen O9 atom of the NO_2 group of ANBF and the allylic-type carbon C11 of Cp has made much less progress than the C7–C10 bond-forming process. A further [3,3] sigmatropic shift on the [4+2] cycloadduct allows its conversion into the thermodynamically more stable [2+4] one. This cycloaddition takes place with a large *endo* selectivity as a consequence of the favorable coulombic interactions on the zwitterionic TS. In this polar DA reaction both the selectivity in the formation of the [4+2] or [2+4] cycloadducts and the *endo*/*exo* stereoselectivity are a consequence of the favorable coulombic interactions on the zwitterionic *endo* TS rather than of molecular orbital interactions. Solvent effects decrease the barrier of the reaction as a consequence of a larger solvation of the zwitterionic TS than of the reactants. The gas-phase *endo* selectivity decreases in dichloromethane as a consequence of a larger solvation of the *exo* TS.

A DFT analysis of the global electrophilicity of ANBF allows to characterize the strong electrophile character of this nitrobenzofuroxan, and its participation in polar DA reactions. Finally, the analysis of the local electrophilicity indicates that the C7 carbon corresponds to the more electrophile site of ANBF, in clear agreement with the regioselectivity observed through covalent hydration.

Acknowledgements

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Chromone derivatives which bind to human hair

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Abstract—Chromone derivatives bearing a quaternary ammonium functionality which bind to human hair were synthesised. The radical scavenging activity, according to the DPPH assay, of the chromone derivatives is considerably lower compared with flavonoids. The compounds show interesting UV absorption properties that depend on the position of a methoxy substituent. A bathochromic shift of 29 nm was observed when the methoxy group on the ammonium salts were shifted from position 7 to position 6.

1. Introduction

Chromones are a group of naturally occurring compounds that are ubiquitous in nature especially in plants.[1] They are oxygen-containing heterocyclic compounds with a benzoannelated γ-pyrone ring, with the parent compound being chromone (4*H*-chromen-4-one, 4*H*-1-benzopyran-4-one).[2] Molecules containing the chromone structure (for example chromones and flavonoids) have a wide range of biological activities including tyrosine and protein kinase C inhibitors, antifungal, antiallergenic, antiviral, antitublin, antihypertensive and anticancer agents, as well being active at benzoazepine receptors, lipoxygenase, cyclooxygenase and modulating P-glycoprotein-mediated multidrug resistance (MDR).[2–5] Due to their abundance in plants and their low mammalian toxicity, chromone derivatives are present in large amounts in the diet of humans (Fig. 1).[6]

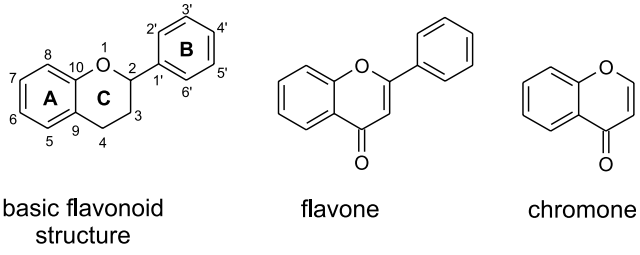

Figure 1. Basic structure of flavonoids, flavones and chromones.

Many of these biological actions are attributed to the ability of flavonoids to transfer electrons, chelate metal catalysts,[7] activate antioxidant enzymes,[8] reduce α-tocopherol radicals,[9] inhibit oxidases[10] as well as through their possible influences on the intracellular redox status, however, the precise mechanisms remain unclear.[11] Recent studies have speculated that the classical hydrogen-donating antioxidant activity of flavonoids[12] is unlikely to be the sole explanation for cellular effects.[13]

The antioxidant characteristics of flavonoids in combination with their favourable UV absorption properties are also exploited by plants to protect them from the suns UV radiation and scavenge UV-generated reactive oxygen species (ROS).[14] For example, there is evidence that flavonoids in leaves, deposited in either the epidermal cells or in the waxy upper leaf surface provide protection from the potential damage of UVB radiation.[15] This use could also be utilised in the protection of human hair from UV-radiation. It is well known that exposure to UV-radiation can damage hair fibres. UVB radiation is the principal radiation responsible for hair protein loss (causing dryness, reduced strength, rough surface texture, decreased luster, stiffness and brittleness), while UVA radiation is responsible for colour changes regardless of hair type.[16] Hair melanins provide some photochemical protection to hair proteins, especially at lower wavelengths where both the hair pigments and proteins absorb radiation.[17] These melanins also immobilise many of the free radicals generated by UV-radiation, however, in the process they are often degraded or bleached.[18] Here we reported the synthesis of new chromone derivatives, bearing a cationic functionality which bind to human hair.

Keywords: Chromone; Hair substantivity; UV activity; Antioxidant; Binding.
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2. Results and discussion

Quaternary ammonium compounds (cationic surfactants, cationic polyelectrolytes and cationic quaternary derivatives of hydrolyzed proteins) have been widely used as hair conditioning agents.[19] The deposition (substantivity) of such compounds can effect the hair fibre friction, stiffness, gloss, anti-static qualities and strength of hair.[17] By synthesising a chromone derivative with quaternary ammonium functionality it was hoped that this compound would show not only activity of cosmetic interest but also hair substantivity.

The synthetic strategy chosen for the preparation of the 2-amido-chromone required the preparation of 2-ethylester-chromone **2**. Condensation of **1** with diethyl oxalate in the presence of sodium ethanoxide in ethanol and followed by acidic cyclisation afforded the ester **2**.[5,20] By reacting different amines with **2**, a variety of chromone amides were synthesised (Scheme 1).

Reacting the ester **2** with either an *n*-alkyl amine (butylamine, octylamine, dodecylamine) or 3-dimethylaminopropylamine gave the amides **3** and **4**, respectively. Treatment of the latter with methyl iodide gave the trimethyl ammonium salt **5**.

Although numerous other methods exist for introducing a new functionality at the C-2 position of chromone,[3,4,21] the chosen synthetic route is short, has two possibilities for introducing diversity (variation of acetophenone and variation of amine) needed to generate a small library and utilises cheap reagents which is an important factor for industrial applications.

In order to increase the radical scavenging activity of **3** and **5**, the 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester was synthesised. Due to the formation of a zwitterion in compound **9** this synthetic route was not expanded for other hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl esters with other substitution patterns (Scheme 2).

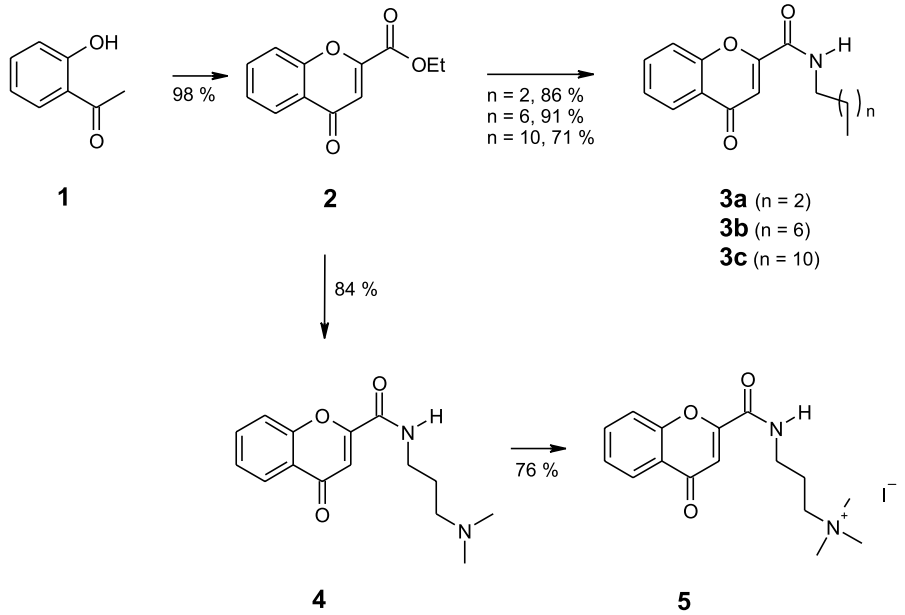
Alternatively, the methoxy derivatives **12** were synthesized (Scheme 3). Although in general hydroxyl groups give a better radical scavenging activity,[22] there are some examples where a hydroxyl group is deleterious and methoxy group beneficial to such activity.[4,23,24] Compound **12** was prepared from the commercially available **11**. The methoxy derivatives **14** could now be easily synthesised and purified without fear of zwitterion formation.

Numerous attempts with various conditions at *O*-demethylating **14a–c** failed.[25] Alternative protecting groups that can withstand the reaction conditions and are industrially feasible in order to readily synthesise hydroxyl derivatives are currently being investigated.

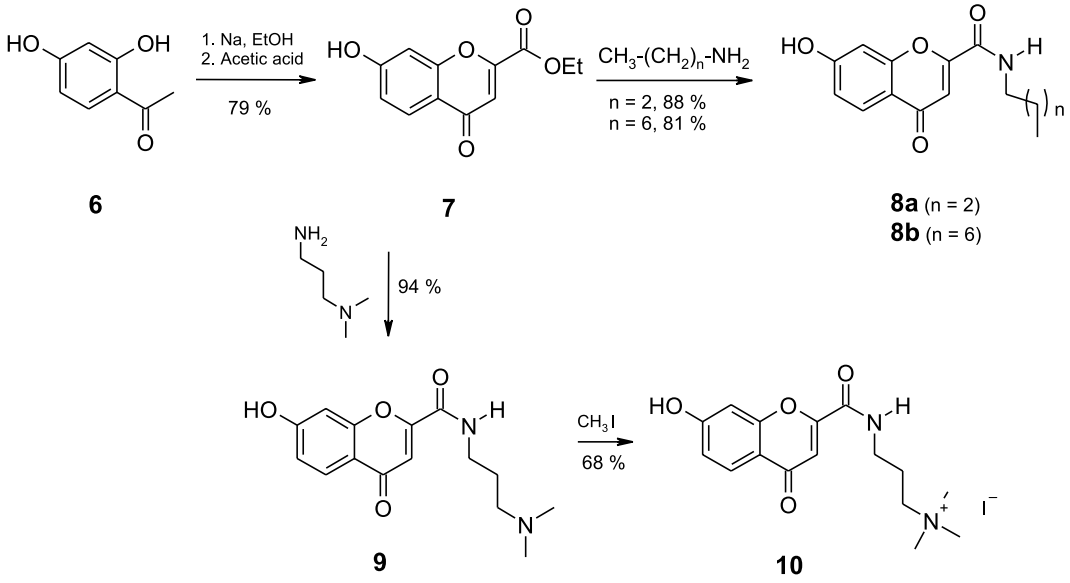
2.1. Hair substantivity

Compounds **2**, **3**, and **5** served as models for hair binding assay. Although there are various methods to measure the substantivity of cationic species to hair,[19,26] many are complicated, require specialized instrumentation and are time-consuming. As many compounds contained in cosmetic products including cationic surfactants[27] and dyes[28] can penetrate into hair fibres, it is important to choose an analytical method which can quantitatively recover the analyte.

Although a number of methods for hair substantivity were tested,[29] only two (MALDI MS[30] and hair digestion followed by HPLC analysis) could confirm the presence of compound bound to hair. The practicality of HPLC analysis and the large number of samples made the HPLC method very feasible. The compound was dissolved in a 70:30 ethanol/water mixture, to which then sterile, washed hair was added and allowed to stir for 1 h. The hair was then



Scheme 1. General synthesis of unsubstituted 2-amido chromones.

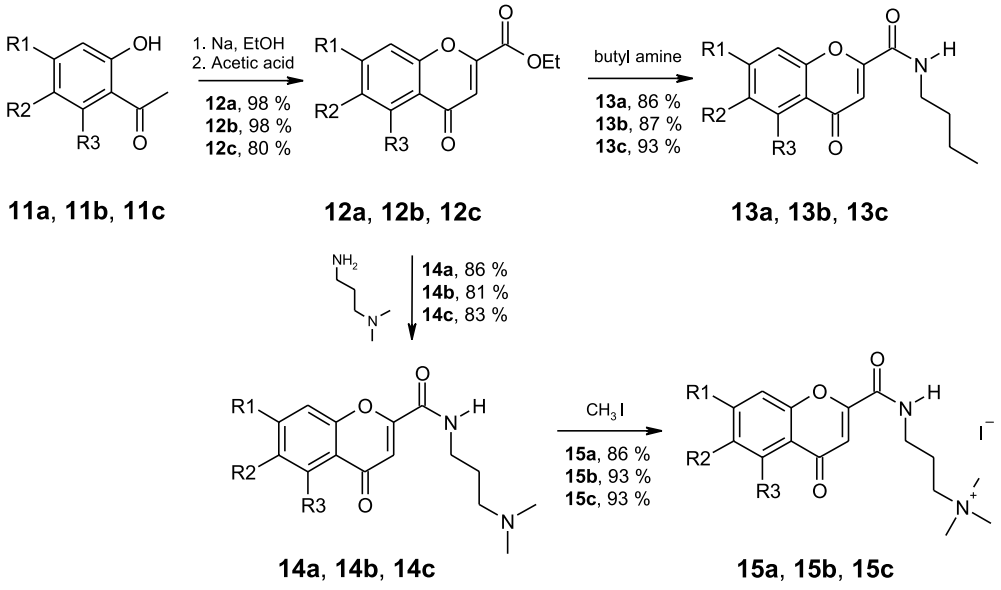


Scheme 2. General synthesis of hydroxyl substituted 2-amido chromones.

thoroughly washed with water and air dried. A sample of this 'treated' hair was dissolved in sodium hydroxide solution at 60 °C and then analysed via HPLC. This method[31] which was adapted from an analytical procedure used for the detection of anabolic steroids in livestock has the advantage that even small amounts of compounds can be quantitatively recovered.

Digested blonde human hair was taken as a reference showing characteristic peaks at 1.54, 1.77, 2.05 and 4.79 min. On digestion of **5** (without coming in contact with any hair), only a peak at 5.47 min corresponding to the carboxylic acid, 4-oxo-4*H*-chromene-2-carboxylic acid (generated due to the strong basic conditions) was observed. Digested hair, which had previously been stirred with **5** and then washed thoroughly, showed a peak at 5.47 min in addition to the characteristic peaks of digested hair. On spiking this sample with a small amount of the digested product of **5**, the peak at 5.47 min increased in intensity confirming that **5** binds to human hair.

In addition to **5**, a number of other compounds were tested for their hair substantivity (Table 1). Tests were carried out on bleached human hair and on brown human hair. The hair substantivity effect of all tested compounds was independent of hair type.



for **11a - 15a** (R1 = OCH_3, R2 = H, R3 = H)
for **11b - 15b** (R1 = H, R2 = OCH_3, R3 = H)
for **11c - 15c** (R1 = H, R2 = H, R3 = OCH_3)

Scheme 3. General synthesis of methoxy substituted 2-amido chromones.

Table 1. Hair substantivity of chromones

Compound	Hair substantivity
2	None
3a	None
3b	Possible
3c	—[a]
8b	Possible
5	Yes
15a	Yes
15b	Yes
15c	Yes

[a] Could not be tested to due solubility problems.

These results show that compounds bearing a quaternary ammonium salt bind well to hair. The HPLC chromatograph shows a definite peak which corresponds to the added compound (see Section 4 for more details concerning analysis). An ester or butyl amide failed to show any binding. There is some evidence to show that an octyl amide functionality may bind to hair, however, due to their poor solubility in ethanol/water mixtures, definite binding could not be confirmed (The observed peak was within experimental error). However, the use of long alkyl chains in hair care products suggests that some interactions must take place.

2.2. Antioxidant activities

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to screen the radical scavenging potential of the compounds. The DPPH radical, $DPPH^{\cdot}$ is a relatively stable paramagnetic free radical, which accepts a hydrogen radical to become a stable diamagnetic molecule. As chromones lack the B-ring of flavonoids, which is responsible for much of their antioxidant power, the radical scavenging ability of chromones remain modest. However, interesting radical scavenging properties were found for the amide derivatives.[32]

2.3. Cyclic voltammetry

In flavonoids the reduction potential is strongly dependant on the electron donating properties of the B-ring as this is generally more electron rich as the A-ring.[33] As chromones do not possess the B-ring, the A-ring should influence the electronic chemical properties of the chromone. The electrochemical properties of compounds **15a**, **15b**, and **15c** were measured by cyclic voltammetry[34] in acetonitrile. All three samples showed an electrochemical irreversible behaviour. Relative to the half wave reduction potential of Fc/Fc^{+}, a shift of $+30$, $+60$ and $+50$ mV were observed for **15a**, **15b**, and **15c**, respectively. Although these shifts are small and close to the experimental error (± 20 mV) the general trend of increased reduction potential with increased electron donating properties of the substitutent is indicated.

2.4. UV absorption of substituted chromones

The UV absorption properties of three different chromone derivatives (ester, alkyl amide and ammonium salt) with varying substitution patterns (methoxy substitution at position 7-, 6- or 5-) were measured. Tables 2–4 summarise the results.

A bathochromic shift was observed for the chromone -esters, -alkyl amides and -ammonium salts upon substitution of a methoxy group at either position 5, 6 or 7. As expected the alkyl chain length has no effect on the UV absorption. When compared to **5**, a red shift of 29 and 15 nm was observed for **15b** and **15c**, respectively, whilst **15a** has the same λ_{max} (304 nm). Thus, by altering the chromone substitution pattern, the UV absorption properties can be tailored to individual needs. For example, **15a** would protect better against UVB radiation and thus hair protein loss, whilst **15b** would protect better against UVA radiation and hair colour changes.

Table 2. Molar extinction coefficients for ethyl ester derivatives

Compound	R1	R2	R3	Wavelength, λ_{max} (nm), Molar extinction coefficient (ε)[a]			
12a	OCH_3	H	H	212 (4.44)	238 (4.29)		310 (4.01)
12b	H	OCH_3	H	206 (4.45)	238 (4.22)	253 (4.31)	343 (3.77)
12c	H	H	OCH_3		238 (4.22)	271 (4.00)	327 (3.64)

[a] Methanol was used as solvent.

Table 3. Molar extinction coefficients for alkyl amide derivatives

Compound	R1	R2	R3	Wavelength, λ_{max} (nm), Molar extinction coefficient (ε)[a]			
13a	OCH_3	H	H	212 (4.42)	236 (4.35)	255 (4.10)[b]	304 (4.04)
13b	H	OCH_3	H	205 (4.43)	230 (4.25)	252 (4.36)	338 (3.75)
13c	H	H	OCH_3		238 (4.29)	260 (4.16)	323 (3.71)

[a] Methanol was used as solvent.
[b] Present as a shoulder.

Table 4. Molar extinction coefficients for quaternary ammonium derivatives

Compound	R1	R2	R3	Wavelength, λ_{max} (nm), Molar extinction coefficient (ε)[a]		
5	H	H	H	204 (4.62)	246 (4.43)	304 (3.83)
15a	OCH_3	H	H	210 (4.47)	242 (4.32)	304 (3.80)
15b	H	OCH_3	H	205 (4.42)	248 (4.34)	333 (3.54)
15c	H	H	OCH_3		242 (4.25)	319 (3.53)

[a] CH_3CN was used as solvent.

3. Conclusion

Chromone derivatives containing a quaternary ammonium functionality which bound to human hair were synthesised. The substitution pattern of the methoxy group was systematically changed to probe its effect on the redox potential and the UV absorption properties. The general radical scavenger activity of all derivatives is lower if compared to flavonoids. The UV absorption of the derivatives depends on the position of the methoxy groups. If the substitutent is shifted from the 7 to 6 position, a bathochromic shift of the UV absorption of 29 nm results.

4. Experimental

4.1. General

Purification and drying according to accepted general procedures.[35] If not otherwise stated, commercially available solvent of the highest purity were used. UV–vis spectra were measured using a Varian Cary BIO 50 UV/VIS/NIR spectrometer, with a 1 cm quartz cell (Hellma) and Uvasol solvents (Merck). Reported as: λ_{max} in nm (ε). IR spectra were measured using a Bio-Rad FT-IR-Spectrometer FTS 155. NMR spectra were measured using a Bruker Avance 300 (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz) and Bruker Avance 600 (^{1}H: 600.1 MHz, ^{13}C: 150.1 MHz). The chemical shifts are in δ-values (ppm) relative to the internal (or external) standard TMS. Reported as: Chemical shift (multiplicity, coupling constant, number of protons, assignment). Reported assignments were determined with the help of COSY, HMQC, HSQC, and NOESY 2D-Spectra. Mass spectra were measured using a Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD) and Finnigan MAT TSQ 7000 (ESI). Melting points are uncorrected and were determined according to Tottoli using instrumentation from Büchi. Elemental analyses were carried out by the microanalytical laboratory of the School of Chemistry and Pharmacy, University of Regensburg.

4.2. HPLC

HPLC analyses were carried out using a Merck Hitachi LaChrom (Interface L-7000, UV detector L-7400, Column Oven L-7350, Autosampler L-7200, Pump L-7100) with a Chromolith RP-18e 100-4.6 column. Wavelength 220–400 nm; column temperature 30 °C; injection volume 50 µl; acetonitrile and water buffered at pH 2.6 served as solvents.

4.3. Cyclic voltammetry

The cyclic voltammetry measurements were conducted in dry acetonitrile with 0.1 mol L^{-1} NBu_4^+ BF_4^- as electrolyte and under argon. The working electrode was a graphite electrode, the counter electrode a platinum wire and the reference electrode Ag/AgCl in LiCl saturated ethanol. The PGSTAT 20 is from the company Eco Chemie and is controlled by the program GPES V 3.0.

4.4. Hair substantitivity

The substance to be tested (1 mg) was dissolved in ethanol/water (70:30, 50 ml) and allowed to stir at room temperature using a magnetic stirrer. Commercially available,[36] washed, sterile human hair (1.00 g, 0.4–0.7 cm pieces) was added to the solution and allowed to stir vigorously for 1 h. The hair was filtered and washed three times with fresh solvent. The hair sample was dried overnight at 40 °C and 200 mbar, yielding 1.00 g of treated hair.

The treated hair (0.5 g) was added to a solution of NaOH (1 M, 4 ml) and allowed to stir at 65 °C for 2 h, forming a brown suspension. The reaction mixture was neutralised with HCl (2 M, 2 ml) after which methanol (3 ml) and THF (1 ml) were added and the mixture allowed to stir for a further hour. A sample (5 mg) of only the substance to be tested (no hair) was subjected to the same conditions to control the stability of the substance. The suspension was centrifuged (4000 rpm, 10 min) and the mother liquor decanted. The mother liquor was then filtered (0.2 µm polypropylene filter) to yield a clear light brown solution. A sample of only hair (no substance) was subjected to the same conditions and used as a reference. The hair substantivity test was carried out using both bleached European and natural light middle brown European hair. No difference was noted in hair substantivity due to hair type.

4.5. X-ray crystallography

X-ray crystallography data for compounds **2**, **13a**, **13b** and **13c** are available under www.dekker.com.

4.6. Synthesis of new compounds

4.6.1. Ethyl-4-oxo-4*H*-chromene-2-carboxylate (2).[37] Sodium (1.49 g, 65 mmol) was dissolved in absolute ethanol (100 ml). Diethyloxalate (5.12 g, 35 mmol) and 2-hydroxyacetophenone (2.04 g, 15 mmol) were dissolved in absolute ethanol (10 ml) and added to the sodium ethanolate solution. The solution was allowed to reflux for 1 h. Concentrated HCl was added dropwise until the reaction was acidic and a white precipitate formed. The white precipitate was filtered and the yellow solution concentrated to a slurry. The slurry was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to give a light yellow solid. The solid was recrystallised from methanol/diisopropylether (4:1) to yield white needles (3.20 g, 14.6 mmol, 98%).

Mp 63 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3447, 3067, 2985, 2938, 1734, 1647, 1466, 758; ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm)=1.43 (t, J=7.1 Hz, 3H, CH_3), 4.46 (q, J=7.1 Hz, 2H, CH_2), 7.11 (s, 1H, H-3), 7.44 (ddd, J=1.2, 7.1, 8.0 Hz, 1H, H-6), 7.61 (ddd, J=0.4, 1.2, 8.5 Hz, 1H, H-8), 7.74 (ddd, J=1.7, 7.1, 8.5 Hz, 1H, H-7), 8.20 (ddd, J=0.4, 1.7, 8.0 Hz, 1H, H-5); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm)= 14.1 (+, CH_3), 63.0 (−, CH_2), 114.8 (+, C-3), 118.8 (+, C-8), 124.4 (C_{quat}, C-9), 125.7 (+, C-5), 125.9 (+, C-6), 134.7 (+, C-7), 152.2 (C_{quat}, C-2), 156.0 (C_{quat}, C-10), 160.6 (C_{quat}, C=O), 178.4 (C_{quat}, C-4); MS (ESI-MS, EtOH/MeOH+10 mmol/l NH_4OAc) m/z (%): 219 (100) $[MH]^+$. Elemental analysis: $C_{12}H_{10}O_4$ Calcd: C, 66.05; H, 4.62. Found: C, 66.07; H, 4.72.

4.6.2. 4-Oxo-4*H*-chromene-2-carboxylic acid butylamide (3a). Ethyl-4-oxo-4*H*-chromene-2-carboxylate (655 mg,

3 mmol) was dissolved in the butylamine (658 mg, 9 mmol) allowed to stir at 50 °C for 10 min. The solvent was evaporated leaving a yellow solid. Glacial acetic acid (5 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water and a white precipitate formed which was filtered, washed with water and dried. The white solid was recrystallised from ethyl acetate to yield white needles (630 mg, 2.6 mmol, 86%).

Mp 130 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3319, 3079, 2954, 2868, 1685, 1642, 1387, 755; UV/vis (MeOH): λ_{max} (nm) (log ε)=202 (4.37), 236 (4.29), 305 (3.85); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=0.91 (t, J=7.2 Hz, 3H, CH_3), 1.34 (qd, J=7.2, 14.1 Hz, 2H, CH_2), 1.54 (XX, J=7.2 Hz, 2H, CH_2), 3.30 (q, J=7.2 Hz, 2H, CH_2), 6.82 (s, 1H, H-3), 7.53 (ddd, J=1.0, 7.1, 8.0 Hz, 1H, H-6), 7.73 (dd, J=1.0, 8.5 Hz, 1H, H-8), 7.89 (ddd, J=1.7, 7.1, 8.5 Hz, 1H, H-7), 8.05 (dd, J=1.7, 8.0 Hz, 1H, H-5), 9.10 (t, J=5.6 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=13.6 (+, CH_3), 19.5 (−, CH_2), 30.8 (−, CH_2), 38.8 (−, CH_2), 110.2 (−, C-3), 118.7 (+, C-8), 123.5 (C_{quat}, C-9), 124.8 (+, C-5), 125.9 (+, C 6), 134.9 (+, C-7), 155.0 (C_{quat}, C-2), 155.7 (C_{quat}, C-10), 158.7 (C_{quat}, C=O), 177.2 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 263.1 (100) $[M+NH_3]^+$, 246.1 (26) $[MH]^+$. Elemental analysis: $C_{14}H_{15}NO_3$ Calcd: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.58; H, 6.04; N, 5.61.

4.6.3. 4-Oxo-4*H*-chromene-2-carboxylic acid octylamide (3b). Ethyl-4-oxo-4*H*-chromene-2-carboxylate (655 mg, 3 mmol) and octylamine (1163 mg, 9 mmol) were dissolved in dichloromethane (5 ml) and allowed to reflux for 10 min. The solvent was evaporated leaving a light yellow solid. Glacial acetic acid (5 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give a white solid. The solid was recrystallised from ethyl acetate to yield white needles (826 mg, 2.7 mmol, 91%).

Mp 131 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3314, 2927, 2849, 1684, 1646, 1529, 1392, 751; UV/vis (MeOH): λ_{max} (nm) (log ε)=202 (4.36), 236 (4.29), 305 (3.84); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=0.85–0.91 (m, 3H, CH_3), 1.24–1.46 (m, 10H, 5×CH_2), 1.61–1.72 (m, 2H, CH_2), 3.49 (tq, J=6.2, 7.2 Hz, 2H, CH_2), 6.91 (s, 1H, N–H), 7.16 (s, 1H, H-3), 7.45 (ddd, J=1.0, 7.2, 8.1 Hz, 1H, H-6), 7.52 (dd, J=0.5, 8.5 Hz, 1H, H-8), 7.73 (ddd, J=1.7, 7.2, 8.5 Hz, 1H, H-7), 8.22 (dd, J=1.7, 8.0 Hz, 1H, H-5); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=14.1 (+, CH_3), 22.6 (−, CH_2), 26.7 (−, CH_2), 27.0 (−, CH_2), 29.1 (−, CH_2), 29.2 (−, CH_2), 29.5 (−, CH_2), 31.8 (−, CH_2), 40.1 (−, CH_2), 112.1 (+, C-3), 118.0 (+, C-8), 124.4 (C_{quat}, C-9), 126.0 (+, C-5), 126.2 (+, C-6), 134.5 (+, C-7), 154.8 (C_{quat}, C-2), 155.3 (C_{quat}, C-10), 159.2 (C_{quat}, C=O), 178.2 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 319.1 (100) $[M+NH_3]^+$, 302.1 (40) $[MH]^+$. Elemental analysis: $C_{18}H_{23}NO_3$ Calcd: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.62; H, 7.58; N, 4.43.

4.6.4. 4-Oxo-4*H*-chromene-2-carboxylic acid dodecylamide (3c). Ethyl-4-oxo-4*H*-chromene-2-carboxylate (1.96 g, 9 mmol) and dodecylamine (5.0 g, 27 mmol) were dissolved in dichloromethane (10 ml) and allowed to reflux for 10 min. The solvent was evaporated giving a light yellow solid. Glacial acetic acid (10 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid. The solid was recrystallised from ethyl acetate to yield white needles (2.30 g, 6.4 mmol, 71%).

Mp 108 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3319, 2959, 2932, 2850, 1982, 1642, 1522, 1387, 753; UV/vis (MeOH): λ_{max} (nm) (log ε)=203 (4.37), 236 (4.29), 305 (3.83); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=0.83 (t, J=6.7 Hz, 3H, CH_3), 1.16–1.33 (m, 18H, 9×CH_2), 1.48–1.60 (m, 2H, CH_2), 3.28 (m, 2H, CH_2), 6.81 (s, 1H, H-3), 7.53 (ddd, J=1.0, 7.2, 8.0 Hz, 1H, H-6), 7.73 (dd, J=0.6, 8.5 Hz, 1H, H-8), 7.89 (ddd, J=1.7, 7.2, 8.5 Hz, 1H, H-7), 8.05 (dd, J=1.7, 8.0 Hz, 1H, H-5), 9.12 (t, J=5.8 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=13.8 (+, CH_3), 22.0 (−, CH_2), 26.3 (−, CH_2), 28.6 (−, CH_2), 28.7 (−, CH_2), 28.8 (−, CH_2), 28.9 (−, CH_2), 28.9 (−, CH_2), 28.9 (−, CH_2), 31.2 (−, CH_2), 39.2 (−, CH_2), 39.4 (−, CH_2), 110.2 (+, C-3), 118.7 (+, C-8), 123.5 (C_{quat}, C-9), 124.8 (+, C-5), 125.9 (+, C-6), 134.9 (+, C-7), 155.0 (C_{quat}, C-2), 155.7 (C_{quat}, C-10), 158.7 (C_{quat}, C=O), 177.2 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 375.2 (100) $[M+NH_3]^+$, 358.2 (56) $[MH]^+$. Elemental analysis: $C_{22}H_{31}NO_3$ Calcd: C 73.92; H 8.74; N, 3.92. Found: C, 73.75; H, 8.75; N, 3.74.

4.6.5. 4-Oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (4). Ethyl-4-oxo-4*H*-chromene-2-carboxylate (655 mg, 3 mmol) and 3-dimethylamino-propylamine (920 mg, 9 mmol) were dissolved in dichloromethane (5 ml) and allowed to reflux for 20 min. The solvent was evaporated, glacial acetic acid (5 ml) was added and the solution allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid. The solid was recrystallised from ethyl acetate to yield white needles (691 mg, 2.5 mmol, 84%).

Mp 125 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3289, 2976, 2946, 2805, 1686, 1640, 1534, 1460, 1392, 759; UV/vis (MeOH): λ_{max} (nm) (log ε)=203 (4.37), 236 (4.29), 305 (3.84); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.69 (p, J=6.9 Hz, 2H, CH_2), 2.15 (s, 6H, 2×CH_3), 2.28 (t, J=6.9 Hz, 2H, CH_2), 3.33 (dd, J=7.2, 12.7 Hz, 2H, CH_2), 6.82 (s, 1H, H-3), 7.53 (ddd, J=1.0, 7.2, 8.0 Hz, 1H, H-6), 7.71 (dd, J=0.7, 8.5 Hz, 1H, H-8), 7.89 (ddd, J=1.7, 7.2, 8.5 Hz, 1H, H-7), 8.05 (dd, J=1.7, 8.0 Hz, 1H, H-5), 9.25 (t, J=5.5 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=26.4 (−, CH_2), 38.0 (−, CH_2), 45.1 (+, 2×CH_3), 56.9 (−, CH_2), 110.2 (+, C-3), 118.6 (+, C-8), 123.5 (C_{quat}, C-9), 124.9 (+, C-5), 125.9 (+, C-6), 134.9 (+, C-7), 155.0 (C_{quat}, C-2), 155.6 (C_{quat}, C-10), 158.7 (C_{quat}, C=O), 177.2 (C_{quat}, C-4); MS (PI-EIMS) m/z (%): 58.1 (100) $[Me_2N{=}CH_2]^+$, 274.1 (11) $[M]^{+\cdot}$. Elemental analysis: $C_{15}H_{18}N_2O_3$ Calcd: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.63; H, 6.41; N, 10.08.

4.6.6. Trimethyl-{3-[(4-oxo-4*H*-chromene-2-carbonyl)-amino]-propyl}-ammonium iodide (5). Iodomethane (260 mg, 1.83 mmol) was dissolved in chloroform (5 ml) and added to a solution of 4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (500 mg, 1.82 mmol)

in chloroform (5 ml). The solution was allowed to stir at room temperature for 30 min and then at reflux for 15 min upon which a precipitate formed. The yellow precipitate was filtered and washed with chloroform yielding a light yellow solid (578 mg, 1.39 mmol, 76%).

Mp 240 °C+; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3404, 3022, 2956, 1673, 1643, 1458, 740; UV/vis (CH_3CN): λ_{max} (nm) (log ε)=204 (4.62), 246 (4.43), 304 (3.83); ^1H NMR (300 MHz, DMSO): δ (ppm)=1.95–2.06 (m, 2H, CH_2), 3.07 (s, 9H, 3×CH_3), 3.34–3.44 (m, 6H, 2×CH_2), 6.86 (s, 1H, H-3), 7.55 (ddd, J=1.0, 7.2, 8.0 Hz, 1H, H-6), 7.75 (d, J=8.5 Hz, 1H, H-8), 7.92 (ddd, J=1.7, 7.2, 8.5 Hz, 1H, H-7), 8.06 (dd, J=1.7, 8.0 Hz, 1H, H-5), 9.25 (t, J=5.9 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=22.6 (−, CH_2), 36.3 (−, CH_2), 52.1 (+, CH_3), 52.2 (+, CH_3), 52.2 (+, CH_3), 63.2 (−, CH_2), 110.5 (+, C-3), 118.6 (+, C-8), 123.5 (C_{quat}, C-9), 124.9 (+, C-5), 126.0 (+, C-6), 135.0 (+, C-7), 155.0 (C_{quat}, C-2), 155.4 (C_{quat}, C-10), 159.2 (C_{quat}, C=O), 177.2 (C_{quat}, C-4); MS (ESI, H_2O/AcN) m/z (%): 289.0 (100) $[K]^+$. Elemental analysis: $C_{16}H_{21}N_2O_3I$ Calcd: C, 46.17; H, 5.08; N, 6.73. Found: C, 45.79; H, 4.70; N, 6.51.

4.6.7. Ethyl-7-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (7).[38] Sodium (1.84 g, 80 mmol) was dissolved in absolute ethanol (100 ml). Diethyloxalate (7.31 g, 50 mmol) and 2,4-dihydroxyacetophenone (2.28 g, 15 mmol) were dissolved in absolute ethanol (10 ml) and added to the sodium ethanolate solution. The solution was allowed to reflux for 1 h. Concentrated HCl was added dropwise until the reaction was acidic and a white precipitate formed. The white precipitate was filtered off and the yellow/brown filtrate concentrated to a slurry. The slurry was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to give a light orange solid. The solid was recrystallised from methanol/diisopropylether (3:1) to yield white needles (2.78 g, 12 mmol, 79%).

Mp 210–211 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3521, 3109, 1742, 1640, 1601, 1570, 1456, 1253, 829; UV/vis (MeOH): λ_{max} (nm) (log ε)=211 (4.44), 239 (4.24), 313 (3.96); ^1H NMR (600 MHz, DMSO): δ (ppm)=1.34 (t, J=7.1 Hz, 3H, CH_3), 4.37 (q, J=7.1 Hz, 2H, CH_2), 6.82 (s, 1H, H-3), 6.90 (d, J=2.2 Hz, 1H, H-8), 6.96 (dd, J=2.2, 8.7 Hz, 1H, H-6), 7.89 (d, J=8.7 Hz, 1H, H-5), 11.02 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO): δ (ppm)=13.8 (+, CH_3), 62.5 (−, CH_2), 102.4 (+, C-8), 113.6 (+, C-3), 115.8 (+, C-6), 116.6 (C_{quat}, C-9), 126.7 (+, C-5), 151.5 (C_{quat}, C-2), 157.1 (C_{quat}, C-10), 159.9 (C_{quat}, C=O), 163.5 (C_{quat}, C-7), 176.1 (C_{quat}, C-4); MS (EI-MS, 70 eV) m/z (%): 234.1 (100) $[M]^{+\cdot}$; HRMS $(C_{12}H_{10}O_5)^{+\cdot}$ Calcd: 234.0528. Found: 234.0528± 0.76 ppm. Elemental analysis: $C_{12}H_{10}O_5$ Calcd: C, 61.54; H, 4.30. Found: C, 60.97; H, 4.21.

4.6.8. 7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid butylamide (8a). Ethyl-7-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (585 mg, 2.5 mmol) and butylamine (512 mg, 7 mmol) were dissolved in dichloromethane (10 ml) and allowed to reflux for 10 min. The solvent was evaporated upon which a light yellow solid formed. Glacial acetic acid (10 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water. The precipitate was filtered off, washed with water and dried. The solid was recrystallised from ethyl acetate/diisopropylether (4:1) to yield a light brown solid (573 mg, 2.2 mmol, 88%).

Mp 240 °C+; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3340, 3195, 3081, 2960, 2875, 1639, 1618, 1394, 829; UV/vis (MeOH): λ_{max} (nm) (log ε)=211 (4.41), 238 (4.31), 307 (3.98); ^1H NMR (300 MHz, DMSO): δ (ppm)=0.90 (t, J=7.2 Hz, 3H, CH_3), 1.32 (qd, J=7.2, 14.2 Hz, 2H, CH_2), 1.47–1.58 (m, 2H, CH_2), 3.28 (dd, J=6.9, 13.2 Hz, 2H, CH_2), 6.69 (s, 1H, H-3), 6.94 (dd, J=2.3, 8.7 Hz, 1H, H-6), 6.99 (d, J=2.2 Hz, 1H, H-8), 7.88 (d, J=8.7 Hz, 1H, H-5), 9.06 (t, J=5.8 Hz, N–H), 10.98 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=13.6 (+, CH_3), 19.5 (−, CH_2), 30.9 (−, CH_2), 38.8 (−, CH_2), 102.6 (+, C-8), 110.1 (+, C-3), 115.6 (+, C-6), 116.4 (C_{quat}, C-9), 126.6 (+, C-5), 155.1 (C_{quat}, C-2), 156.9 (C_{quat}, C-10), 158.8 (C_{quat}, C=O), 163.1 (C_{quat}, C-7), 176.3 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 262.2 (100) $[MH]^+$. Elemental analysis: $C_{14}H_{15}NO_4$ Calcd: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.12; H, 5.48; N, 5.11.

4.6.9. 7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid octylamide (8b). Ethyl-7-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (585 mg, 2.5 mmol) and octylamine (905 mg, 7 mmol) were dissolved in dichloromethane (10 ml) and allowed to reflux for 10 min. The solvent was evaporated upon which a brown solid formed. Glacial acetic acid (10 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water yielding a precipitate, which was filtered off, washed with water and dried. The solid was recrystallised from ethyl acetate to yield a white solid (642 mg, 2.0 mmol, 81%).

Mp 215 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3256, 3155, 2947, 2919, 2853, 1634, 1598, 1530, 1388, 1242, 838; UV/vis (MeOH): λ_{max} (nm) (log ε)=210 (4.44), 238 (4.33), 308 (3.99); ^1H NMR (600 MHz, DMSO): δ (ppm)=0.84 (t, J=6.7 Hz, 3H, CH_3), 1.20–1.31 (m, 10H, 5×CH_2), 1.50–1.56 (m, 2H, CH_2), 3.27 (dd, J=6.7, 13.4 Hz, 2H, CH_2), 6.69 (s, 1H, H-3), 6.94 (dd, J=1.9, 8.7 Hz, 1H, H-6), 6.99 (d, J=1.9 Hz, 1H, H-8), 7.88 (d, J=8.7 Hz, 1H, H-5), 9.03 (t, J=5.5 Hz, 1H, N–H), 10.96 (s, 1H, OH); ^{13}C NMR (150 MHz, DMSO): δ (ppm)=13.8 (+, CH_3), 22.0 (−, CH_2), 26.3 (−, CH_2), 28.5 (−, CH_2), 28.6 (−, CH_2), 28.7 (−, CH_2), 31.1 (−, CH_2), 39.1 (−, CH_2), 102.6 (+, C-8), 110.1 (+, C-3), 115.6 (+, C-6), 116.4 (C_{quat}, C-9), 126.6 (+, C-5), 155.1 (C_{quat}, C-2), 156.9 (C_{quat}, C-10), 158.8 (C_{quat}, C=O), 163.1 (C_{quat}, C-7), 176.2 (C_{quat}, C-4); MS (ESI, DCM/MeOH +10 mmol/l NH_4Ac) m/z (%): 318.1 (100) $[MH]^+$. Elemental analysis: $C_{18}H_{23}NO_4$ Calcd: C, 68.12; H, 7.30; N, 4.41. Found: C, 67.85; H, 7.30; N, 4.24.

4.6.10. 7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (9). Ethyl-7-hydroxy-4-oxo-4*H*-chromene-2-carboxylate (1.17 g, 5 mmol) and 3-dimethylaminopropylamine (1.53 g, 15 mmol) were dissolved in dichloromethane (10 ml) and allowed to reflux for 1 h. The solvent was evaporated, glacial acetic acid (10 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The aqueous layer was neutralised with sodium hydrogen carbonate and extracted with ethyl acetate. The aqueous layer was made basic with sodium carbonate and

extracted with ethyl acetate. The aqueous layer was made acidic with concentrated HCl and then evaporated to dryness giving a yellow solid. The yellow solid was added to methanol and a white solid crystallised out, which was filtered off. The yellow filtrate was evaporated to dryness and repeatedly dissolved in methanol until no further white solid crystallised. The yellow mother liquor was evaporated to dryness yielding a yellow solid, which was dissolved in boiling ethanol, filtered and evaporated to dryness yielding a yellow solid (1.37 g, 4.7 mmol, 94%).

Mp 240 °C+; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3455, 3273, 3064, 2999, 1683, 1650, 1619, 1548, 1468, 1272, 786; UV/vis (MeOH): λ_{max} (nm) (log ε)=210 (3.92), 237 (3.80), 307 (3.46); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=2.00 (t, J=7.2 Hz, 2H, CH_2), 2.72 (s, 6H, 2×CH_3), 2.89 (t, J=7.2 Hz, 2H, CH_2), 3.02–3.18 (m, 2H, CH_2), 6.72 (s, 1H, H-3), 7.00 (dd, J=2.2, 8.8 Hz, 1H, H-6), 7.16 (d, J=2.2 Hz, 1H, H-5), 7.86 (d, J=8.8 Hz, 1H, H-8), 9.40 (t, J=5.9 Hz, 1H, N–H), 11.36 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=21.8 (−, CH_2), 36.0 (−, CH_2), 41.8 (+, 2×N–CH_3), 53.3 (−, CH_2), 102.7 (+, C-8), 110.2 (+, C-3), 115.8 (+, C-6), 116.3 (C_{quat}, C-9), 126.5 (+, C-5), 154.8 (C_{quat}, C-2), 156.9 (C_{quat}, C-10), 159.2 (C_{quat}, C=O), 163.4 (C_{quat}, C-7), 176.3 (C_{quat}, C-4); MS (ESI, DCM/MeOH +10 mmol/l NH_4Ac) m/z (%): 291.1 (100) $[MH]^+$. HRMS $(C_{15}H_{18}N_2O_4)^{+\cdot}$ Calcd: 290.1266. Found: 290.1266±0.7 ppm.

4.6.11. {3-[(7-Hydroxy-4-oxo-4*H*-chromene-2-carbonyl)-amino]-propyl}-trimethyl-ammonium iodide (10). 7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (436 mg, 1.5 mmol) was added to acetonitrile (30 ml) containing sodium carbonate (415 mg, 5 mmol). Iodomethane (227 mg, 1.6 mmol) was added to the suspension and allowed to stir at room temperature overnight. The reaction mixture was evaporated to dryness and resuspended in methanol. A white precipitate was filtered off and the resulting yellow mother liquor evaporated to dryness yielding a light yellow solid (439 mg, 1 mmol, 68%).

Mp 240 °C+; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3445, 3266, 3064, 2979, 1681, 1645, 1252, 832; ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.90–2.02 (m, 2H, CH_2), 3.07 (s, 9H, 3×CH_3), 3.27–3.40 (m, 4H, 2×CH_2), 6.66 (s, 1H, H-3), 6.80 (d, J=2.2 Hz, 1H, H-8), 6.83 (dd, J=2.2, 8.6 Hz, 1H, H-6), 7.79 (d, J=8.6 Hz, 1H, H-5), 9.15 (t, J=5.9 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=22.7 (−, CH_2), 36.2 (−, CH_2), 52.1 (+, 3×N–CH_3), 63.2 (−, CH_2), 102.3 (+, C-8), 110.2 (+, C-3), 114.5 (C_{quat}, C-9), 117.2 (+, C-6), 126.2 (+, C-5), 154.3 (C_{quat}, C-2), 157.4 (C_{quat}, C-10), 159.4 (C_{quat}, C=O), 167.1 (C_{quat}, C-7), 175.9 (C_{quat}, C-4); MS (ESI, H_2O/AcN) m/z (%): 305.0 (100) $[M]^+$; HRMS $(C_{16}H_{21}N_2O_4)^+$ Calcd: 305.1501. Found: 305.1500±1.23 ppm.

4.6.12. Ethyl-7-methoxy-4-oxo-4*H*-chromene-2-carboxylate (12a).[37] Sodium (1.49 g, 65 mmol) was dissolved in absolute ethanol (100 ml). Diethyloxalate (5.12 g, 35 mmol) and 2-hydroxy-4-methoxyacetophenone (2.49 g, 15 mmol) were dissolved in absolute ethanol (10 ml) and added to the sodium ethanolate solution. The solution was allowed to reflux for 1 h. Concentrated HCl was added dropwise until the reaction was acidic and a white precipitate formed. The white precipitate was filtered and the yellow solution concentrated to a slurry. The slurry was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to give a light yellow solid. The solid was recrystallised from methanol/diisopropylether (4:1) to yield a yellow solid (3.65 g, 14.7 mmol, 98%).

Mp 109 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3459, 3110, 2997, 2856, 1742, 1664, 1628, 1442, 1258, 838; UV/vis (MeOH): λ_{max} (nm) (log ε)=212 (4.44), 238 (4.29), 310 (4.01); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.34 (t, J=7.1 Hz, 3H, CH_3), 3.91 (s, 3H, O–CH_3), 4.38 (q, J=7.1 Hz, 2H, CH_2), 6.85 (s, 1H, H-3), 7.07 (dd, J=2.4, 8.9 Hz, 1H, H-6), 7.19 (d, J=2.4 Hz, 1H, H-8), 7.91 (d, J=8.9 Hz, 1H, H-5); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=13.8 (+, CH_3), 56.2 (+, O–CH_3), 62.5 (−, CH_2), 100.8 (+, C-8), 113.8 (+, C-3), 115.6 (+, C-6), 117.5 (C_{quat}, C-9), 126.7 (+, C-5), 151.5 (C_{quat}, C-2), 157.1 (C_{quat}, C-10), 159.9 (C_{quat}, C=O), 163.5 (C_{quat}, C-7), 176.1 (C_{quat}, C-4); MS (EI-MS, 70 eV) m/z (%): 248.1 (100) $[M]^{+\cdot}$. Elemental analysis: $C_{13}H_{12}O_5$ Calcd: C, 62.9; H, 4.87. Found: C, 62.67; H, 4.67.

4.6.13. 7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid butylamide (13a). Ethyl-7-methoxy-4-oxo-4*H*-chromene-2-carboxylate (590 mg, 2.4 mmol) and butylamine (512 mg, 7 mmol) were dissolved in dichloromethane (10 ml) and allowed to reflux for 10 min. The solvent was evaporated yielding a light yellow solid. Glacial acetic acid (10 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was poured into ice water, the precipitate was filtered off, washed with water and dried. The solid was recrystallised from dichloromethane/diisopropylether (1:1) to yield white needles (562 mg, 2.0 mmol, 86%).

Mp 130 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3349, 2959, 2869, 1655, 1610, 1357, 843; UV/vis (MeOH): λ_{max} (nm) (log ε)=212 (4.42), 236 (4.35), 255 (sh 4.10), 304 (4.04); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=0.97 (t, J=7.3 Hz, 3H, CH_3), 1.43 (qd, J=7.3, 14.4 Hz, 2H, CH_2), 1.64 (td, J=7.3, 14.9 Hz, 2H, CH_2), 3.48 (dd, J=6.9, 13.4 Hz, 2H, CH_2), 3.91 (s, 3H, O–CH_3), 6.89 (d, J=2.3 Hz, 1H, H-8), 6.99 (dd, J=1.9, 8.9 Hz, 1H, H-6), 7.09 (s, 1H, H-3), 8.10 (d, J=8.9 Hz, 1H, H-5); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=13.8 (+, CH_3), 20.1 (−, CH_2), 31.5 (−, CH_2), 39.8 (−, CH_2), 56.0 (+, O–CH_3), 100.4 (+, C-8), 112.2 (+, C-3), 115.0 (+, C-6), 118.2 (C_{quat}, C-9), 127.4 (+, C-5), 154.6 (C_{quat}, C 2), 157.0 (C_{quat}, C-10), 159.3 (C_{quat}, C=O), 164.7 (C_{quat}, C-7), 177.5 (C_{quat}, C 4); MS (CI-MS, NH_3) m/z (%): 276.2 (100) $[MH]^+$. Elemental analysis: $C_{15}H_{17}NO_4$ Calcd: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.43; H, 5.82; N, 4.91.

4.6.14. 7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (14a). Ethyl-7-methoxy-4-oxo-4*H*-chromene-2-carboxylate (620 mg, 2.5 mmol) and 3 dimethylaminopropylamine (766 mg, 7.5 mmol) were dissolved in dichloromethane (10 ml) and the reaction mixture was refluxed for 1 h. The solvent was evaporated, glacial acetic acid (10 ml) was added and the solution allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The aqueous layer was neutralised with sodium hydrogen carbonate and extracted with ethyl acetate. The aqueous

layer was made basic with sodium carbonate and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid (654 mg, 2.1 mmol, 86%).

Mp 127–128 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3440, 3257, 3032, 2950, 1667, 1630, 1442, 845; ^1H NMR (300 MHz, $CDCl_3$): δ (ppm)=1.79 (td, J=5.9, 11.8 Hz, 2H, CH_2), 2.36 (s, 6H, 2×CH_3), 2.51–2.58 (m, 2H, CH_2), 3.58 (td, J=5.2, 5.9 Hz, 2H, CH_2), 3.91 (s, 3H, O–CH_3), 6.80 (d, J=2.3 Hz, 1H, H-5), 6.99 (dd, J=2.3, 8.9 Hz, H-6), 7.05 (s, 1H, H-3), 8.11 (d, J=8.9 Hz, 1H, H-8), 9.28 (s, 1H, N–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=24.8 (−, CH_2), 40.6 (−, CH_2), 45.6 (+, 2×N–CH_3), 55.8 (+, O–CH_3), 59.2 (−, CH_2), 100.5 (+, C-8), 111.8 (+, C-3), 114.5 (+, C-6), 118.3 (C_{quat}, C-9), 127.5 (+, C-5), 155.1 (C_{quat}, C-2), 157.1 (C_{quat}, C-10), 159.2 (C_{quat}, C=O), 164.6 (C_{quat}, C-7), 177.7 (C_{quat}, C-4); MS (ESI, H_2O/AcN) m/z (%): 305.0 (100) $[MH]^+$; HRMS $(C_{16}H_{20}N_2O_4)^{+\cdot}$ Calcd: 304.1423. Found: 304.1421±0.44 ppm.

4.6.15. {3-[(7-Methoxy-4-oxo-4*H*-chromene-2-carbonyl)-amino]-propyl}-trimethyl-ammonium iodide (15a). 7-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (456 mg, 1.5 mmol) was dissolved in chloroform (10 ml), iodomethane (284 mg, 2 mmol) added and the reaction mixture was allowed to stir at room temperature for 1 h. The solution was heated to reflux for 10 min, the formed precipitate filtered off and washed with chloroform. The precipitate was dried yielding a white solid (575 mg, 1.3 mmol, 86%).

Mp 208 °C (decomposition); IR (KBr): $\tilde{\nu}$ (cm^{-1})=3490, 3411, 3257, 3012, 2954, 1676, 1633, 1604, 1442, 848; UV/vis (CH_3CN): λ_{max} (nm) (log ε)=210 (4.47), 242 (4.32), 304 (3.80); ^1H NMR (600 MHz, DMSO): δ (ppm)=1.97–2.03 (m, 2H, CH_2), 3.07 (s, 9H, 3×CH_3), 3.35–3.39 (m, 2H, CH_2), 3.36–3.41 (m, 2H, CH_2), 3.93 (s, 3H, O–CH_3), 6.78 (s, 1H, H-3), 7.13 (dd, J=2.4, 8.9 Hz, H-6), 7.18 (d, J=2.4 Hz, 1H, H-8), 7.97 (d, J=8.9 Hz, 1H, H-5), 9.19 (s, 1H, N–H); ^{13}C NMR (150 MHz, DMSO): δ (ppm)=22.7 (−, CH_2), 36.3 (−, CH_2), 52.2 (+, 3×N–CH_3), 56.1 (+, O–CH_3), 63.3 (−, CH_2), 100.8 (+, C-8), 110.6 (+, C-3), 115.3 (+, C-6), 117.5 (C_{quat}, C-9), 126.5 (+, C-5), 155.1 (C_{quat}, C-2), 156.9 (C_{quat}, C-10), 159.3 (C_{quat}, C=O), 164.3 (C_{quat}, C-7), 176.4 (C_{quat}, C-4); MS (ESI, H_2O/AcN) m/z (%): 319.0 (100) $[M]^+$; HRMS $(C_{17}H_{23}N_2O_4)^+$ Calcd: 319.1657. Found: 319.1661±1.16 ppm.

4.6.16. Ethyl-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate (12b). Sodium (1.49 g, 65 mmol) was dissolved in absolute ethanol (100 ml). Diethyloxalate (5.12 g, 35 mmol) and 2-hydroxy-5-methoxyacetophenone (2.49 g, 15 mmol) were dissolved in absolute ethanol (10 ml) and added to the sodium ethanolate solution. The solution was allowed to reflux for 1 h. Concentrated HCl was added dropwise until the reaction was acidic and a white precipitate formed. The white precipitate was filtered off and the yellow solution was concentrated to a slurry. The slurry was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to give a light yellow solid. The solid was recrystallised from methanol/diisopropylether (3:1) to yield a yellow solid (3.66 g, 14.8 mmol, 98%).

Mp 98 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3455, 3114, 3078, 2984, 2844, 1740, 1657, 1610, 1488, 1288, 839; UV/vis (MeOH): λ_{max} (nm) (log ε)=206 (4.45), 238 (4.22), 253 (4.31), 343 (3.77); ^1H NMR (600 MHz, $CDCl_3$): δ (ppm)=1.42 (t, J=7.1 Hz, 3H, CH_3), 3.89 (s, 3H, O–CH_3), 4.45 (q, J=7.1 Hz, 2H, CH_2), 7.10 (s, 1H, H-3), 7.32 (dd, J=3.2, 9.2 Hz, 1H, H-7), 7.53 (dd, J=0.4, 3.2 Hz, 1H, H-5), 7.54 (dd, J=0.4, 9.2 Hz, 1H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm)=14.1 (+, CH_3), 56.0 (+, O–CH_3), 62.9 (−, CH_2), 104.6 (+, C-5), 113.8 (+, C-3), 120.2 (+, C-8), 125.0 (+, C-7), 125.2 (C_{quat}, C-9), 150.8 (C_{quat}, C-10), 152.0 (C_{quat}, C-2), 157.5 (C_{quat}, C-6), 160.6 (C_{quat}, C=O), 178.3 (C_{quat}, C 4); MS (PI-EIMS, 70 eV) m/z (%): 248.0 (100) $[M]^{+\cdot}$. Elemental analysis: $C_{13}H_{12}O_5$ Calcd: C, 62.90; H, 4.87. Found: C, 62.67; H, 4.66.

4.6.17. 6-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid butylamide (13b). Ethyl-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate (590 mg, 2.4 mmol) and butylamine (512 mg, 7 mmol) were dissolved in dichloromethane (10 ml) and the reaction mixture was refluxed for 10 min. The solvent was evaporated upon which a light yellow solid formed. Glacial acetic acid (10 ml) was added and allowed to stir at 70 °C for 10 min. The mixture was added to ice water, the precipitate was filtered off, washed with water and dried. The solid was recrystallised from dichloromethane/diisopropylether (1:3) to yield white needles (572 mg, 2.1 mmol, 87%).

Mp 146 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3305, 3091, 2956, 2869, 1639, 1610, 1359, 833; UV/vis (MeOH): λ_{max} (nm) (log ε)=205 (4.43), 230 (4.25), 252 (4.36), 338 (3.75); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm)=0.98 (t, J=7.3 Hz, 3H, CH_3), 1.43 (qd, J=7.3, 14.3 Hz, 2H, CH_2), 1.65 (td, J=7.3, 14.9 Hz, 2H, CH_2), 3.49 (dd, J=7.1, 13.3 Hz, 2H, CH_2), 3.90 (s, 3H, O–CH_3), 7.15 (s, 1H, H-3), 7.31 (dd, J=3.1, 9.2 Hz, 1H, H-7), 7.45 (d, J=9.2 Hz, 1H, H-8), 7.56 (d, J=3.0 Hz, 1H, H-5); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=13.8 (+, CH_3), 20.1 (−, CH_2), 31.5 (−, CH_2), 39.8 (−, CH_2), 56.0 (+, O–CH_3), 105.1 (+, C-5), 111.2 (+, C-3), 119.4 (+, C-8), 124.6 (+, C-7), 125.1 (C_{quat}, C-9), 150.0 (C_{quat}, C-10), 154.6 (C_{quat}, C-2), 157.5 (C_{quat}, C-6), 159.3 (C_{quat}, C=O), 178.1 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 276.2 (100) $[MH]^+$. Elemental analysis: $C_{15}H_{17}NO_4$ Calcd: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.33; H, 5.92; N, 4.80.

4.6.18. 6-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (14b). Ethyl-6-methoxy-4-oxo-4*H*-chromene-2-carboxylate (620 mg, 2.5 mmol) and 3-dimethylaminopropylamine (766 mg, 7.5 mmol) were dissolved in dichloromethane (10 ml) and the reaction mixture was refluxed for 1 h. The solvent was evaporated, glacial acetic acid (10 ml) was added and the solution allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The aqueous layer was neutralised with sodium hydrogen carbonate and extracted with ethyl acetate. The aqueous layer was made basic with sodium carbonate and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid (615 mg, 2.1 mmol, 81%).

Mp 108–109 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3434, 3305, 3041, 2945, 1680, 1641, 1385, 830; ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.68 (p, J=6.9 Hz, 2H, CH_2), 2.15 (s, 6H, 2×CH_3), 2.28 (t, J=6.9 Hz, 2H, CH_2), 3.32 (dd, J=6.9, 13.3 Hz, 2H, CH_2), 3.86 (s, 3H, O–CH_3), 6.79 (s, 1H, H-3), 7.40 (d, J=3.1 Hz, 1H, H-5), 7.48 (dd, J=3.1, 9.1 Hz, 1H, H-7), 7.66 (d, J=9.1 Hz, 1H, H-8), 9.23 (t, J=5.6 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=26.4 (−, CH_2), 37.9 (−, CH_2), 45.0 (+, 2×CH_3), 55.7 (+, O–CH_3), 56.8 (−, CH_2), 104.5 (+, C-5), 109.3 (+, C-3), 120.2 (+, C-8), 124.1 (+, C-7), 124.3 (C_{quat}, C-9), 149.6 (C_{quat}, C-10), 155.4 (C_{quat}, C-2), 156.8 (C_{quat}, C-6), 158.7 (C_{quat}, C=O), 176.9 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 305.2 (100) $[MH]^+$; HRMS $(C_{16}H_{20}N_2O_4)^{+\cdot}$ Calcd: 304.1423. Found: 304.1425±0.71 ppm.

4.6.19. {3-[(6-Methoxy-4-oxo-4*H*-chromene-2-carbonyl)-amino]-propyl}-trimethyl-ammonium iodide (15b). 6-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (456 mg, 1.5 mmol) was dissolved in chloroform (10 ml), iodomethane (284 mg, 2 mmol) was added and the reaction mixture was allowed to stir at room temperature for 1 h. The solution was heated to reflux for 10 min, the formed precipitate filtered off and washed with chloroform. The precipitate was dried yielding a white solid (620 mg, 1.4 mmol, 93%).

Mp 240 °C+; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3422, 3298, 3031, 3013, 2945, 1680, 1651, 1615, 1485, 828; UV/vis (CH_3CN): λ_{max} (nm) (log ε)=205 (4.42), 248 (4.34), 333 (3.54); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.93–2.06 (m, 2H, CH_2), 3.07 (s, 9H, 3×CH_3), 3.30–3.41 (m, 4H, 2×CH_2), 3.87 (s, 3H, O–CH_3), 6.83 (s, 1H, H-3), 7.42 (d, J=3.1 Hz, 1H, H-5), 7.51 (dd, J=3.1, 9.2 Hz, 1H, H-7), 7.70 (d, J=9.2 Hz, 1H, H-8), 9.24 (t, J=5.9 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=22.6 (−, CH_2), 36.2 (−, CH_2), 52.1 (+, 3×CH_3), 55.7 (+, O–CH_3), 63.2 (−, CH_2), 104.5 (+, C-5), 109.5 (+, C-3), 120.3 (+, C-8), 124.2 (+, C-7), 124.3 (C_{quat}, C-9), 149.6 (C_{quat}, C-10), 155.1 (C_{quat}, C-2), 156.8 (C_{quat}, C-6), 159.2 (C_{quat}, C=O), 176.9 (C_{quat}, C-4); MS (ESI, H_2O/AcN) m/z (%): 319.0 (100) $[M]^+$. Elemental analysis: $C_{17}H_{23}N_2O_4I$ Calcd: C, 45.75; H, 5.19; N, 6.28. Found: C, 45.40; H, 5.14; N, 6.51. HRMS $(C_{17}H_{23}N_2O_4)^+$ Calcd: 319.1658. Found: 319.1660± 0.76 ppm.

4.6.20. Ethyl-5-methoxy-4-oxo-4*H*-chromene-2-carboxylate (12c).[37] Sodium (1.49 g, 65 mmol) was dissolved in absolute ethanol (100 ml). Diethyloxalate (5.12 g, 35 mmol) and 2-hydroxy-6-methoxyacetophenone (2.49 g, 15 mmol) were dissolved in absolute ethanol (10 ml) and added to the sodium ethanolate solution. The solution was allowed to reflux for 1 h. Concentrated HCl was added dropwise until the reaction was acidic and a white precipitate formed. The white precipitate was filtered off and the yellow solution concentrated to a slurry. The slurry was extracted with ethyl acetate, dried over Na_2SO_4 and evaporated to give a light yellow solid. The solid was recrystallised from methanol/diisopropylether (2:1) to yield a yellow solid (2.99 g, 12.1 mmol, 80%).

Mp 124 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3433, 3084, 2988, 2844, 1728, 1659, 1507, 1478, 1269, 800; UV/vis (MeOH): λ_{max} (nm) (log ε)=238 (4.22), 271 (4.00), 327 (3.64); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=1.40 (t, J=7.1 Hz, 3H, CH_3), 3.97 (s, 3H, O–CH_3), 4.42 (q, J=7.1 Hz, 2H, CH_2), 6.83 (d, J=8.5 Hz, 1H, H-6), 6.99 (s, 1H, H-3), 7.14 (dd, J=0.9, 8.5 Hz, 1H, H-8), 7.59 (t, J=8.5 Hz, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=14.1 (+, CH_3), 56.5 (+, O–CH_3), 62.8 (−, CH_2), 106.9 (+, C-6), 110.6 (+, C-8), 115.3 (C_{quat}, C-9), 116.5 (+, C-3), 134.7 (+, C-7), 150.3 (C_{quat}, C-2), 158.0 (C_{quat}, C 10), 159.8 (C_{quat}, C-5), 160.6 (C_{quat}, C=O), 178.0 (C_{quat}, C-4); MS (PI-EIMS, 70 eV) m/z (%): 248.0 (100) $[M]^{+\cdot}$. Elemental analysis: $C_{13}H_{12}O_5$ Calcd: C, 62.90; H, 4.87. Found: C, 62.84; H, 4.75.

4.6.21. 5-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid butylamide (13c). Ethyl-5-methoxy-4-oxo-4*H*-chromene-2-carboxylate (590 mg, 2.4 mmol) and butylamine (512 mg, 7 mmol) were dissolved in dichloromethane (10 ml) and the reaction mixture was allowed to reflux for 10 min. The solvent was evaporated giving a light yellow solid. Glacial acetic acid (10 ml) was added, the solution was allowed to stir at 70 °C for 10 min, added to ice water, the precipitate filtered off, washed with water and dried. The solid was recrystallised from dichloromethane/diisopropylether (2:1) to yield white needles (607 mg, 2.2 mmol, 93%).

Mp 128 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3314, 3090, 2961, 1650, 1605, 1387, 794; UV/vis (MeOH): λ_{max} (nm) (log ε)=238 (4.29), 260 (4.16), 323 (3.71); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=0.96 (t, J=7.3 Hz, 3H, CH_3), 1.35–1.47 (m, 2H, CH_2), 1.63 (td, J=7.3, 15.0 Hz, 2H, CH_2), 3.47 (dd, J=6.8, 13.6 Hz, 2H, CH_2), 3.97 (s, 3H, O–CH_3), 6.84 (d, J=8.4 Hz, 1H, H-6), 7.03 (s, 1H, H-3), 7.05 (d, J=8.4 Hz, 1H, H-8), 7.60 (t, J=8.4 Hz, 1H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm)=13.8 (+, CH_3), 20.1 (−, CH_2), 31.5 (−, CH_2), 39.7 (−, CH_2), 56.6 (+, O–CH_3), 107.0 (+, C-6), 109.8 (+, C-8), 113.7 (+, C 3), 115.0 (C_{quat}, C-9), 134.5 (+, C-7), 152.8 (C_{quat}, C-2), 157.3 (C_{quat}, C-10), 159.2 (C_{quat}, C-5), 160.1 (C_{quat}, C=O), 177.9 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 276.1 (100) $[MH]^+$. Elemental analysis: $C_{15}H_{17}NO_4$ Calcd: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.40; H, 6.00; N, 4.96.

4.6.22. 5-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (14c). Ethyl-5-methoxy-4-oxo-4*H*-chromene-2-carboxylate (620 mg, 2.5 mmol) and 3-dimethylaminopropylamine (766 mg, 7.5 mmol) were dissolved in dichloromethane (10 ml) and the reaction mixture was refluxed for 1 h. The solvent was evaporated, glacial acetic acid (10 ml) was added and the solution allowed to stir at 70 °C for 10 min. The mixture was added to ice water and extracted with ethyl acetate. The aqueous layer was neutralised with sodium hydrogen carbonate and extracted with ethyl acetate. The aqueous layer was made basic with sodium carbonate and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give a yellow solid (627 mg, 2.1 mmol, 83%).

Mp 87–88 °C; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3420, 3312, 3080, 2955, 1650, 1603, 798; ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.67 (p, J=6.9 Hz, 2H, CH_2), 2.15 (s, 6H, 2×CH_3), 2.27 (t, J=6.9 Hz, 2H, CH_2), 3.31 (dd, J=6.9, 13.3 Hz, 2H,

CH_2), 3.86 (s, 3H, O–CH_3), 6.61 (s, 1H, H-3), 7.02 (d, J= 8.4 Hz, 1H, H-6), 7.20 (dd, J=0.7, 8.4 Hz, 1H, H-8), 7.75 (t, J=8.4 Hz, 1H, H-7), 9.17 (t, J=5.6 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=26.4 (−, CH_2), 37.9 (−, CH_2), 45.0 (+, 2×CH_3), 56.0 (+, O–CH_3), 56.8 (−, CH_2), 107.4 (+, C-6), 109.9 (+, C-8), 111.8 (+, C-3), 114.0 (C_{quat}, C-9), 134.9 (+, C-7), 153.3 (C_{quat}, C-2), 156.9 (C_{quat}, C-10), 158.7 (C_{quat}, C-5), 159.1 (C_{quat}, C=O), 176.4 (C_{quat}, C-4); MS (CI-MS, NH_3) m/z (%): 305.2 (100) $[MH]^+$. HRMS $(C_{16}H_{20}N_2O_4)^{+\cdot}$ Calcd: 304.1424. Found: 304.1425±1.1 ppm.

4.6.23. {3-[(5-Methoxy-4-oxo-4*H*-chromene-2-carbonyl)-amino]-propyl}-trimethyl-ammonium iodide (15c). 5-Methoxy-4-oxo-4*H*-chromene-2-carboxylic acid (3-dimethylamino-propyl)-amide (456 mg, 1.5 mmol) was dissolved in chloroform (10 ml), iodomethane (284 mg, 2 mmol) was added and the reaction mixture was allowed to stir at room temperature for 1 h. The solution was heated to reflux for 10 min, the formed precipitate filtered off and washed with chloroform. The precipitate was dried yielding a white solid (625 mg, 1.4 mmol, 93%).

Mp 240 °C+; IR (KBr): $\tilde{\nu}$ (cm^{-1})=3433, 3244, 3018, 2940, 1683, 1650, 1602, 1475, 802; UV/vis (CH_3CN): λ_{max} (nm) (log ε)=242 (4.25), 319 (3.53); ^{1}H NMR (300 MHz, DMSO): δ (ppm)=1.92–2.04 (m, 2H, CH_2), 3.06 (s, 9H, 3×CH_3), 3.30–3.41 (m, 4H, 2×CH_2), 3.87 (s, 3H, O–CH_3), 6.65 (s, 1H, H-3), 7.04 (d, J=8.4 Hz, 1H, H-6), 7.23 (dd, J=0.7, 8.4 Hz, 1H, H-8), 7.77 (t, J=8.4 Hz, 1H, H-7), 9.17 (t, J=5.9 Hz, 1H, N–H); ^{13}C NMR (75 MHz, DMSO): δ (ppm)=22.6 (−, CH_2), 36.2 (−, CH_2), 52.1 (+, 3×CH_3), 56.1 (+, O–CH_3), 63.2 (−, CH_2), 107.5 (+, C-6), 109.9 (+, C-8), 112.0 (+, C-3), 114.0 (C_{quat}, C-9), 135.0 (+, C-7), 153.1 (C_{quat}, C-2), 156.9 (C_{quat}, C-10), 159.1 (C_{quat}, C-5), 159.2 (C_{quat}, C=O), 176.3 (C_{quat}, C-4); MS ESI, H_2O/AcN) m/z (%): 319.0 (100) $[M]^+$. HRMS $(C_{17}H_{23}N_2O_4)^+$ Calcd: 319.1658. Found: 319.1660±1.02 ppm.

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changes in UV absorption within experimental error were noted, regardless of substance tested or hair type (brown or bleached hair). After 60 min the hair was filtered and washed three times with fresh solvent. The hair sample was dried overnight at 40 °C and 200 mbar, placed into a crucible containing liquid nitrogen and ground to a fine powder. Due to the small quantities of substance on the hair, IR analysis of the ground sample could not confirm the presence of the substance when compared to the reference (for neither brown nor bleached hair).

30. A sample of the treated hair (bleached) and the corresponding reference sample were analysed using MALDI-MS. Here a definite peak at M^+ can be seen, when compared to the reference sample.
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Stereoselective synthesis of novel benzoins catalysed by benzaldehyde lyase in a gel-stabilised two-phase system

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Abstract—Asymmetric benzoin condensation was performed using recombinant benzaldehyde lyase (BAL) from *Pseudomonas fluorescens* Biovar I. To enable the conversion of hydrophobic substrates, the enzyme was entrapped in polyvinyl alcohol and suspended in hexane. Compared to the reported application of the biocatalyst in an aqueous phase containing 20% DMSO, the productivity of the resulting gel-stabilised two-phase system was 3-fold better. The entrapment process had an efficiency of >90%, no enzyme or cofactor was lost during reaction or storage. The entrapped enzyme was stable in hexane for 1 week at 4 °C and more than 1 month at −20 °C. Without preceding optimisation the novel benzoins (*R*)-1,2-di(3-furanyl)-2-hydroxyethanone, (*R*)-2-hydroxy-1,2-di(3-thienyl) ethanone, (*R*)-1,2-di(4-ethoxyphenyl)-2-hydroxyethanone, (*R*)-1,2-di(3-ethoxyphenyl)-2-hydroxyethanone, (*R*)-2-hydroxy-1,2-di(3-tolyl)ethanone, and (*R*)-1,2-di(benzofuran-2-yl)-2-hydroxyethanone were prepared with yields up to 31.8% and enantiomeric excess >99%.

1. Introduction

α-Hydroxyketones are important building blocks for the synthesis of several drugs and natural products.[1–12] For this reason, many chemical methods for their synthesis are described in literature.[13–21] Benzoins, derivatives of 1,2-diaryl-2-hydroxyethanone of special interest, are frequently derived by benzoin condensation, a traditional C–C bond forming reaction in organic chemistry[22,23] that uses either non-chiral catalysts[24–28] or chiral thiazolium and triazolium salts in a biomimetic manner.[29–32] Enzymatically, chiral benzoins can be obtained by reduction of α-diketones,[33–36] or kinetic resolution of racemic 2-peroxo-,[37] 2-hydroxy-[38–40] or 2-acetoxyketones.[7,41–43] Also, an enzyme-catalysed benzoin condensation using the benzaldehyde lyase from *Pseudomonas fluorescens* Biovar I (BAL) was recently established.[44–46] This last reaction has become of profound interest because of the outstanding stereoselectivity of the biocatalyst, which yields (*R*)-benzoins with 99% ee.[44] Moreover, symmetrical as well as unsymmetrical benzoin condensations[47] starting from a broad substrate range can be catalysed with BAL. However, a drawback of the BAL-catalysed synthesis of benzoins so far is the low solubility of many benzaldehyde substrates in aqueous media. To a certain extent, this solubility can be improved by the addition of 20% (v/v) of DMSO,[45] but the resulting reaction productivity is usually low.

In this study, BAL-catalysed benzoin condensations were performed using an alternative reaction system with two immiscible phases of water and hexane. The enzyme was entrapped in the aqueous phase, which was solidified by polyvinyl alcohol[48] to ease handling and recovery of BAL. The organic solvent was simultaneously used as reservoir for substrates and extracting agent for products. With the achieved gel-stabilised two-phase system, the synthesis of a couple of novel (*R*)-benzoins starting from substrates with moderate to low solubility in water was performed. For evaluation the synthesis of 2-furoin was compared to published data.[45]

2. Results and discussion

2.1. Performance of the PVA-stabilised two-phase system

The benefit of using BAL for the conversion of hydrophobic

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Table 1. BAL-catalysed syntheses of 2-furoin

BAL in	c_0 (mM)	c_t (mM)	t (h)	Conv. (%)	Pd. ($mg_{Product}/U_{BAL}$ h)
PVA-stabilised two-phase system	48.4	30.1	17.0	15.6[a]	0.3[b]
Buffer/20% DMSO	30.0	3.6	62.0	88.0	0.1[c]

[a] Calculated according to formula 1.
[b] Calculated according to formula 2.
[c] Calculated from data published by Demir et al.[45]

compounds in a gel-stabilised two-phase system was evaluated by comparing the synthesis of 2-furoin from 2-furaldehyde to data published for the same reaction by Demir et al.[45] It was found that conversion of substrate after 17 h was 15.6% (calculated from 2-furaldehyde concentration in the hexane phase according to formula 1), and a productivity of 0.3 mg 2-furoin per unit of BAL and hour of reaction was achieved (calculated according to formula 2). This was three times higher than the productivity that was calculated from published data[45] for synthesis in water/DMSO (Table 1). Evidently, the gel-stabilised two-phase system reveals favourable features for continuous synthesis of benzoins with BAL.

$$\text{Conv.} = 1 - \left[\frac{C^t_{\text{Hexane}}}{C^0_{\text{Hexane}}} + \frac{C^t_{\text{Hexane}} V_{\text{PVA}}}{P C^0_{\text{Hexane}} V_{\text{Hexane}}}\right] \quad (1)$$

where C^0 is the initial concentration of substrate in hexane or PVA; V, volume of the appropriate phases; P, partition of substrate between hexane and water. For the chosen concentration range the partition coefficient was constant ($P_{\text{2-furaldehyde}} = 0.7$), data not shown.

$$\text{Pd.} = 0.5 \frac{C^0_{\text{Hexane}} V_{\text{Hexane}} \text{Conv.}}{\text{Akt}_{\text{BAL}} t} \text{MW}_{\text{Benzoin}} \quad (2)$$

where C^0 is the initial concentration of substrate; V, volume; Conv., conversion of substrate; MW, molecular weight of reaction product; Akt., amount of employed enzyme; t, reaction time.

Investigation of the immobilisates revealed an immobilisation efficiency of 90.8%, no leaking of enzyme or cofactor during storage or use in hexane, and a storage stability of 1 week at 4 °C or more than 1 month at −20 °C. Immobilisates that were re-used after having completed a production cycle showed considerably lower activity than fresh immobilisates. Their activity, however, could be significantly improved by repeated washing with hexane. This finding indicates that residual product from the first reaction cycle remains in the matrix, which has a negative effect on the enzyme activity. Consequently, the performance of recycled immobilisates and even of the entire process should be improved, if hexane was replaced by another suitable solvent as extracting agent. Such optimisation is currently in progress.

2.2. Synthesis of novel benzoins

The PVA-stabilised BAL in hexane catalysed the asymmetric formation of the novel benzoin derivatives (*R*)-1,2-di(3-furanyl)-2-hydroxyethanone (**2a**), (*R*)-1,2-di(benzofuran-2-yl)-2-hydroxyethanone (**2b**), (*R*)-2-hydroxy-1,2-di(3-thienyl)ethanone (**2c**), (*R*)-1,2-di(3-ethoxyphenyl)-2-hydroxyethanone (**2e**), (*R*)-1,2-di(4-ethoxyphenyl)-2-hydroxyethanone (**2f**), and (*R*)-2-hydroxy-1,2-di(3-tolyl)-ethanone (**2h**) from the corresponding substituted aryl aldehydes (Table 2). The productivities of these reactions were at least comparable to the synthesis of 2-furoin. The synthesis of 3-furoin from **2a** ran with an even 4-fold higher productivity. Obviously, the continuous supply of the entrapped enzyme with fresh substrate from the surrounding organic solvent countervailed the overall low concentrations in the aqueous PVA-matrix itself. Limitations were found for substrates with a $\log P_{\text{OW}}$ higher than 3, like 4-isopropylbenzaldehyde (**1i**). For this compound only a very slight conversion within 72 h was observed in TLC analysis. It should be possible, however, to improve the conversion of such hydrophobic compounds by optimising the phase composition of the gel-stabilised two-phase system. In contrast, the also observed low or absent conversion of the *ortho*-substituted substrates 2-tolualdehyde (**1g**) and 2-ethoxybenzaldehyde (**1d**) can be attributed to steric hindrances of the respective

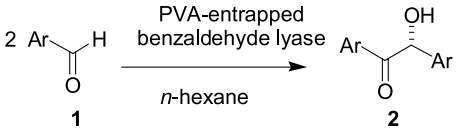
Table 2. Benzoin condensation of hydrophobic aromatic aldehydes with entrapped BAL in *n*-hexane

(*R*)-Benzoins **2**	Ar	$\log P_{\text{OW}}$[a]	P[b]	c_0 (mM)	c_t (mM)	t (h)	Conv.[c] (%)	Pd.[d] ($mg_{Product}/U_{BAL}$ h)	$Y_{Product}$ (%)	ee (%)[e]
2a	3-Furanyl–	0.34	0.7	42.8	7.3	20	76.9	1.3 (0.4)	16.4	94.7
2b	2-Benzofuranyl–	1.45	6.0	50.0	32.0	31.25	33.3	0.6 (0.2)	6.6	74.7
2c	3-Thienyl–	1.71	2.1	39.2	18.1	49.75	48.3	0.3 (0.1)	6.9	>99
2d	2-$EtOC_6H_4$–	1.99	n.d.	50.0	50.0	72	—	—	—	—
2e	3-$EtOC_6H_4$–	1.99	18.5	27.1	18.8	31.25	29.7	0.3 (0.1)	26.0	>99
2f	4-$EtOC_6H_4$–	1.99	18.5	50.7	37.2	50.25	25.6	0.3 (0.1)	17.7	>99
2g	2-MeC_6H_4–	2.27	n.d.	50.0	>48.0	72	—	—	—	—
2h	3-MeC_6H_4–	2.27	18.5	50.0	30.5	31.25	38.2	0.6 (0.2)	31.8	>99
2i	4-^{i}PrC$_6$H$_4$–	3.02	n.d.	50.0	>48.0	72	—	—	—	—

n.d., not determined.
[a] Calculated by Ghose and Crippen's[52] fragmentation methodology.
[b] Calculated according to formula 3 at a volume ratio of hexane/PVA 4:1.
[c] Calculated according to formula 1.
[d] Calculated according to formula 2.
[e] Determined by HPLC analysis as described in Section 3.

substituents in the active site of the biocatalyst, which is in accordance to published data.[45]

The BAL-catalysed synthesis of **2c**, **2e**, **2f** and **2h** ran according to the known high selectivity of the biocatalyst for production of (*R*)-configurated benzoins.[44] In contrast, a minor enantiomeric excess was achieved in the synthesis of **2a** and **2b**. This decrease in enantiopurity, however, is not the result of a worse selectivity of BAL towards the corresponding aldehydes **1a** and **1b**, but rather to a spontaneous racemisation of furoins. In fact, it has been described[50] that for compounds with a 3-furancarbonyl moiety, the racemisation proceeds very quickly because of the favourable resonance interaction of the carbonyl group with the oxygen of the 3-furyl ring. Minor enantiopurity has also been described before in the synthesis of 2-furoin (Scheme 1).[45]



Scheme 1.

The racemisation of **2a** during the production process is demonstrated in Figure 1. While **2a** was detected within 1.5 h after initiation of the reaction, the corresponding (*S*)-enantiomer was observed for the first time after about 24 h. Subsequently, the total amount of both enantiomers increased only slightly, while the ratio between (*R*)- and (*S*)-enantiomer changed rapidly. After 94 h, the enantiomeric excess of **2a** had decreased to 72%, after a reaction time of 200 h an enantiomeric excess of only 42.0% was determined. Additional to racemisation the oxidation product of **2a**, 1,2-di(furan-3-yl)ethane-1,2-dione (**4a**, Scheme 2)[45,51] was detected after about 3 h of reaction. An analogous side reaction occurred in the synthesis of **2g**, but none of the other benzoins was oxidised during BAL-mediated carboligation. However, a complete oxidation of **2b** occurred at the attempt of concentrating the purified product, which accounts for the missing analytical data of this compound.

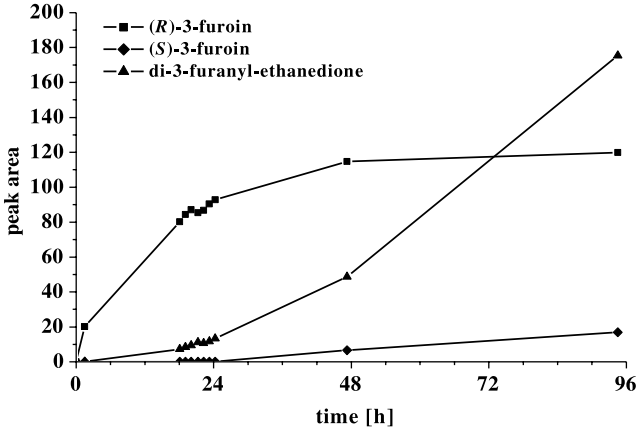
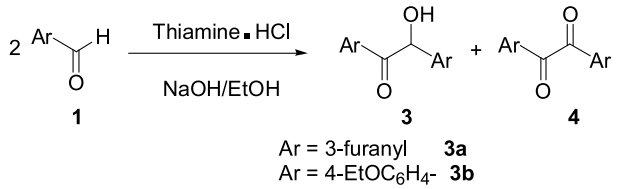


Figure 1. Time course of the formation of (*R*)-3-furoin and side products from 3-furaldehyde in a batch reaction containing 1 g of BAL-immobilisate and 4 mL of hexane.



Scheme 2.

It should easily be possible to prevent both racemisation as well as oxidation of the reaction products by appropriate adaptation of the production process, for example, by running a continuous synthesis under argon atmosphere with direct downstream processing of the products. Thus, the method described here for synthesis of benzoins with BAL provides a promising system for the stereoselective synthesis of benzoins overcoming solubility limitations. The easy transfer to the synthesis of hydrophobic cross-benzoins[47] and α-hydroxy ketones[44–46] can be expected.

3. Experimental

3.1. General methods

Enzymatic syntheses were performed in *n*-hexane (96%, HPLC grade, Scharlau). NMR spectra were recorded on a Bruker 500. Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^{1}H: δ 7.27) and $CDCl_3$ (^{13}C: δ 77.0). Column chromatography purifications were conducted on silica gel 60 (40–63 μm). TLC was carried out on aluminium sheets precoated with silica gel $60F_{254}$ (Macherey-Nagel, Merck), the spots were visualised with UV light (λ=254 nm). Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Elementary microanalyses were carried out in the corresponding 'Centro de Apoyo a la Investigación' of the Complutense University, Madrid, using a Leco® CHNS 932 equipment.

HPLC analysis was performed with a chiral column Chiralcel OD (cellulose carbamate, 25 cm×0.46 cm i.d.) at room temperature using a Thermo/Separation Consta Metric® 4100 Quaternary Solvent Delivery Systems, a SpectroMonitor® 5000 equipped with a Photo Diode Array Detector and a Knauer Chiral Detector A1000, and a mobile phase of *n*-hexane/2-propanol 90:10 at a flow rate of 0.8 mL/min; alternatively the analysis was performed on a chiral column Chiralpak AD-H (amylose tris(3,5-dimethylphenylcarbamate), 25 cm×4.6 cm i.d.) at 20 °C using a Beckman Coulter System Gold equipped with an autosampler Triathlon and a Diode Array Detector Module 168, and a mobile phase of *n*-hexane/2-propanol 90:10 at a flow rate of 0.75 mL/min.

3.2. Preparation of immobilised BAL

Expression and purification of BAL were performed using the recombinant *E. coli* $SG13009_{prep4}$[pBAL-His_6] according to Iding et al.[49] One unit of activity is defined as the amount of enzyme, which catalyses the cleavage of 1 μmol benzoin per min at 30 °C and pH 8.0 in aqueous solution. For the immobilisation in polyvinyl alcohol (PVA), a mixture of 10% (w/v) PVA Mowiol 10-98 (degree of

polymerisation: 1.400) and 10% (w/v) PEG 1000 was dissolved in bi-distilled water at 90 °C, cooled down to room temperature, reacted with 30 mg NaOH/g PVA and stirred for 30 min. The pH was adjusted to 8.0 with concentrated HCl, then 0.5 U/mL of BAL, 0.1 mmol/L of thiamine diphosphate and 2.5 mmol/L of $MgSO_4$ were added. The solution with a final PVA-concentration of 8.9% (w/v) was dropped into chilled silicon oil (−24 °C) and converted to a hydrogel by slowly thawing to room temperature. The obtained PVA-beads were washed with and stored in hexane.

3.3. Determination of partition coefficients

The partition of substrates between the aqueous PVA phase (10 mL) and hexane (40 mL) was determined by measuring the concentration of the respective compound in the solvent before and after 48 h of equilibration. All beads were free of enzyme. The partition coefficient (P) was calculated according to Eq. 3.

$$P = \frac{C^{\infty}_{\text{Hexane}} V_{\text{PVA}}}{\left(C^{0}_{\text{Hexane}} - C^{\infty}_{\text{Hexane}}\right) V_{\text{PVA}}} = \frac{C^{\infty}_{\text{Hexane}}}{C^{\infty}_{\text{PVA}}} \quad (3)$$

where C is the initial (0) or equilibrium (∞) concentration of the partitioning compound; V, volume of the appropriate phases.

3.4. Enzymatic carboligation

Ten grams of immobilisate were added to 40 mL hexane containing the respective substrate. The two-phase system was set under argon atmosphere and stirred on a rotary shaker at 30 °C. Samples drawn from the organic solvent were qualitatively analysed by TLC using *n*-hexane/ethyl acetate 7:3 or petrol ether/AcOEt 4:1 as mobile phase. Quantitative analysis was performed by HPLC. At the end of conversion the immobilisates were filtered out, and products were purified and analysed as described above.

3.5. Chemical synthesis of racemic benzoins

Racemic benzoins of 3-furancarbaldehyde (**1b**) and 4-ethoxybenzaldehyde (**1g**), which were used as reference compounds for analysis of enantiomeric excess, were synthesised using a variation of the classical chemical thiamine-catalysed benzoin synthesis methodology,[29–32] as depicted in Scheme 2.

For synthesising 1,2-di(furan-3-yl)-2-hydroxyethanone (**3a**), thiamine hydrochloride (1 g, 3 mmol) was dissolved in 3 mL of distilled-water. In a further step, 12.5 mL of 95% EtOH, 2 mL of NaOH 3 M, and 5 mL of **1b** (60 mmol) were added. The reaction was carried out in a Branson Sonifier 450 (Tunner 14, Dutycycle 20, Output control 2) for 48 h, and followed by TLC (hexane/ethyl acetate 5:1, v/v; **3a**, R_f=0.35; **1b**, R_f=0.20; **4a**, R_f=0.06). After that time, the reaction mixture was cooled in an ice bath, and was extracted by a continuous extraction system with *n*-hexane. The extract was dried over Na_2SO_4 and concentrated under low pressure, giving crystals of **3a** and **4a**. Residual **3a** dissolved in the water was recovered by adding HCl and extracting the mixture with $CHCl_3$ (3×50 mL). All the extracts were combined, dried over Na_2SO_4 and concentrated under low pressure. The resulting mixture was then passed through a column of SiO_2 using *n*-hexane/AcOEt 5:1 (v/v), as mobile phase to afford **4a** (20 mg) and **3a** (110 mg, 0.57 mmol) as a white crystalline solid. The analysis (^{1}H NMR and ^{13}C NMR) of **3a** were concordant with those obtained for **2b**.

For synthesising 1,2-bis(4-ethoxyphenyl)-2-hydroxyethanone (**3b**), a mixture of thiamine hydrochloride (1 g, 3 mmol) and H_2O (3 mL), EtOH (12.5 mL) and NaOH 3 M (2 mL) was placed in a 50 mL flask. 4-Ethoxybenzaldehyde (**1g**) 8.34 mL were added and the reaction was carried out in Branson Sonifier 450 (Tunner 14, Dutycycle 20, Output control 2). After 2 days, an extra amount thiamine hydrochloride (1 g) and NaOH 3 M (2 mL) were added. The reaction was followed by TLC (*n*-hexane/AcOEt 7:3, (v/v); **4b**, R_f=0.525; **1g**, R_f=0.43; **3b**, R_f=0.23). After 3 days, the reaction mixture was extracted with *n*-hexane (8×25 mL); the extracts were combined, dried over Na_2SO_4 and concentrated under low pressure. The resulting oil was dissolved in $HCCl_3$ and passed through a column of SiO_2 using the above mentioned mobile phase to afford **4b**, **1g** and **3b**, as well as some amount of 4-ethoxybenzoic acid. All the fractions were concentrated under low pressure, and added to 100 mL of basic water, and 100 mL of $HCCl_3$ were added to the fractions containing **3b** and 4-ethoxybenzoic acid. Finally, the $HCCl_3$ was dried over Na_2SO_4 and concentrated, giving **3b** (0.1965, 0.65 mmol) as a white crystalline solid, with analysis (^{1}H NMR and ^{13}C NMR) concordant with those carried out for **2g**.

3.5.1. (*R*)-1,2-Di(furan-3-yl)-2-hydroxyethanone (2a). White solid, ee>97%, $[\alpha]_D^{25}$ −110.8 (*c* 0.0066, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ: 4.09 (1H, d, J=5.18 Hz, OH), 5.48 (1H, d, J=4.55 Hz, H-2), 6.24 (1H, 2d, J=0.78, 0.73 Hz, H-4″), 6.70 (1H, 2d, J=0.79 Hz, H-4′), 7.32 (1H, t, J=1.58 Hz, H-5″), 7.36 (1H, t, J=1.81 Hz, H-5′), 7.47 (1H, s, H-2″), 7.91 (1H, dd, J=0.44, 0.87 Hz, H-2′); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 68.59 (C-2), 107.81 (C-4″), 107.89 (C-4′), 122.40 (C-3″), 123.44 (C-3′), 140.05 (C-2″), 143.01 (C-5″), 143.17 (C-5′), 147.59 (C-2′), 192.19 (C-2). Anal. Calcd for $C_{10}H_8O_4$: C, 62.50; H, 4.20. Found: C, 62.06; H, 4.26.

3.5.2. (*R*)-2-Hydroxy 1,2-di(3-thienyl) ethanone ((*R*)-3,3′-thenoin) (2c). Yellowish oil, ee>99%, $[\alpha]_D^{25}$ −103.96 (*c* 0.005, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.34 (1H, d, J=6.03 Hz, OH), 5.84 (1H, d, J=6.01 Hz, H-2), 6.99 (1H, dd, J=4.99, 1.21 Hz, H-4″), 7.28 (1H, d, J=2.83 Hz, H-2″), 7.29 (1H, dd, J=2.91, 0.69 Hz, H-5″), 7.33 (1H, dd, J=2.70, 1.09 Hz, H-5′), 7.51 (1H, dd, J=5.13, 1.17 Hz, H-4′), 8.04 (1H, dd, J=2.84, 1.20 Hz, H-2′); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 72.42 (C-2), 124.21 (C-2″), 124.24 (C-4″), 126.57 (C-5″), 127.06 (C-4′), 127.25 (C-5′), 134.21 (C-2′), 192.39 (C-1). Anal. Calcd for $C_{10}H_8O_2S_2$: C, 53.55; H, 3.59; S, 28.59. Found: C, 53.52; H, 3.92; S, 27.36.

3.5.3. (*R*)-1,2-Bis(3-ethoxyphenyl)-2-hydroxyethanone (2e). Yellowish oil, ee>99%, $[\alpha]_D^{25}$ −128.2 (*c* 0.028, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.36 (3H, t,

$J=6.03$ Hz, CH_3), 1.38 (3H, t, $J=6.03$ Hz, CH_3), 3.96 (2H, m, CH_2), 4.07 (2H, m, CH_2), 4.59 (1H, d, $J=6.11$ Hz, OH), 5.86 (1H, d, $J=5.82$ Hz, H-2), 6.78 (1H, m, H-4″), 6.83 (1H, m, H-2″), 6.90 (1H, m, H-6″), 7.04 (1H, m, H-4′), 7.21 (1H, t, $J=7.92$ Hz, H-5″), 7.26 (1H, t, $J=7.96$ Hz, H-5′), 7.43 (1H, m, H-2′), 7.46 (1H, m, H-6′); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 14.44 (C–CH_3), 14.66 (C–CH_3), 63.66 (C–CH_2), 66.42 (C–CH_2), 73.20 (C-2), 113.70 (C-2′), 113.87 (C-4″), 114.66 (C-2″), 120 (C-4′), 120.93 (C-6′), 121.60 (C-6″), 129.62 (C-5′), 130.12 (C-5″), 134.71 (C-1″), 140.41 (C-1′), 159.06 (C-3′), 159.43 (C-3″), 198.71 (C-1). Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.91; H, 6.65.

3.5.4. (*R*)-1,2-Bis(4-ethoxyphenyl)-2-hydroxyethanone (2f). White solid, ee>99%, $[\alpha]_D^{25}$ −94.61 (*c* 0.010 g, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.36. (3H, t, $J=$ 7 Hz, CH_3), 1.39 (3H, t, $J=7.01$ Hz, CH_3), 3.96 (2H, c, $J=$ 6.99 Hz, CH_2), 4.03 (2H, c, $J=7$ Hz, CH_2), 4.57 (1H, d, $J=$ 6.02 Hz, OH), 5.83. (1H, d, $J=6.01$ Hz, H-2), 6.81 (2H, m, H-3′ and H-5′), 6.83 (2H, m, H-3″ and H-5″), 7.21 (1H, m, H-6″), 7.23 (1H, m, H-2″), 7.86 (1H, m, H-6′), 7.88 (1H, m, H-2′); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 14.58 (C–CH_3), 14.78 (C–CH_3), 63.40 (C–CH_2), 63.79 (C–CH_2), 75.23 (C-2), 114.28 (C-3′ and C-5′), 114.98 (C-3″ and C-5″), 128.98 (C-2′ and C-6′), 131.56 (C-2″ and C-4″), 126.10 (C-1′ and C-1″), 163.41 (C-4′), 197.32 (C-1). Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.79; H, 6.70.

3.5.5. (*R*)-2-Hydroxy-1,2-di-*m*-tolylethanone (2h). Yellowish oil, ee>99%, $[\alpha]_D^{25}$ −123.6 (*c* 0.00275, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.29 (3H, s, CH_3), 2.34 (3H, s, CH_3), 4.53 (1H, d, $J=5.77$ Hz, OH), 5.89 (1H, d, $J=$ 5.46 Hz, H-2), 7.13 (1H, m, H-4″), 7.13 (1H, m, H-2″), 7.06 (1H, m, H-6″), 7.19 (1H, m, H-5″), 7.25 (1H, m, H-4′), 7.32 (1H, m, H-5′), 7.68 (1H, m, H-6′), 7.76 (1H, m, H-2′); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 21.27 (C–CH_3), 21.30 (C–CH_3), 76.15 (C-2), 124.93 (C-6′), 126.43 (C-6″), 128.23 (C-4″), 128.46 (C-5′), 128.93 (C-2′), 129.32 (C-5″), 129.57 (C-4′), 133.50 (C-1″), 134.69 (C-2″), 138.47 (C-1′), 138.67 (C-3′), 138.87 (C-3″), 199.14 (C-1). Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 79.86; H, 6.75.

3.5.6. 1,2-Di(furan-3-yl)ethane-1,2-dione (4a). Yellow solid, ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.53 (2H, s, H-1), 7.49 (2H, t, $J=1.60$ Hz, H-5), 6.93 (2H, d, $J=$ 1.51 Hz, H-4); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 109.5 (C4′), 123.35 (C3′), 144.37 (C5′), 152.30 (C2′), 184.49 (C=O). Anal. Calcd for $C_{10}H_6O_4$: C, 63.16; H, 3.18. Found: C, 63.24; H, 3.21.

3.5.7. 1,2-Bis(4-ethoxyphenyl)ethane-1,2-dione (4b). Yellow solid, ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.95 (4H, d, $J=8.88$ Hz, H-2′, H-6′, H-2″ and H-6″), 6.97 (4H, d, $J=8.91$ Hz, H-3′,H-5′, H-3″ and H-5″), 4.14 (4H, c, $J=$ 6.98 Hz, CH_2), 1.47 (6H, d, $J=6.98$ Hz, CH_3); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 14.99 (CH_3), 64.38 (CH_2), 115.08 (C3′, C5′, C3″ and C5″), 126.56 (C1′ and C1″), 132. 77 (C2′, C6′, C2″ and C6″), 164.68 (C4′ and C4″), 193.94 (C=O). Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08. Found: C, 72.42; H, 6.11.

3.5.8. 1,2-Di(benzofuran-2-yl)ethane-1,2-dione. Yellow solid, ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 8.04 (2H, d, H-3′ and H-3″), 7.79 (2H, d, $J=12.22$ Hz, H-4′ and H-4″), 7.66 (2H, dd, $J=8.5$, 0.85 Hz, H-7′ and H7″), 7.57 (2H, m, H-6′ and H-6″), 7.37 (2H, m, H-5′ and H-5″); ^{13}C NMR (250 MHz, $CDCl_3$) δ: 113.08 (C7′ and C7″), 121.52 (C3′ and C3″), 124.67 (C6′ and C6″), 124.85 (C5′ and C5″), 127.38 (C3a′ and C3a″), 130.43 (C4′ and C4″), 149.45 (C2′ and C2″), 157.05 (C7a′ and C7a″), 178.98 (both C=O). Anal. Calcd for $C_{18}H_{10}O_4$: C, 74.48; H, 3.47. Found: C, 74.49; H, 3.50.

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Synthesis and properties of 4,9-methanoundecafulvenes and their transformation to 3-substituted 7,12-methanocycloundeca[4,5]furo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones: photo-induced autorecycling oxidizing reaction toward amines

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Abstract—The synthesis and properties of 4,9-methanoundecafulvene [5-(4,9-methanocycloundeca-2′,4′,6′,8′,10′-pentaenylidene) pyrimidine-2,4,6(1,3,5*H*)-trione] derivatives **8a**,**b** were studied. Their structural characteristics were investigated on the basis of the ^{1}H and ^{13}C NMR and UV–vis spectra. The rotational barrier ($\Delta G^{\ddagger}$) around the exocyclic double bond of **8a** was found to be 12.55 kcal mol^{-1} by the variable temperature ^{1}H NMR measurement. The electrochemical properties of **8a**,**b** were also studied by CV measurement. Furthermore, the transformation of **8a**,**b** to 3-substituted 7,12-methanocycloundeca[4,5]furo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **16a**,**b** was accomplished by oxidative cyclization using DDQ and subsequent ring-opening and ring-closure. The structural details and chemical properties of **16a**,**b** were clarified. Reaction of **16a** with deuteride afforded C13-adduct **19** as the single product, and thus, the methano-bridge controls the nucleophilic attack to prefer endo-selectivity. The photo-induced oxidation reaction of **16a** and a vinylogous compound, 3-methylcyclohepta[4,5]furo[2,3-*d*]pyrimidine-2,4(3*H*)-dione **2a**, toward some amines under aerobic conditions were carried out to give the corresponding imines (isolated by converting to the corresponding 2,4-dinitrophenylhydrazones) with the recycling number of 6.1–64.0 (for **16a**) and 2.7–17.2 (for **2a**), respectively.

1. Introduction

Flavins are known to play an important role as co-factors in a wide variety of biological redox reactions.[1] The flavin-redox systems have been investigated extensively through synthetic model systems and theoretical calculations.[2] Among these, 5-deazaflavins[3] and 5-deaza-10-oxaflavins[4] (2*H*-chromeno[2,3-*d*]pyrimidine-2,4(3*H*)-diones) have also been studied extensively in the hope of gaining mechanistic insight into flavin-catalyzed reactions. On the basis of the above observations, we have previously studied convenient preparations of 3,10-disubstituted cyclohepta[4,5]-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-diones **1a**–**c** and 3-substituted cyclohepta[4,5]furo[2,3-*d*]pyrimidine-2,4(3*H*)-diones **2a**–**c** (Fig. 1), which are the structural isomers of 5-deazaflavin and 5-deaza-10-oxaflavin, respectively, and their ability to oxidize some alcohols to the corresponding carbonyl compounds.[5,6] Furthermore, the synthesis, properties, and reactivity of cationic derivative

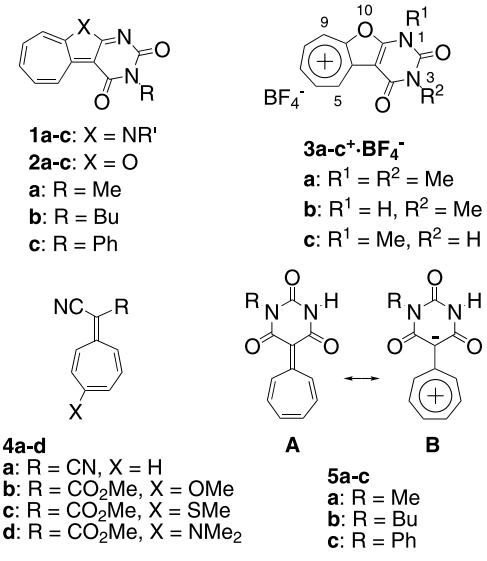

Figure 1.

Keywords: 5-(4,9-Methanocycloundeca-2′,4′,6′,8′,10′-pentaenylidene)-pyrimidine-2,4,6(1,3,5*H*)-trione; Rotational barrier; 7,12-Methanocycloundeca[4,5]furo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones; Photo-induced autorecycling oxidation.
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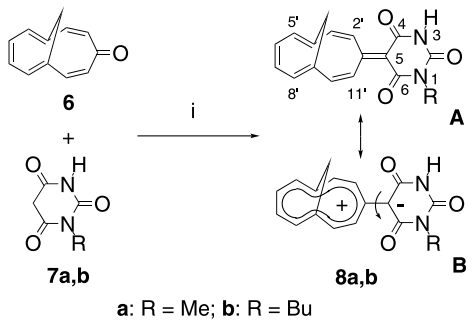


Scheme 1. Reagents and conditions: (i) Ac_2O, 120 °C, 1 h.

$\mathbf{3a}^+\cdot\mathbf{BF}_4^-$,[7] and its sulfur and nitrogen analogues[8,9] as well as their novel photo-induced autorecycling oxidizing reactions toward some alcohols were investigated.

On the other hand, heptafulvenes have intrigued chemists for several decades, especially in the context of the concept of aromaticity.[10–12] The properties and chemical behavior of substituted heptafulvenes are usually rationalized in terms of the differing contribution of the zwitterionic canonical structure. Thus, various heptafulvenes, such as 8,8-dicyanoheptafulvene (**4a**) (Fig. 1) and its derivatives **4b–d** have been synthesized and their properties and X-ray structure analyses have been studied.[13–15] Furthermore, the chemistry of the heptafulvenes derived by insertion of more complicated conjugated π-systems has also been studied in relation to the molecular design of organic dyes, highly polarized compounds, and electron acceptors or electron donors.[16,17] In this context, we have reported the synthesis and properties of novel heptafulvenes **5a–c**.[18] The contributions of their zwitterionic canonical structures and rotational barrier ($\Delta G^{\ddagger}$) around the exocyclic double bond have been clarified. Thus, the structurally modified heptafulvenes are interesting from the viewpoint of their novel properties and molecular functions. Based on this concept, we have now investigated the synthesis of 4,9-methanoundecafulvene derivatives **8a**,**b** (Scheme 1), which are vinylogous compounds of **5a–c**. From the variable temperature NMR and CV measurements, their properties and structural characteristics were studied. Furthermore, the transformation of **8a**,**b** to novel methano-bridged compounds **16a**,**b**, which are vinylogous compounds of **2a–c**, was accomplished by the oxidative cyclization and subsequent ring-opening and ring-closure. The structural details and chemical properties of **16a**,**b** were investigated, and the photo-induced oxidizing reaction of **16a** and a vinylogous compound **2a** toward some amines were studied as well. We report herein the results in detail.

2. Results and discussion

2.1. 4,9-Methanoundecafulvenes (8a,b)

Since heptafulvenes **5a–c** were synthesized by the reaction of tropone with corresponding barbituric acids,[18] the method was applied to synthesize novel 4,9-methanoundecafulvenes **8a**,**b** (Scheme 1). Condensation reactions of 4,9-methano[11]annulenone **6**[19] with monosubstituted barbituric acids **7a**,**b** in Ac_2O afforded **8a**,**b** as dark reddish or reddish powder in modest yields (Table 1). Compounds **8a**,**b** were fully characterized on the basis of the ^{1}H NMR, ^{13}C NMR, IR. UV–vis, and mass spectral data as well as elemental analyses. In the ^{13}C NMR spectra, the C5 carbon signals of the barbituric acid moiety in **8a**,**b** appear at low field (δ_C 108.2 and 108.0), suggesting the low electron density as compared with those of the C5 of **5a**,**b** (**5a**: δ_C 101.7, **5b**: δ_C 102.2) (Table 2).[18] Thus, contribution of the zwitterionic canonical structure **B** of **8a**,**b** (Scheme 1) seems to be less important as compared with those of **5a**,**b**; however, the 1,6-methano[11]annulenylium ion $\mathbf{9}^+$ (pK_{R+}, 6.2)[20] is more stable than the tropylium ion $\mathbf{10}^+$ (pK_{R+}, 3.9) (Fig. 2).[21] This feature is similar to the cases of dicyano-substituted heptafulvene and 4,9-methanoundecafulvene; the C12 carbon signal of 12,12-dicyano-4,9-methanoundecafulvene (δ_c 83.6)[22] is shifted to lower field as compared with the C8 carbon signal of 8,8-dicyanoheptafulvene **4a** (δ_c

Table 1. Results for the preparation of **8a**,**b**, cations $\mathbf{13a}^+\cdot\mathbf{BF}_4^-$ and $\mathbf{14a}^+\cdot\mathbf{BF}_4^-$, and **16a**,**b**

Run	Compound	Condensation	Oxidative cyclization		Ring-opening and ring-closure
		Product (yield/%)	Product (yield/%)	Ratio of $\mathbf{13}^+/\mathbf{14}^+$	Product (yield/%)
1	**7a**	**8a** (23)	$\mathbf{13a}^+\cdot\mathbf{BF}_4^-$ (28), $\mathbf{14a}^+\cdot\mathbf{BF}_4^-$ (56)	1: 2	**16a** (61)
2	**7b**	**8b** (30)	$\mathbf{13b}^+\cdot\mathbf{BF}_4^-$ (23), $\mathbf{14b}^+\cdot\mathbf{BF}_4^-$ (47)	1:2	**16b** (44)

Table 2. ^{1}H and ^{13}C NMR spectral data and rotational barriers ($\Delta G^{\ddagger}$) of methanoundecafulvenes **8a**,**b** and reference compounds **5a**,**b**

Compound	NMR data		Rotational barrier	
	^{1}H/δ	^{13}C/δ[a]	T_c/K	$\Delta G^{\ddagger}$/kcal/mol
8a	7.24[b]	108.2	258	12.55
8b	7.19[b]	108.0	—	—
5a[c]	9.31, 9.33[d]	101.7	300	14.51
5b[c]	9.28, 9.39[d]	102.2	308	14.70

[a] C5 of the barbituric acid moiety.
[b] H2′ and H11′.
[c] Ref. 18.
[d] H2′ and H7′.

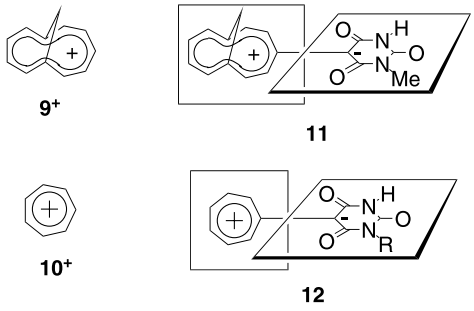


Figure 2.

Table 3. UV–vis spectral data of **8a**,**b** and **16a**,**b** in CH_3CN

Compound	λ_{max}/nm (log ε/dm^3 mol^{-1} cm^{-1})
8a	473 (3.99), 339 (4.05), 304 (4.11)
8b	472 (3.67), 339 (3.79), 304 (3.88)
16a	546 (4.04), 506 (3.70), 358 (3.82), 307 (3.67), 253 (3.58)
16b	545 (4.44), 507 (4.12), 358 (4.22), 306 (4.08), 251 (3.94)

Table 4. Redox potentials of **8a**,**b** and **16a**,**b** and reference compounds **2a**,**b** and **5a**,**b**

Compound	Redox potential[a]		Compound	Redox potential[a]	
	$E1_{red}$	$E1_{ox}$		$E1_{red}$	$E1_{ox}$
8a	−1.16	+0.83	**16a**	−0.89	—
8b	−1.15	+0.78	**16b**	−0.90	—
5a[b]	−1.15	+1.08	**2a**[c]	−1.12	—
5b[b]	−1.19	+1.09	**2b**[c]	−1.12	—

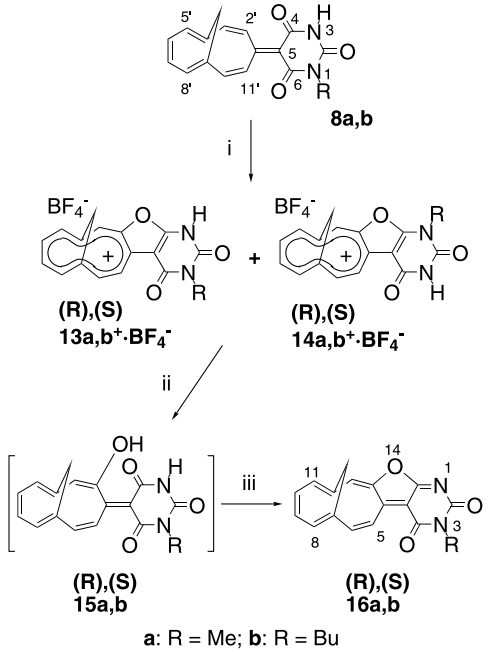
[a] V versus $Ag/AgNO_3$; cathodic and anodic peak potentials.
[b] Ref. 18.
[c] This work.

70.1).[23] Furthermore, the proton signals of the H2′ and the H11′ of **8a**,**b** appearing at δ 7.19–7.24 are remarkably shifted to higher field as compared with those of **5a**,**b** (δ 9.28–9.39).[18] These features are ascribed to the bending structure of the 11-membered ring of compounds **8a**,**b**, which do not allow the H2′ and the H11′ to locate in the deshielding region of the carbonyl groups in the barbituric acid moiety. In the ^{1}H NMR spectra of **8a**,**b** at room temperature, the signals of H2′ and H11′ (Scheme 1) appear as equivalent, while they appear as two sharp doublets at low-temperature (−60 °C). Thus, rapid free rotation around the exocyclic double bond of **8a**,**b** clearly occurs on the NMR time scale at room temperature. The fact shows that the enantiomers (*R*)-**8a**,**b** and (*S*)-**8a**,**b** isomerize each other. Through variable temperature ^{1}H NMR measurement of **8a**, the coalescence temperature was determined to be 258 K. In addition, the chemical shift difference between H-2′ and H-11′ of **8a** was 20.0 Hz. Consequently, rotational barrier ($\Delta G^{\ddagger}$) around the exocyclic double bond for **8a** was determined to be 12.55 kcal mol^{-1} (Table 2). The signals of H2′, H3′, H10′, and H11′ overlap for **8b** at low temperature, and thus, their rotational barriers ($\Delta G^{\ddagger}$) could not be determined. The value ($\Delta G^{\ddagger}$) of **8a** is smaller by 1.96 kcal mol^{-1} than that of **5a** (14.51 kcal mol^{-1}).[18] The feature is rationalized on the basis of the structural change of **8a** in the transition state of the rotation around the exocyclic double bond. At the transition state, the 11-membered ring and the barbituric acid moiety have to be twisted to become perpendicular to each other, and thus, the 11-membered ring would have a positive charge as the depicted structure **11** in Figure 2. Since the 1,6-methano[11]annulenylium ion **9**$^+$ (pK_{R+}, 6.2)[20] is more stable than the tropylium ion **10**$^+$ (pK_{R+}, 3.9),[21] the transition state of the rotation of **8a** would be more stabilized than that of **5a**. Thus, the rotational barrier ($\Delta G^{\ddagger}$) of **8a** would be smaller than that of **5a**.

The UV–vis spectra of **8a**,**b** are similar and the absorption maxima are summarized in Table 3. The longest wavelength absorption maxima of **8a**,**b** are longer by ca. 40 nm than that of **5a** (432 nm),[18] suggesting the elongated π-conjugation of compounds **8a**,**b** as compared with that of **5a**. Furthermore, the redox property of **8a**,**b** was determined by cyclic voltammetry (CV) in acetonitrile. The oxidation and reduction waves of **8a**,**b** were irreversible under the conditions of CV measurements, and thus, the peak potentials are summarized in Table 4. The first reduction potentials ($E1_{red}$) of **8a**,**b** are similar to those of **5a**,**b**, while the first oxidation potentials ($E1_{ox}$) of **8a**,**b** are less positive than those of **5a**,**b**.[18] These features support that compounds **8a**,**b** have the elongated π-conjugation as the vinylogous compounds of **5a**,**b**.

2.2. 7,12-Methanocycloundeca[4,5]furo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones (16a,b)

As novel methodology for synthesizing the furan-ring, we have previously reported the oxidative cyclization of **5a** by using DDQ to give a mixture of **3b**$^+\cdot$**BF**$_4^-$ and **3c**$^+\cdot$**BF**$_4^-$ (Fig. 1), and thus, the transformation of **8a**,**b** to **16a**,**b** was investigated. Oxidation reactions of **8a**,**b** with DDQ in CH_2Cl_2 at room temperature and subsequent anion exchange reaction by using 42% aq HBF_4 in Ac_2O afforded possible racemic mixtures of **13a**,**b**$^+\cdot$**BF**$_4^-$ and **14a**,**b**$^+\cdot$**BF**$_4^-$, respectively (Scheme 2). The yields and ratios of **13a**,**b**$^+\cdot$**BF**$_4^-$ and **14a**,**b**$^+\cdot$**BF**$_4^-$ are summarized in Table 1. Upon treatment with an alkaline solution, the mixtures of **13a**,**b**$^+\cdot$**BF**$_4^-$ and **14a**,**b**$^+\cdot$**BF**$_4^-$ were hydrolyzed to give **15a**,**b**, which were recyclized by using TFA to



Scheme 2. Reagents and conditions: (i) (a) DDQ, CH_2Cl_2, rt, 2 h, (b) 42% aq HBF_4, Ac_2O, 0 °C, 1 h; (ii) aq K_2CO_3, EtOH, rt, 16 h, (iii) TFA, CH_2Cl_2, rt, 1 h.

afford **16a**,**b** in moderate to good yields, respectively.[6] Apparently, the oxidative cyclization of **8a**,**b** at the C2′ position and the C4-carbonyl group gives (*R*)-**13a**,**b**$^{+}\cdot$**BF**$_4^-$, while the cyclization at the C11′ position and the C4-carbonyl group affords (*S*)-**13a**,**b**$^{+}\cdot$**BF**$_4^-$. Furthermore, the oxidative cyclization of **8a**,**b** at the C2′ position and the C6-carbonyl group gives (*R*)-**14a**,**b**$^{+}\cdot$**BF**$_4^-$, while the cyclization at the C11′ position and the C6-carbonyl group affords (*S*)-**14a**,**b**$^{+}\cdot$**BF**$_4^-$. Thus, the oxidative cyclization results in the formation of racemic compounds (*R*),(*S*)-**13a**,**b**$^{+}\cdot$**BF**$_4^-$ and (*R*),(*S*)-**14a**,**b**$^{+}\cdot$**BF**$_4^-$. Accordingly, compounds **16a**,**b** are also racemic compounds (*R*),(*S*)-**16a**,**b**. Unfortunately, attempted optical resolution by means of recrystallization and chromatography is unsuccessful. Thus, racemic compounds (*R*),(*S*)-**13a**,**b**$^{+}\cdot$**BF**$_4^-$, (*R*),(*S*)-**14a**,**b**$^{+}\cdot$**BF**$_4^-$, and (*R*),(*S*)-**16a**,**b** are represented as **13a**,**b**$^{+}\cdot$**BF**$_4^-$, **14a**,**b**$^{+}\cdot$**BF**$_4^-$, and **16a**,**b**.

Compounds **16a**,**b** were fully characterized on the basis of the ^{1}H and ^{13}C NMR, IR, UV–vis, and mass spectral data, as well as elemental analyses. The UV–vis spectra of **16a**,**b** in CH_3CN are similar and the absorption maxima are summarized also in Table 3. The longest wave length absorption maxima (λ_{max}) of **16a**,**b** show a red-shift by ca. 107 nm as compared with those of **2a** (439 nm), suggesting the elongated π-conjugation of **16a**,**b**: the HOMO becomes higher and the LUMO becomes lower as compared with those of **2a**, respectively. The ^{1}H NMR spectra of **16a**,**b** are noteworthy, since the chemical shifts of bridged-annulene systems are quite useful in determining such structural properties as diatropicity and bond alternation. Unambiguous proton assignment was made by analyzing ^{1}H NMR, H–H COSY, and NOE spectra. Since a similar feature is observed in a series of **16a**,**b**, the chemical shifts of the bridge protons and selected coupling constants of the peripheral protons of **16a** are shown in Figure 3 together with those of the reference compounds **2a**,[6] **17**$^{+}\cdot$**BF**$_4^-$,[24] and **18**.[25] The large geminal coupling constant of the methylene protons ($J_{E,Z}$=11.3 Hz) supports the absence of a norcaradiene structure for **16a**. The bridge protons of **16a** appear at high-field (δ −0.05 and δ 0.47), and the peripheral protons appear in the aromatic region (δ 7.68 to 8.97), suggesting a large diatropic ring current.[26] The differences in vicinal coupling constants in **16a** are larger than those found in vinylogous compound **2a** and delocalized cationic species **17**$^{+}\cdot$**BF**$_4^-$, while they are smaller than those found in **18**. The fact suggests that the bond alternation of **16a** is larger than those of **2a** and **17**$^{+}\cdot$**BF**$_4^-$ and is smaller than that of **18**.

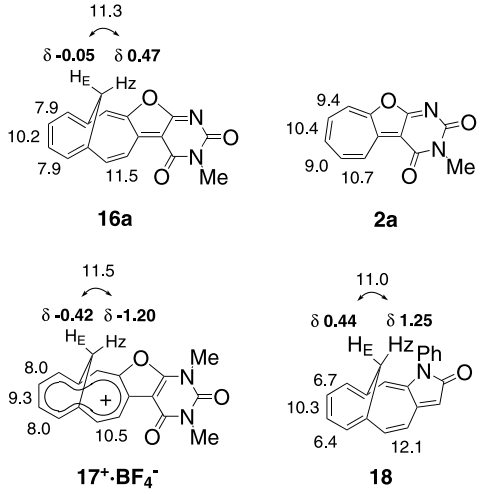
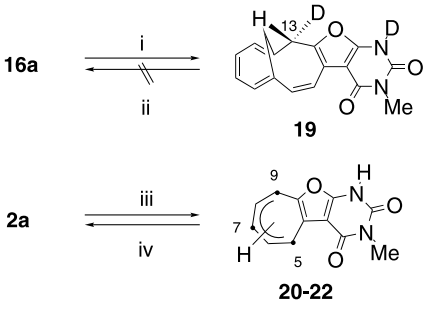


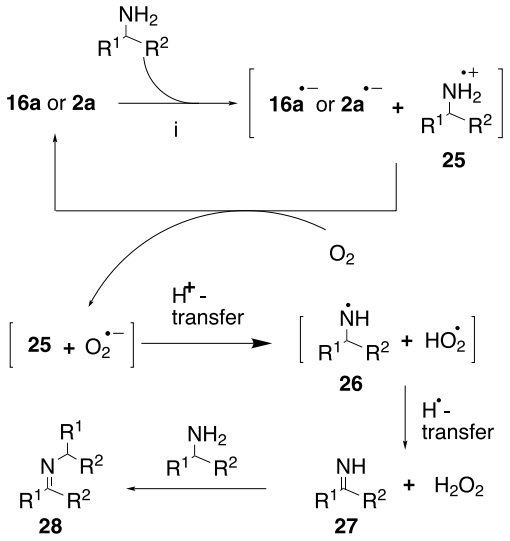
Figure 3. Chemical shifts and coupling constants of **16a**, **2a**, **17**$^{+}\cdot$**BF**$_4^-$, and **18**.

The reduction potentials of **16a**,**b** were determined by cyclic voltammentry (CV) in CH_3CN. The reduction waves of **16a**,**b** were irreversible under the conditions of the CV measurements; the peak potentials are summarized in Table 4, together with those of the vinylogous compound **2a**,**b**. The $E1_{red}$ of **16a**,**b** are less negative by 0.23 and 0.22 V than that of **2a**,**b**, respectively. This feature is rationalized by the elongated π-conjugation of **16a**,**b** as compared with **2a**,**b**. The irreversible nature is probably due to the formation of a radical species and its dimerization, as reported to be a typical property of uracil-annulated heteroazulenes.[5,6]

In order to clarify the reactivity, the reactions of **16a** with some nucleophiles were investigated. The reaction of **2a** with $NaBH_4$ proceeded at the C5, C7, and C9 to afford a mixture of three regio-isomers **20**–**22** (Scheme 3).[6] In contrast, the reaction of **16a** with $NaBD_4$ in CD_3OD in an NMR tube indicated clean formation of **19**, the deuterium of which locates at endo-position of the C13, and thus, the methano-bridge controls the nucleophilic attacks to prefer endo-selectivity. The results show that the methano[11]-annulene system has a possibility for a novel chiral auxiliary. Compound **19** is unstable under aerobic conditions and could not be isolated in pure form; however, satisfactory ^{1}H NMR spectral data are obtained. The mixture of three regio-isomers **20**–**22** was oxidized under aerobic conditions to regenerate **2a** quantitatively.[6] The CD_3OD solution of **19** was stirred at room temperature under aerobic conditions for 24 h; however, **19** was decomposed to result in the formation of a trace amount of **16a** in addition to a substantial quantity of unidentified materials. While we have reported the reactions of **2a** and **17**$^{+}\cdot$**BF**$_4^-$ with some amines,[5,24] reactions of **16a** with diethylamine and benzylamine did not proceed and the starting **16a** was recovered quantitatively.



Scheme 3. Reagents and conditions: (i) $NaBD_4$, CD_3OD, rt, 1 h; (ii) aerobic, CD_3OD, rt, 24 h; (iii) $NaBH_4$, CH_3OH, rt, 1 h; (iv) aerobic, CH_2Cl_2, rt, 24 h.



Scheme 4. Reagents and conditions: (i) hν, aerobic, CH_3CN, rt, 16 h.

2.3. Autorecycling oxidation of amines

We have previously reported that compounds **1a**,[5] $\mathbf{3a}^{+}\cdot\mathbf{BF}_4^{-}$,[27] and $\mathbf{17}^{+}\cdot\mathbf{BF}_4^{-}$[24] undergo autorecycling oxidation toward some amines under photo-irradiation. In this context and in a search for the functions of **16a**, we examined the oxidation of some amines (Scheme 4). We found that compound **16a** has oxidizing ability toward benzylamine, 1-phenylethylamine, hexylamine and cyclohexylamine to give the corresponding imines under aerobic and photo-irradiation conditions. Imine $R^1R^2C{=}NH$ **25** is produced at first; however, it reacts with another amine to result in the formation of $R^1R^2C{=}N{-}CHR^1R^2$ **26**. Oxidation reaction of some amines using **2a** was also carried out under similar conditions. The results are summarized in Table 5. Direct irradiation of the amines in the absence of **16a** and **2a** (named 'blank') gives the corresponding imines in low to modest yields. Thus, the yields are calculated by subtraction of the blank yield from the yield of the imine in the presence of **16a** and **2a**. The recycling numbers are more than one (Table 5, runs 1–8), and thus, autorecycling oxidation clearly proceeds. In the photo-induced oxidation of each amine, the yields of the imines by using **16a** are larger than the yields of the imines by using **2a**. Furthermore, the yields of photo-induced oxidation of normal amines are larger than those of secondary alkyl amines by using compounds **16a** (Table 5, runs 1–4). A similar feature is observed in the oxidation by using **2a**. Thus, the photo-induced oxidizing reaction by using **16a** and **2a** is more effective for the normal amines. We propose that the present autorecycling oxidation proceeds via electron-transfer from amine to the excited compounds **16a** and **2a** as shown in Scheme 4[5,27] The electron-transfer from amine to the excited state of **16a** and **2a** would occur to produce anion radicals of **16a** and **2a** and a cation radical **23**. An electron transfer from anion radical species **16a** and **2a** to molecular oxygen may give the superoxide anion radical and **16a** and **2a**, since tropyl radical derivatives are known to be oxidized readily by molecular oxygen.[28] Then, a proton-transfer from cation radical **23** to the superoxide anion radical may occur, and followed by formation of the products **25** and H_2O_2. Compound **25** reacts with excess amine to give imine **26**.

Table 5. Autorecycling oxidation of some amines by **16a** and **2a** under photo-irradiation[a]

Run	Compound	Amine	Yield/ μmol[b,c]	Recycling no.[d]
1	**16a**	$PhCH_2NH_2$	320.0	64.0
2	**16a**	$PhCH(Me)NH_2$	227.0	45.4
3	**16a**	Hexylamine	237.5	47.5
4	**16a**	Cyclohexylamine	30.6	6.1
5	**2a**	$PhCH_2NH_2$	86.0	17.2
6	**2a**	$PhCH(Me)NH_2$	79.5	15.9
7	**2a**	Hexylamine	66.0	13.2
8	**2a**	Cyclohexylamine	13.6	2.7

[a] An CH_3CN (16 mL) solution of compound **16a** or **2a** (5 μmol) and amines (2.5 mmol, 500 equiv) was irradiated by RPR-100, 350 nm lamps under aerobic conditions for 16 h.
[b] Isolated by converting to the corresponding 2,4-dinitrophenylhydrazone.
[c] The yield is calculated by subtraction of the 'blank' yield from the total yield.
[d] Recycling number of **16a** and **2a**.

3. Conclusion

Novel 4,9-methanoundecafulvene derivatives **8a**,**b** were synthesized for the first time. Their structural characteristics were clarified on the basis of the ^1H and ^{13}C NMR and UV–vis spectra. Through the variable temperature ^1H NMR measurement, the rotational barrier ($\Delta G^{\ddagger}$) around the exocyclic double bond of **8a** was determined to be 12.55 kcal mol^{-1}, which is smaller by 1.96 kcal mol^{-1} than that of the seven-membered vinylogue **5a** due to the higher stability of 1,6-methano[11]annulenylium ion $\mathbf{9}^{+}$. The electrochemical properties of **8a**,**b** were also clarified by CV measurement. Furthermore, the transformation of **8a**,**b** to 3-substituted 7,12-methanocycloundeca[4,5]-furo[2,3-*d*]pyrimidin-2,4(1*H*,3*H*)-diones **16a**,**b** was accomplished by oxidative cyclization and subsequent ring-opening and ring-closure. The physical properties of **16a** were studied by measurement of the UV–vis spectra, ^1H NMR, and redox potentials. Reaction of **16a** with deuteride afforded C13-adduct **19** as the single product, and thus, the methano-bridge controls the nucleophilic attacks to prefer endo-selectivity. The results show that the methano[11]-annulene system has a possibility for a novel chiral auxiliary. The photo-induced autorecycling oxidation of **16a** and **2a** toward some amines under aerobic conditions was carried out to give the corresponding imines (isolated by converting to the corresponding 2,4-dinitrophenyl-hydrazones) with the recycling number 6.1–64.0 (for **16a**) and 2.7–17.2 (for **2a**), respectively.

4. Experimental

4.1. General

IR spectra were recorded on a HORIBA FT-710 spectrometer. UV–vis spectra were recorded on a Shimadzu UV-3101PC spectrometer. Mass spectra and high-resolution mass spectra were run on JMS-AUTOMASS 150 and JMS-SX102A spectrometers. Unless otherwise specified, ^1H and ^{13}C NMR spectra were recorded on JNM-lambda500 and

AVANCE600 spectrometers, and the chemical shifts are given relative to internal $SiMe_4$ standard; *J*-values are given in Hz. Mps were recorded on a Yamato MP-21 apparatus and were uncorrected.

4.2. Preparation of 8a,b

A solution of each barbituric acid **7a** (568 mg, 4 mmol) and **7b** (736 mg, 4 mmol) and 4,9-methano[11]annulenone **6** (170 mg, 1 mmol) in Ac_2O (2 mL) was heated at 120 °C for 1 h. After the reaction was completed, the reaction mixture was concentrated in vacuo. The resulting residue was purified by column chromatography on Al_2O_3 by using AcOEt as the eluent to give the products **8a** (68 mg, 23%) and **8b** (101 mg, 30%).

4.2.1. Compound 8a. Dark reddish powder; mp 271–272 °C (from AcOEt); ^{1}H NMR (500 MHz, $CDCl_3$) δ −0.27 (1H, d, J=11.0 Hz, H_E), 1.58 (1H, d, J=11.0 Hz, H_Z), 3.34 (3H, s, NMe), 7.08–7.10 (2H, m, H-5′, H-8′), 7.19 (2H, d, J=11.5 Hz, H-3′, H-10′), 7.24 (2H, d, J=11.5 Hz, H-2′, H-11′), 7.41 (2H, m, H-6′, H-7′), 7.86 (1H, s. NH); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 27.6, 34.1, 108.2, 116.2, 122.5, 123.5, 127.0, 131.6, 132.2, 135.0, 135.1, 147.0, 150.3, 161.1, 162.7, 165.3 (one carbon overlapping); IR ($CHCl_3$) ν_{max} 1729, 1686, 1652, 1599, 1486, 1406, 1317 cm^{-1}; EIMS (70 eV) *m/z* (rel. intens) 294 (M^+, 1.4), 58 (100). Anal. Calcd for $C_{17}H_{14}N_2O_3\cdot H_2O$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.20; H, 4.96; N, 8.82.

4.2.2. Compound 8b. Reddish powder; mp 119–120 °C (from AcOEt); ^{1}H NMR (500 MHz, $CDCl_3$ 60 °C) δ −0.23 (1H, d, J=10.9 Hz, H_E), 0.96 (3H, t, J=7.4 Hz, Bu-4), 1.41 (2H, sext., J=7.4 Hz, Bu-3), 1.64 (2H, quint., J=7.4 Hz, Bu-2), 1.67 (1H, d, J=11.5 Hz, H_Z), 3.91 (2H, t, J=7.4 Hz, Bu-1), 7.03–7.05 (2H, m, H-5′, H-8′), 7.14 (2H, d, J=11.9 Hz, H-3′, H-10′), 7.19 (2H, d, J=11.9, Hz, H-2′, H-11′), 7.38 (2H, m, H-6′, H-7′), 7.62 (1H, s, NH); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 13.8, 20.2, 30.2, 34.1, 41.0, 108.0, 111.4, 116.3, 122.5, 131.6, 132.0, 133.9, 136.5, 136.8, 138.2, 150.0, 161.1, 162.5, 165.2 (one carbon overlapping); IR ($CHCl_3$) ν_{max} 2966, 2369, 2326, 1714, 1683, 1651, 1560, 1538, 1489, 1457, 1405 cm^{-1}; MS (MALDI) *m/z* 336 (M^+). Anal. Calcd for $C_{20}H_{20}N_2O_3\cdot$ AcOEt: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.77; H, 6.01; N, 7.36.

4.3. Preparation of mixtures of 13a,b$^+\cdot$BF$_4^-$ and 14a,b$^+\cdot$BF$_4^-$

To a solution of each **8a** (59 mg, 0.2 mmol) and **8b** (67 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) was added DDQ (70 mg, 0.3 mmol) and the mixture was stirred at rt for 2 h until the reaction was completed. After evaporation of the CH_2Cl_2, the residue was dissolved in Ac_2O (5 mL) and 42% HBF_4 (1 mL) at 0 °C, and the mixture was stirred for 1 h. To the mixture was added Et_2O (100 mL), and the precipitates were collected by filtration to give a mixture of **13a**$^+\cdot$BF$_4^-$ and **14a**$^+\cdot$BF$_4^-$ (64 mg, 84%) and a mixture of **13b**$^+\cdot$BF$_4^-$ and **14b**$^+\cdot$BF$_4^-$ (59 mg, 70%).

4.3.1. A mixture of compounds 13a$^+\cdot$BF$_4^-$ and 14a$^+\cdot$BF$_4^-$. Dark brown powder; (**13a**$^+\cdot$BF$_4^-$) ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.31 (1H, d, J=11.2 Hz, H_Z), −0.47 (1H, d, J=11.2 Hz, H_E), 3.37 (3H, s, NMe), 8.35–8.44 (2H, m, H-8 and H-11), 8.49 (1H, d, J=7.8 Hz, H-8 or H-11), 8.60 (1H, d, J=8.1 Hz, H-11 or H-8), 9.18 (1H, d, J=10.7 Hz, H-6), 9.63 (1H, d, J=10.7 Hz, H-5), 9.66 (1H, s, H-13), 9.81 (1H, bs, NH). (**14a**$^+\cdot$BF$_4^-$) ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.26 (1H, d, J=11.2 Hz, H_E), −0.46 (1H, d, J=11.2 Hz, H_E), 3.67 (3H, s, NMe), 8.35–8.44 (2H, m, H-8 and H-11), 8.49 (1H, d, J=7.8 Hz, H-8 or H-11), 8.60 (1H, d, J=8.1 Hz, H-11 or H-8), 9.16 (1H, d, J=10.7 Hz, H-6), 9.52 (1H, d, J=10.7 Hz, H-5), 9.68 (1H, s, H-13), 9.81 (1H, bs, NH).

4.3.2. A mixture of compounds 13b$^+\cdot$BF$_4^-$ and 14b$^+\cdot$BF$_4^-$. Dark brown powder; (**13b**$^+\cdot$BF$_4^-$) ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.26 (1H, dt, J=11.5, 1.7 Hz, H_Z), −0.42 (1H, d, J=11.5 Hz, H_E), 1.12 (3H, t, J=7.3 Hz, Bu-4), 1.43 (2H, sext., J=7.3 Hz, Bu-3), 1.68 (2H, quint., J=7.3 Hz, Bu-2), 4.04 (2H, t, J=7.3 Hz, Bu-1), 8.38–8.47 (2H, m, H-9 and H-10), 8.53 (1H, d, J=7.8 Hz, H-8 or H-11), 8.61 (1H, d, J=8.1 Hz, H-11 or H-8), 9.22 (1H, d, J=10.6 Hz, H-6), 9.69 (1H, s, H-13), 9.69 (1H, d, J=10.6 Hz, H-5). (**14b**$^+\cdot$BF$_4^-$) ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.21 (1H, dt, J=11.5, 1.7 Hz, H_Z), −0.41 (1H, d, J=11.5 Hz, H_E), 1.01 (3H, t, J=7.3 Hz, Bu-4), 1.50 (2H, sext., J=7.3 Hz, Bu-3), 1.86 (2H, quint., J=7.3 Hz, Bu-2), 4.24 (2H, td, J=7.3, 2.0 Hz, Bu-1), 8.38–8.47 (2H, m, H-9 and H-10), 8.53 (1H, d, J=7.8 Hz, H-8 or H-11), 8.61 (1H, d, J=8.1 Hz, H-11 or H-8), 9.19 (1H, d, J=10.5 Hz, H-6), 9.58 (1H, d, J=10.5 Hz, H-5), 9.69 (1H, s, H-13).

4.4. Preparation of 16a,b

To a solution of each mixture of **13a**,**b**$^+\cdot$BF$_4^-$ and **14a**,**b**$^+\cdot$BF$_4^-$ (0.05 mmol) in EtOH (2 mL) was added 2 M aq K_2CO_3 (1 mL), and the mixture was stirred at rt for 16 h. To the mixture was added satd aq NH_4Cl, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over Na_2SO_4 and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (2 mL) and TFA (0.2 mL), the mixture was stirred at rt for 1 h until the reaction was completed. To the solution was added satd aq $NaHCO_3$, and the mixture was extracted with CH_2Cl_2, and the extract was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by column chromatography on SiO_2 by using MeOH as the eluent to give the products **16a** (8.9 mg, 61%) and **16b** (7.3 mg, 44%).

4.4.1. Compound 16a. Reddish powder; mp>280 °C (from AcOEt); ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.05 (1H, d, J=11.3 Hz, H_E), 0.47 (1H, d, J=11.3 Hz, H_Z), 3.47 (3H, s, NMe), 7.68–7.71 (1H, m, H-8), 7.73–7.76 (1H, m, H-11), 7.83–7.87 (2H, m, H-9, H-10), 8.24 (1H, d, J=11.5 Hz, H-6), 8.58 (1H, s, H-13), 8.97 (1H, d, J=11.5 Hz, H-5); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 27.8, 35.0, 103.7, 13.6, 125.0, 131.7, 132.2, 132.3, 135.0, 135.2, 145.3, 147.0, 152.6, 158.6, 161.4, 174.1 (one carbon overlapping); IR ($CHCl_3$) ν_{max} 1696, 1621, 1577, 1313 cm^{-1}; MS (FAB) *m/z* 293 (M^++H). HRMS calcd for $C_{17}H_{12}N_2O_3$: 293.0950 (M+H). Found: 293.0943 (M^++H).

4.4.2. Compound 16b. Reddish powder; mp>280 °C (from AcOEt); ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.02 (1H,

d, $J=11.4$ Hz, H_E), 0.55 (1H, dt, $J=11.4$, 1.8 Hz, H_Z), 0.97 (3H, t, $J=7.3$ Hz, Bu-4), 1.43 (2H, sext., $J=7.3$ Hz, Bu-3), 1.70 (2H, quint., $J=7.3$ Hz, Bu-2), 4.08 (2H, m, Bu-1), 7.64–7.67 (1H, m, H-8), 7.69–7.72 (1H, m, H-11), 7.81–7.84 (2H, m, H-9, H-10), 8.19 (1H, d, $J=11.4$ Hz, H-6), 8.54 (1H, s, H-13), 8.96 (1H, d, $J=11.4$ Hz, H-5); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ 13.9, 20.4, 30.2, 35.1, 41.0, 77.4, 101.0, 123.5, 124.9, 131.5, 131.7, 132.0, 134.9, 135.0, 145.3, 146.8, 152.7, 158.0, 161.3, 174.1, (one carbon overlapping); IR ($CHCl_3$) ν_{max} 2361, 2333, 1712, 1624, 1575, 1546, 1510, 1303 cm^{-1}; MS (FAB) m/z 335 ($M^+ +$ H). HRMS calcd for $C_{20}H_{18}N_2O_3$: 335.1430 (M+H). Found: 335.1375 ($M^+ +$H). Anal Calcd for $C_{20}H_{18}N_2O_3 \cdot 2$AcOEt: C, 65.87; H, 6.71; N, 5.49. Found: C, 65.13; H, 6.57; N, 5.22.

4.5. Cyclic voltammetry of 8a,b and 16a,b

The redox potentials of **8a**,**b** and **16a**,**b** were determined by means of CV-27 voltammetry controller (BAS Co). A three-electrode cell was used, consisting of Pt working and counter electrodes and a reference $Ag/AgNO_3$ electrode. Nitrogen was bubbled through an acetonitrile solution (4 mL) of each compound (0.5 mmol dm^{-3}) and Bu_4NClO_4 (0.1 mol dm^{-3}) to deaerate it. The measurements were made at a scan rate of 0.1 V s^{-1} and the voltammograms were recorded on a WX-1000-UM-019 (Graphtec Co) X-Y recorder. Immediately after the measurements, ferrocene (0.1 mmol) ($E_{1/2}=+0.083$) was added as the internal standard, and the observed peak potentials were corrected with reference to this standard. The compounds exhibited no reversible redox wave: each of the redox potentials was measured through independent scan, and they are summarized in Table 4.

4.6. Reduction of 16a with $NaBD_4$ in CD_3OD

A solution of **16a** (8.7 mg, 0.03 mmol) and $NaBD_4$ (1.2 mg, 0.03 mmol) in CD_3OD (0.75 mL) in an NMR tube was shaken at rt for 5 min, and the ^{1}H NMR spectrum of the solution of **19** was recorded using Me_4Si as the internal standard. The reaction mixture was shaken under aerobic conditions for 24 h, and partial regeneration **16a** and decomposition were observed.

4.6.1. Compound 19. ^{1}H NMR (600 MHz, CD_3OD) δ 1.65 (1H, d, $J=11.4$ Hz, H_E), 4.01 (1H, s, H_{10}), 4.34 (1H, d, $J=11.4$ Hz, H_Z), 6.01 (1H, d, $J=5.0$ Hz, H_8), 6.15 (1H, d, $J=4.8$ Hz, H_{11}), 6.16 (1H, d, $J=12.8$ Hz, H_6), 6.52 (1H, dd, $J=10.8$, 5.0 Hz, H_9), 6.55 (1H, dd, $J=10.8$, 4.8 Hz, H_{10}), 6.77 (1H, d, $J=12.8$ Hz, H_5).

4.7. Reactions of 16a with diethylamine and benzylamine

Solutions of **16a** (3 mg, 0.01 mmol) and diethylamine or benzylamine (0.02 mmol) in CD_3OD (0.75 mL) in an NMR tube were heated at 65 °C, and the ^{1}H NMR spectra were recorded using Me_4Si as the internal standard. No reactions were observed. The resulting mixtures were purified by column chromatography on SiO_2 by using MeOH as the eluent to recover **16a** (diethylamine: 3 mg, 100%, benzylamine: 3 mg, 100%).

4.8. General procedure for autorecycling oxidation of amines in the presence of 16a and 2a

An CH_3CN (16 mL) solution of compound **16a** (1.47 mg, 5 μmol) or **2a** (1.14 mg, 5 μmol) and amines (2.5 mmol, 500 equiv) in a Pyrex tube was irradiated by RPR-100, 350 nm lamps under aerobic conditions for 16 h. The reaction mixture was concentrated in vacuo and diluted with Et_2O and filtered. The ^{1}H NMR spectra of the filtrates revealed the formation of the corresponding imines. The filtrate was treated with a saturated solution of 2,4-dinitrophenylhydrazine in 6% HCl to give 2,4-dinitrophenylhydrazone of the corresponding carbonyl compounds. The results are summarized in Table 5.

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Synthesis of the EF-ring segment of ciguatoxin CTX1B based on novel regioselective reduction of unsaturated cyanohydrins and ring-closing olefin metathesis

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Abstract—Aiming at a convergent total synthesis of ciguatoxin CTX1B, its EF-ring segment has been synthesized. During the synthesis, a novel method for the construction of branched ethers, based on regioselective reduction of γ-alkoxy β,γ-unsaturated α-silyloxy nitriles with borontrifluoride etherate and trialkyl silane or tributyltin hydride, has been developed. Combination use of the method and ring-closing olefin metathesis successfully provided medium-sized cyclic ethers. Efficient site-selective reduction of vinyl epoxides into homoallyl alcohols has also been developed.

1. Introduction

Ciguatoxin CTX1B is a causative toxin of ciguatera fish poisoning,[1,2] which afflicts more than 20,000 people in tropical and subtropical areas annually. The toxin is produced originally by the epiphytic dinoflagellate, *Gambierdiscus toxicus*, transferred to herbivorous fish, and accumulated subsequently in carnivorous fish through the food chain, thus causing human intoxication.[3] The symptoms of ciguatera are represented by diarrhea, vomiting, joint pain, prostration and unusual temperature perception disturbance called 'dry-ice sensation'. Generally, patients need several months to recover completely from these symptoms, which has resulted in serious social problems.

CTX1B was first isolated from the moray eel, *Gymnothorax javanicus*, by Scheuer and co-workers in 1967 and characterized as a polycyclic ether compound in 1980.[4] The whole structure of CTX1B, except for the absolute configuration and the relative configuration at C2, was elucidated from a purified sample of only 0.35 mg of CTX1B isolated from 4 t of *G. javanicus* by Yasumoto and co-workers in 1989.[5] They reported that the structure of CTX1B consisted of 12 *trans*-fused cyclic ethers, ranging from six- to nine-membered, and a five-membered spirocyclic ether at one end. Moreover, the absolute configuration of CTX1B was determined in 1997 as shown in Figure 1 by collaboration of Yasumoto, Hirama and Harada.[6]

The potent bioactivity of CTX1B is thought to result from the activation of voltage-sensitive sodium channels (VSSCs) in neuron cells by the strong binding of CTX1B to site 5 on the channel.[7] While it is known that the binding site on VSSC was shared by brevetoxins or another class of structurally related marine toxins,[8] the precise location of the receptor site for these toxins and the binding mode of CTX1B to the channel protein has not yet been elucidated.[9] However, the studies of ciguatoxin in neurology and hygiene have been impeded by the extremely limited availability of CTX1B from natural sources. Therefore, a synthetic supply of CTX1B on a practical scale is essential in order to solve the problem.

From the synthetic viewpoint, its unique *trans*-fused polycyclic ether structure and strong bioactivity have attracted the attention of synthetic chemists. The convergent construction of such large fused polyether structure has been a significant challenge. Intensive efforts by synthetic chemists aiming at the concise construction of the polyether framework as well as completion of the total synthesis of CTX1B and its congeners are ongoing.[10–13] In the course of

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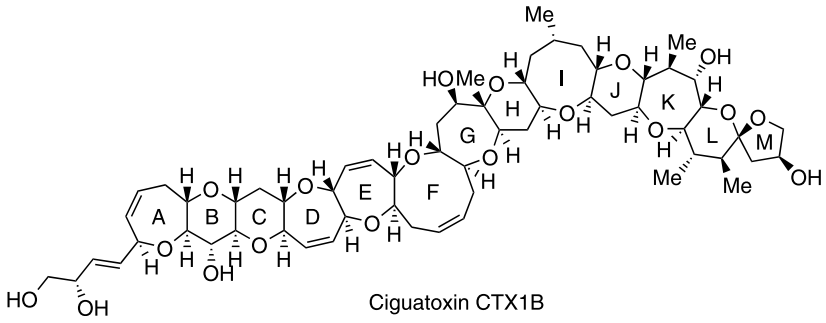
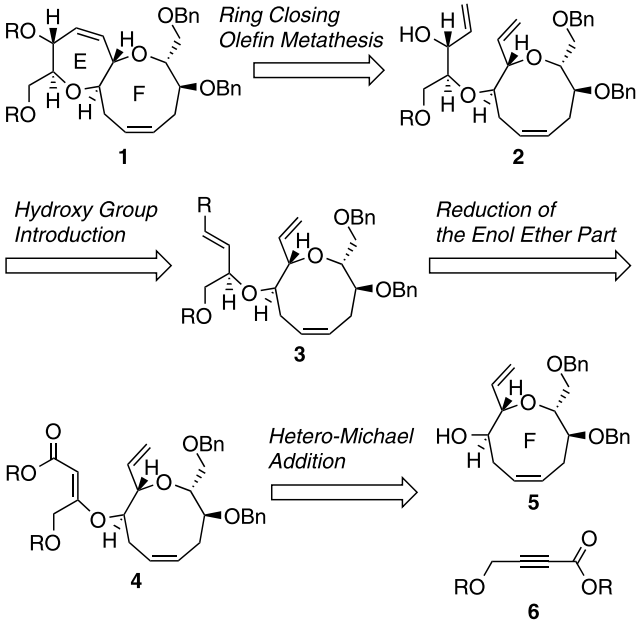


Figure 1.

our synthetic studies on CTX1B and its congeners,[14] we have established a method based on the coupling reaction of an acyl anion equivalent with an aldehyde for the convergent construction of a *trans*-fused 6/6 or 6/7 cyclic ether system in the middle part.[14g,i,k,15] Therefore, we designed the synthetic segments of CTX1B corresponding to the AB-, EF-, I-, and LM-ring parts, and planned to connect them at the CD-,[14i,k] GH-, and JK-ring[14i] parts at the later stage of total synthesis. So far, the AB-,[14l] I-,[14j] and LM-ring[14e] segments have been constructed. Here, synthesis of the remaining EF-ring segment based on a novel branched ether formation using regioselective reduction of unsaturated cyanohydrins and ring-closing olefin metathesis (RCM) is described.[14n] An efficient transformation reaction of a vinyl epoxide into a homoallyl alcohol, developed during the synthesis, is also disclosed.

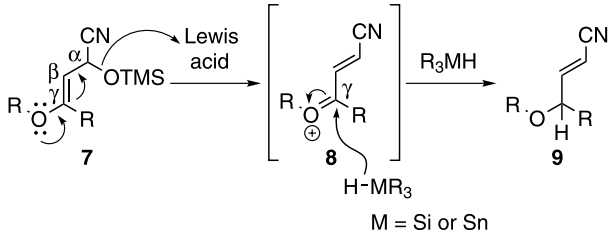
2. First generation synthesis of the EF-ring segment

First, we planned to synthesize the EF-ring segment **1** from the F-ring part **5** (Scheme 1). The synthetic route consisted of the following four processes: (i) construction of the E-ring in **1** from precursor diene **2** by Grubbs' RCM;[16,17] (ii) introduction of a hydroxy group to the β-position of the branched ether part of **3**; (iii) reduction of the 3-alkoxy-2-butenoate part of **4** and (iv) hetero-Michael addition of 2-butynoate ester **6** with the F-ring part **5** according to Paintner's procedure.[18]



Scheme 1. Retrosynthetic analysis for the EF-ring segment **1**.

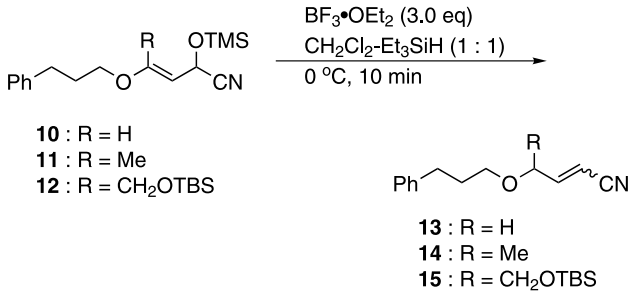
Grubbs' RCM has now become one of the most reliable methods among a number of approaches for the construction of medium-sized cyclic ethers, because it realizes efficient ring-closure under mild catalytic conditions and tolerates a wide variety of functional groups in its substrates.[10,16] Accordingly, we applied Grubbs' RCM to the construction of the E-ring in the process (i). On the other hand, preparation of the precursors for RCM involves a serious difficulty of the stereoselective construction of an acyclic branched ether part in each substrate. Recently, several successful methods based on an alkylation or an aldol reaction of a glycolate ester derivative,[11q,r,19] allyl[11a,f,t,y] or hydride[11e,w] addition to an acetal group, an addition reaction of an α-alkoxy carbon radical to a β-alkoxy propenoate ester,[11h,j,v] or ring cleavage of *C*-glycosides,[14j,20] have been developed to solve the problem. However, the number of methods is insufficient to meet the requirements for the synthesis of a variety of complex natural cyclic ethers. Therefore, a reductive transformation reaction shown in Scheme 2 was newly designed for the process (iii).[14n,21] We expected that γ-alkoxy β,γ-unsaturated α-silyloxy nitrile **7** would be activated by an appropriate Lewis acid to generate oxonium ion **8**, which would be selectively reduced at the γ-position into γ-alkoxy α,β-unsaturated nitrile **9** by a proper reducing agent. The nitrile group of **9** would be available for the synthesis of **2** (process (ii)).



Scheme 2. Lewis-acid-promoted γ-position selective reduction of a γ-alkoxy β,γ-unsaturated α-silyloxy nitrile system.

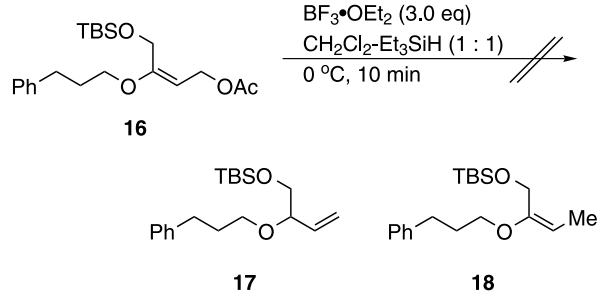
First, γ-position selective reduction of simple acyclic cyanohydrin derivatives **10**, **11**, and **12** was examined. We selected Et_3SiH as a reducing agent because of its efficient reducing ability toward the oxonium ion intermediate. After extensive exploration for effective activators of the cyanohydrins, $BF_3 \cdot OEt_2$ was found to give the best result. Selected successful examples are shown in Table 1. Every

Table 1. $BF_3 \cdot OEt_2$ promoted γ-position selective reduction of model compounds



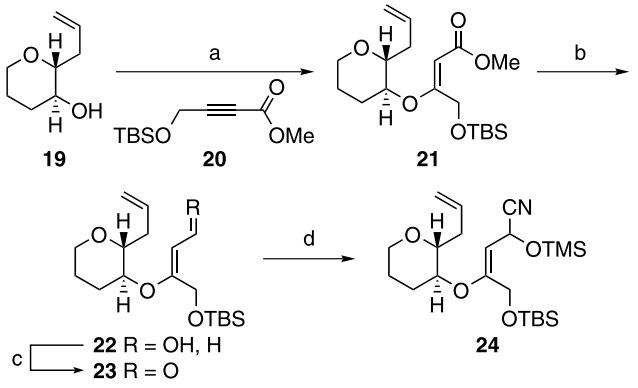
Entry	R	Yield (%)
1	H	64% (*E*:*Z*=6.2:1.0)
2	Me	62% (only *E*)
3	CH_2OTBS	64% (only *E*)

reaction was carried out in a 1:1 (v/v) mixture of Et_3SiH and CH_2Cl_2 in the presence $BF_3 \cdot OEt_2$ (3.0 equiv) at 0 °C and afforded the corresponding γ-alkoxy α,β-unsaturated nitrile (**13**, **14**, or **15**) in good yield. On the other hand, simple and easily available 3-alkoxy-2-propenyl acetate **16** did not produce the corresponding allyl or vinyl ether (**17** or **18**) under the same conditions. It only resulted in decomposition of the substrate. Thus, the γ-alkoxy β,γ-unsaturated α-silyloxy nitrile was found to be a good substrate (Scheme 3).



Scheme 3. An attempt to reduce 3-alkoxy-2-propenyl acetate **16**.

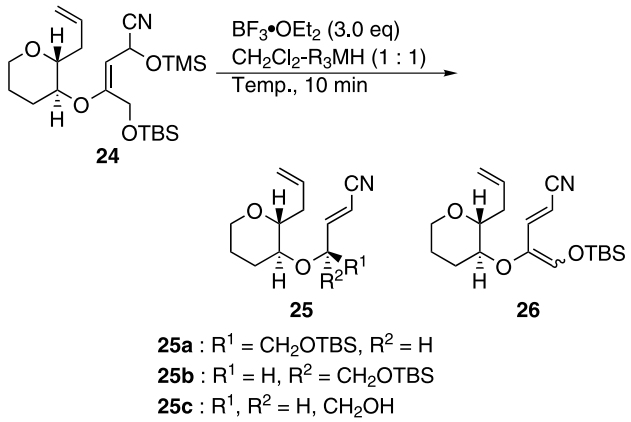
Next, encouraged by the results, we planned to synthesize a *trans*-fused 6/8 cyclic ether model, which has a side chain and a hydroxyl group with proper stereochemistry available for further construction of a *trans*-fused ether ring, from **19**[14g] and **20** according to the above strategy. Reduction precursor **24** was synthesized according to Scheme 4. Hetero-Michael addition of **19** to butynoate **20** in the presence of Me_3P afforded **21** in 95% yield.[22] Reduction of the ester **21** with DIBAH followed by oxidation with TPAP gave the aldehyde **23** in 88% yield. Treatment of **23** with TMSCN (2.5 equiv) in the presence of Me_3Al in benzene at ambient temperature gave **24** as a 1:1 mixture of diastereomers in 74% yield.



Scheme 4. Preparation of reduction precursor **24**. Reagents and conditions: (a) PMe_3 (1.1 equiv), **20** (1.7 equiv), CH_2Cl_2, 0 °C, →24 °C, 1 h, 95% (only *E*); (b) DIBAH (4.0 equiv), CH_2Cl_2, −78 °C, 10 min, ~100%; (c) TPAP (0.1 equiv), NMO (2.0 equiv), MS 4A, CH_2Cl_2, 24 °C, 50 min, 88%; (d) Me_3Al (1.1 equiv), TMSCN (2.5 equiv), benzene, 24 °C, 1 h, 74%.

The regioselective reduction of **24** under the same conditions as the case of acyclic models produced a mixture of **25a**,**b**, and **26** (3.6:6.4:1.0) in 49% yield along with alcohol **25c** in 14% yield (entry 1, Table 2).[23] Although the by-production of **25c** was reduced by lowering reaction temperature to −18 °C, the ratio of diene **26** increased (**25a**:**25b**:**26**=1.9:3.8:1.0, entry 2). In order to suppress the diene, we examined several organometallic hydrides. Trialkyl silanes (Et_3SiH, Et_2MeSiH, $EtMe_2SiH$, and Me_2PhSiH) gave almost the same result, and changing the bulkiness of their alkyl substituents did not affect the diene-formation (entries 2–5). On the other hand, Bu_3SnH provided a good result, where no production of **26** was observed, and the ratio of **25a** to **25b** slightly increased (entry 6). The stereochemistry at the newly formed stereocenters of **25a** and **25b** was determined after transformation of **25b** into bicyclic ether **31** (vide infra). Thus, efficient conditions for the γ-selective reduction of α-silyloxy nitrile **24** were found.

Table 2. $BF_3 \cdot OEt_2$ promoted reduction of **24** with several reducing reagents

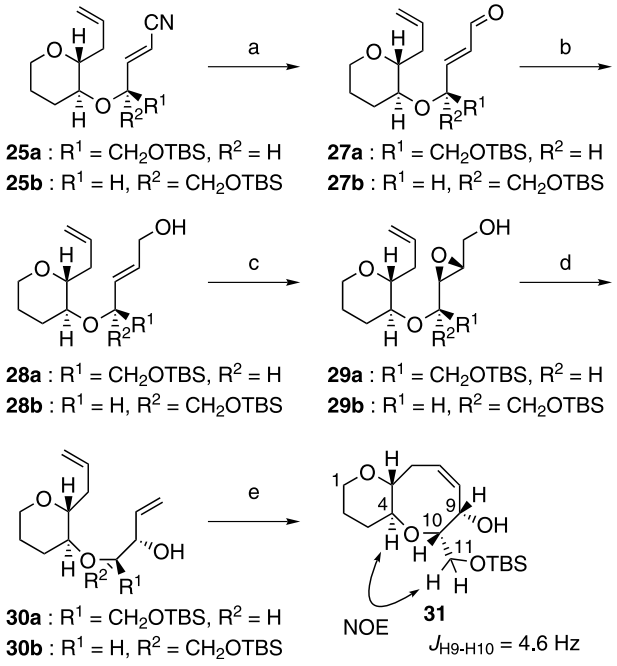


Entry	R_3MH	Temp.	**25a**:**25b**:**26** (Yield) **25c**
1	Et_3SiH	0 °C	3.6:6.4:1.0 (49%) 14%
2[a]	Et_3SiH	−18 °C	1.9:3.8:1.0 (71%)
3	Et_2MeSiH	−18 °C	1.8:3.9:1.0 (65%)
4	$EtMe_2SiH$	−18 °C	1.4:3.5:1.0 (74%)
5	Me_2PhSiH	−18 °C	2.0:4.2:1.0 (61%)
6	Bu_3SnH	−18 °C	1.0:1.3:0 (64%)

[a] Reaction period was 17 min.

Synthesis of a 6/8 bicyclic system from **25a** and **25b** is shown in Scheme 5. A ca. 1:2 mixture of **25a** and **25b** was converted to a mixture of allyl alcohols **28a** and **28b** (68%) by repeated reduction with DIBAH. The Katsuki-Sharpless asymmetric epoxidation[24] of the allyl alcohols using

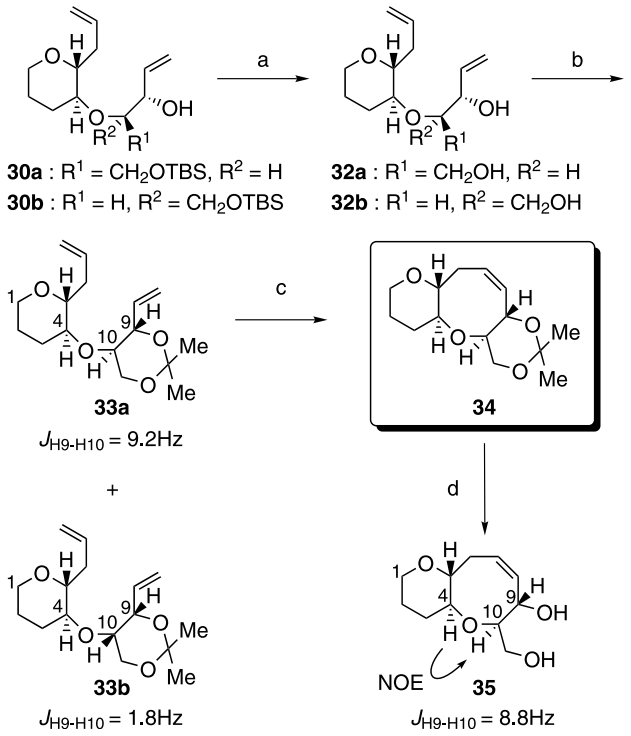
(−)-DET stereoselectively produced a mixture of epoxides **29a** and **29b** (~100%), which was treated with PPh_3 and I_2 in the presence of imidazole at ambient temperature to give allyl alcohols **30a** and **30b** (61%) as a 1:2 mixture.[25,26] Ring closure of dienes **30a** and **30b** in refluxing CH_2Cl_2 by Grubbs' second-generation catalyst[27] gave a mixture of products, in which only **31** was isolated as a bicyclic product (44%), and the desired *trans*-fused 6/8 bicyclic ether was not detected.[28] Stereochemistry of **31** was confirmed by the presence of NOE between H4 and H11 as well as the small *J* value between H9 and H10 (4.6 Hz). From the fact that the 1:2 ratio of the diastereomers was maintained throughout the transformation process from **25** to **30**, and that the yield of **31** (44%) was apparently higher than the ideal yield (33%) of the cyclization product from minor diastereomer **30a**, it was concluded that **31** was produced from **30b** and originated from **25b**.



Scheme 5. Synthesis of bicyclic ether **31**. Reagents and conditions: (a) DIBAH (2.5 equiv), CH_2Cl_2, −78 °C, 10 min, 68%; (b) DIBAH (3.0 equiv), CH_2Cl_2, −78 °C, 8 min, ~100%; (c) D-(−)-DET (0.8 equiv), $Ti(O^iPr)_4$ (0.7 equiv), TBHP (5.0 equiv), MS 4A, CH_2Cl_2, −40 °C, 30 min → −25 °C, 24 h, ~100%; (d) Ph_3P (5.0 equiv), imidazole (5.0 equiv), I_2 (4.0 equiv), THF, 25 °C, 45 min, 61%; (e) $(H_2IMes)(PCy_3)Cl_2Ru$=CHPh (10 mol%), CH_2Cl_2 (5 mM), reflux, 6 h, 44%.

The results from the final RCM step suggested that the vinyl groups of **30a** were apart from each other in the stable conformation of **30a**, and such orientation of the vinyl groups was inappropriate for the cyclization of **30a**. Therefore, we decided to prepare *bicyclic* RCM precursor **33a**, of which the vinyl groups would be placed in close proximity to each other, for successful ring closure in the synthesis of *trans*-fused-bicyclic ether **34** (Scheme 6).

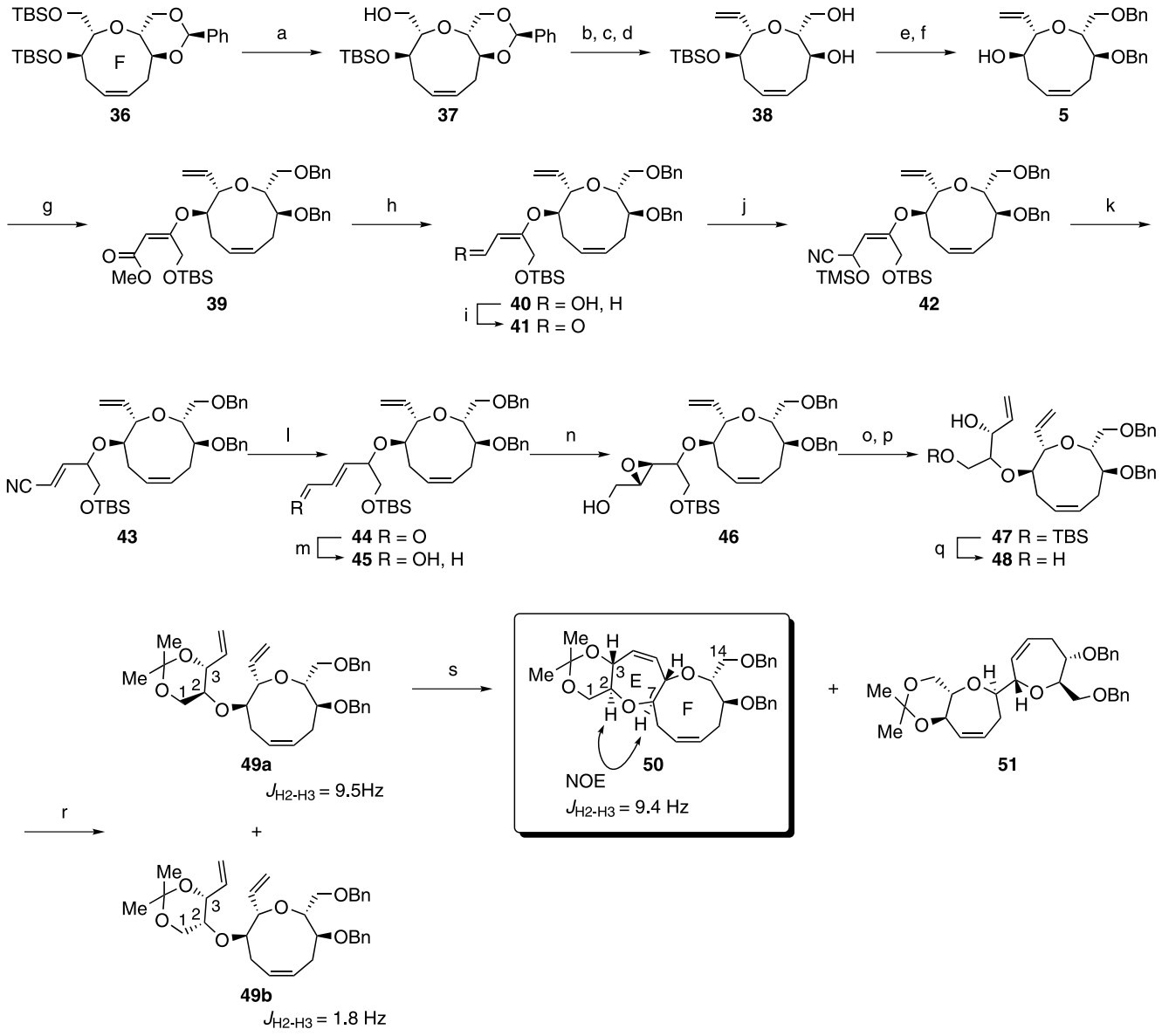
A ca. 1:2 mixture of **30a** and **30b** was converted to a mixture of diols **32a** and **32b** by removal of the TBS group (99%). The acetonide protection of the diols under the standard conditions produced *bicyclic* RCM precursor **33a** (24%) and **33b** (60%), which were easily separated by silica gel column chromatography. The stereochemistry of each



Scheme 6. Synthesis of *trans*-fused bicyclic ether **34**. Reagents and conditions: (a) TBAF (2.0 equiv), THF, 24 °C, 20.5 h, 99%; (b) 2,2-dimethoxypropane (10 equiv), CSA (0.5 equiv), CH_2Cl_2, 24 °C, 7.5 h, **33a**: 24%, **33b**: 60%; (c) $(H_2IMes)(PCy_3)Cl_2Ru$=CHPh (10 mol%), CH_2Cl_2 (4 mM), reflux, 4.5 h, 83%; (d) THF–H_2O–TFA (10:10:1), 23 °C, 11.5 h, 71%.

compound was determined by the *J* value between H9 and H10. RCM[27] of desired **33a** with Grubbs' second-generation catalyst in refluxing CH_2Cl_2 expectedly gave the desired *trans*-fused 6/8 cyclic ether **34** in 83% yield. Since the structure of **34** was difficult to be confirmed, the acetonide **34** was converted to diol **35**. The stereochemistry of **35** was verified by the presence of NOE between H4 and H10 as well as the large *J* value between H9 and H10 (8.8 Hz). Thus, a *bicyclic* precursor was proved to promote efficient ring closure by RCM.

Then, according to the above results, we examined the synthesis of the EF-ring segment **50** from the F-ring part **36**[14j] (Scheme 7). Treatment of **36** with TFA gave primary alcohol **37** selectively in 68% yield along with **36** in 24% recovery. Swern oxidation of the primary alcohol and Wittig reaction followed by removal of benzylidene acetal afforded the diol **38** in 58% yield for three steps. The benzyl (Bn) protection of the diol and removal of the TBS group gave the alcohol **5** in 100% yield. Hetero-Michael addition of the alcohol to 2-butynoate ester **20** produced **39** in good yield (90%),[18] which was converted to α-silyloxy nitrile **42** through reduction, oxidation, and addition of TMSCN. The reduction of **42** with Bu_3SnH in CH_2Cl_2 in the presence of $BF_3{\cdot}OEt_2$ (3.0 equiv) at −18 °C smoothly afforded nitrile **43** as a 1:1 mixture of diastereomers in 55% yield along with **42** in 18% recovery. Conversion of nitrile **43** to allyl alcohol **45** followed by a three-step transformation [(i) Katsuki-Sharpless asymmetric epoxidation[24] using (+)-DET; (ii) iodation of the hydroxy group[25] and (iii) reduction of the resulting epoxy iodide with Zn] gave the corresponding



Scheme 7. Synthesis of the EF-ring segment **50**. Reagents and conditions: (a) THF–H_2O–TFA (10:10:1), 0 °C, 4 h, 68%, 24% recovery of **36**; (b) $(COCl)_2$ (3.0 equiv), DMSO (5.0 equiv), CH_2Cl_2, −78 °C, 15 min then NEt_3 (10 equiv), −20 °C, 10 min; (c) Ph_3PCH_3Br (5.0 equiv), NaHMDS (4.8 equiv), THF, −78 °C, 2.5 h→ 24 °C, 2.5 h; (d) 1,2-ethanedithiol (20 equiv), $NaHCO_3$ (10 equiv), $Zn(OTf)_2$ (1.0 equiv), CH_2Cl_2, 0 °C, 2.5 h, 58% for three steps; (e) NaH (12 equiv), BnBr (6.0 equiv), TBAI (0.1 equiv), THF, 24 °C, 15 h; (f) TBAF (9.0 equiv), THF, 25 °C, 18 h, 100% for two steps; (g) PMe_3 (1.5 equiv), **20** (3.0 equiv), CH_2Cl_2, 0 °C, →24 °C, 1 h, 90% (only *E*); (h) DIBAH (4.0 equiv), CH_2Cl_2, −78 °C, 10 min, 99%; (i) TPAP (0.2 equiv), NMO (2.0 equiv), MS 4A, CH_2Cl_2, 24 °C, 1.5 h, 76%; (j) Me_3Al (1.1 equiv), TMSCN (5.0 equiv), benzene, 24 °C, 1 h, 79%; (k) $BF_3{\cdot}OEt_2$ (3.0 equiv), CH_2Cl_2–Bu_3SnH (1:1), −18 °C, 15 min, 55%, 18% recovery of **42**; (l) DIBAH (2.5 equiv), CH_2Cl_2, −78 °C, 10 min; (m) DIBAH (6.0 equiv), CH_2Cl_2, −78 °C, 20 min, 72% for two steps; (n) L-(+)-DET (1.5 equiv), $Ti(O^iPr)_4$ (1.3 equiv), TBHP (10 equiv), MS 4A, CH_2Cl_2, −40 °C, 30 min→ −25 °C, 26 h; (o) Ph_3P (5.0 equiv), imidazole (5.0 equiv), I_2 (4.0 equiv), THF, 25 °C, 35 min; (p) Zn (7.0 equiv), EtOH-satd. NH_4Claq. (40:1), 25 °C, 2.5 h, 68% for three steps; (q) TBAF (1.5 equiv), THF, 25 °C, 11.5 h, 68%; (r) 2,2-dimethoxypropane (4.0 equiv), CSA (0.5 equiv), CH_2Cl_2, 24 °C, 3 h then acetone (5.0 equiv), 11.5 h, **49a**: 50%, **49b**: 46%; (s) $(Cy_3P)_2Cl_2Ru$=CHPh (25 mol%), CH_2Cl_2 (3 mM), 24 °C, 15 h, **49a**:**50**:**51**=1:1:0.5; $(Cy_3P)_2Cl_2Ru$=CHPh (30 mol%), CH_2Cl_2 (3 mM), 24 °C, 24 h, **50**: 67%, **51**: 24%, after two cycles.

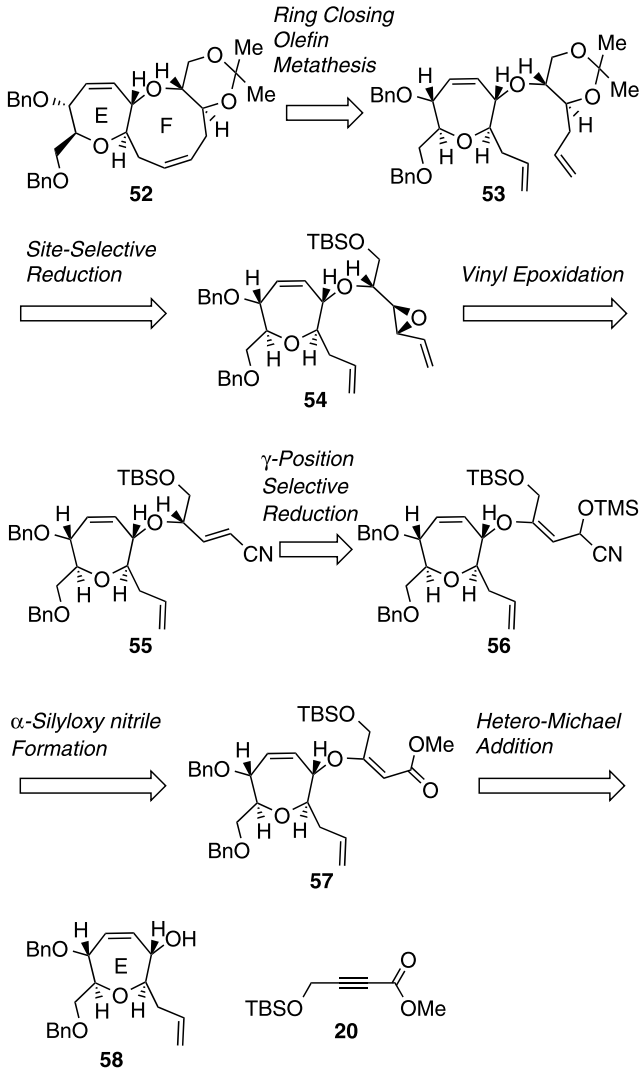
allyl alcohol **47** as a 1:1 mixture of diastereomers in 68% yield. In order to facilitate the closure of the *trans*-fused medium ring by RCM and to confirm the stereochemistry of each diastereomer, acetonides **49a** and **49b** were synthesized from **47** through removal of the TBS group followed by protection of the resulting diol. Diastereomers **49a** and **49b** were easily separated by silica gel column chromatography, and the stereochemistry of each compound was determined by the *J* value between H2 and H3. RCM[16,17] of **49a** with Grubbs' first-generation catalyst in CH_2Cl_2 at ambient temperature successfully produced the desired *trans*-fused EF-ring segment **50** of CTX1B in 67% yield along with **51** in 24% yield. The stereochemistry of **50** was confirmed by the presence of NOE between H2 and H7 as well as the large *J* value between H2 and H3 (9.4 Hz).

Although the synthesis of **50** was thus achieved, two problems arose at the final RCM stage. One was the low reactivity of **49a** under the RCM conditions using Grubbs' first-generation catalyst. The reaction often stopped before completion, and had to be repeated in order to consume **49a** completely. The other problem was a significant

by-production of **51**. It meant that the desired RCM between the terminal vinyl groups making the E-ring was a slow process, and that the metathesis at the olefin part of the F-ring competed with the RCM. These problems would be caused by the severe strain of the E-ring part. In order to solve the problems, we decided to design an alternative improved synthesis of the EF-ring segment.

3. Second generation synthesis of the EF-ring segment

The revised plan for the synthesis of the EF-ring segment of CTX1B is shown in Scheme 8. We intended to cyclize the F-ring by RCM[16,17] at the final stage of the synthesis, and to use the E-ring part as a Michael donor in the initial stage. Therefore, the EF-ring segment **52** was planned to be constructed from the bicyclic diene **53** by RCM.[16,17] The diene would be synthesized via site-selective reduction of vinyl epoxide **54**, which would be prepared from α,β-unsaturated nitrile **55**. According to our established process, the nitrile was designed to be synthesized through hetero-Michael reaction of the E-ring part **58** with **20**,[18] cyanohydrin synthesis from **57**, and regioselective reduction of **56**.[14n]

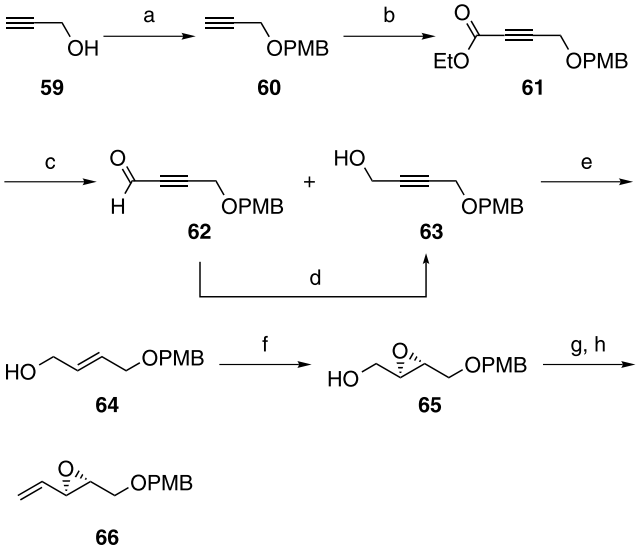


Scheme 8. Retrosynthetic analysis for the EF-ring segment **52**.

In the revised plan, site-selective reduction of vinyl epoxide **54** to the corresponding homoallyl alcohol was a key step. Tsuji and Shimizu reported efficient site-selective hydrogenolysis of epoxides adjacent to an alkene group using a Pd-catalyst and formic acid.[29,30] Therefore, we decided to apply the Pd-catalyzed reduction to our synthesis. Although many trisubstituted epoxides were successfully reduced under the conditions,[29] there were few applications to simple vinyl epoxides.[29a,30] In order to optimize the reduction conditions, epoxide **66** was first examined as a model substrate.

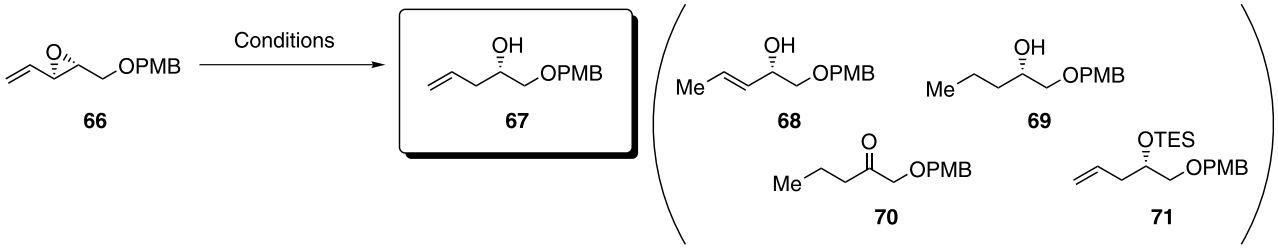
Model compound **66** was synthesized from propargyl alcohol **59**, shown in Scheme 9. Protection of **59** as *p*-methoxy benzyl (PMB) ether (99%) followed by lithiation and addition to ethyl chloroformate produced 2-butynoate ester **61** (79%), which was reduced with DIBAH to give alcohol **63** (26%) and aldehyde **62** (74%). The aldehyde **62** was reduced again with DIBAH to **63** (100%). Reduction of **63** with LAH in THF provided *trans*-allyl alcohol **64**, which was subjected to Katsuki-Sharpless asymmetric epoxidation[24] using (−)-DET to give epoxide **65** (71% yield, 93% ee). Oxidation of **65** followed by Wittig reaction synthesized the desired vinyl epoxide **66** (overall yield 78%).

With the model compound **66** in hand, we examined the site-selective reduction of **66** into homoallyl alcohol **67** in the presence of a Pd-catalyst. First, according to the Tsuji–Shimizu procedure,[29] we attempted the reduction of **66** with formic acid in the presence of triethylamine (entry 1). Although the epoxide **66** was selectively cleaved at the desired site, over-reduced alcohol **69** was mainly produced



Scheme 9. Synthesis of model compound **66**. Reagents and conditions: (a) NaH (1.8 equiv), PMBCl (1.5 equiv), TBAI (0.13 equiv), THF, 24 °C, 16 h, 99%; (b) *n*-BuLi (1.5 equiv), EtOCOCl (1.5 equiv), THF, −78 °C, 1 h→0 °C, 15 min, 79%; (c) DIBAH (2.5 equiv), CH_2Cl_2, −78 °C, 45 min, **62**: 74%, **63**: 26%; (d) DIBAH (2.5 equiv), CH_2Cl_2, −78 °C, 10 min, 100%; (e) LAH (4.0 equiv), THF, −78 °C, 13 min→−20 °C, 26 h, 64%, 24% recovery of **63**; (f) D-(−)-DET (0.20 equiv), $Ti(O^iPr)_4$ (0.15 equiv), TBHP (2.5 equiv), MS 4A, CH_2Cl_2, −40 °C, 30 min→−20 °C, 2.5 d, 71%, 93% ee; (g) SO_3·pyridine (10 equiv), DMSO–NEt_3–CH_2Cl_2 (1:1.4:6), 0 °C→24 °C, 1 h; (h) Ph_3PCH_3Br (5.0 equiv), NaHMDS (4.7 equiv), THF, −78 °C, 2 h→24 °C, 17 h, 78% for two steps.

Table 3. The site-selective reduction of vinyl epoxide **66**



Entry	Conditions	Product
1	$Pd_2(dba)_3\cdot CHCl_3$ (0.1 equiv), n-Bu_3P (0.1 equiv), HCOOH (5.0 equiv), NEt_3 (2.0 equiv), THF, 24 °C, 2.5 h	**69** (83%), **70** (13%)
2	$Pd(PPh_3)_4$ (0.1 equiv), $Me_2NH\cdot BH_3$ (1.1 equiv), AcOH (3.0 equiv), CH_2Cl_2, 23 °C, 1.5 h	**67**:**68**=5.6: 1.0 (97%)
3	$Pd(PPh_3)_4$ (0.1 equiv), Et_3SiH (1.1 equiv), CH_2Cl_2, 24 °C, 1 h	**71** (75%)
4	$Pd(PPh_3)_4$ (0.1 equiv), Bu_3SnH (1.1 equiv), CH_2Cl_2, 24 °C, 20 min	**67** (95%)

along with ketone **70**. Next, as described by Guibé,[31] who reported an improved version of the Tsuji–Shimizu procedure,[29] vinyl epoxide **66** was treated with a catalytic amount of $Pd(PPh_3)_4$ and $Me_2NH\cdot BH_3$ in the presence of acetic acid to give an inseparable 5.6:1.0 mixture of the desired **67** and the isomer **68** (entry 2). On the other hand, when the site-selective reduction was examined with Et_3SiH instead of $Me_2NH\cdot BH_3$ in the absence of acetic acid, not only the desired site selective reduction but also silylation occurred to afford TES ether **71** in 75% yield (entry 3). Eventually, the site-selective reduction with 1.1 equiv of Bu_3SnH gave the best result, where the desired homoallyl alcohol **67** was afforded as the sole product in 95% yield (entry 4). Thus, we found the effective conditions in the site-selective reduction of vinyl epoxide (Table 3).

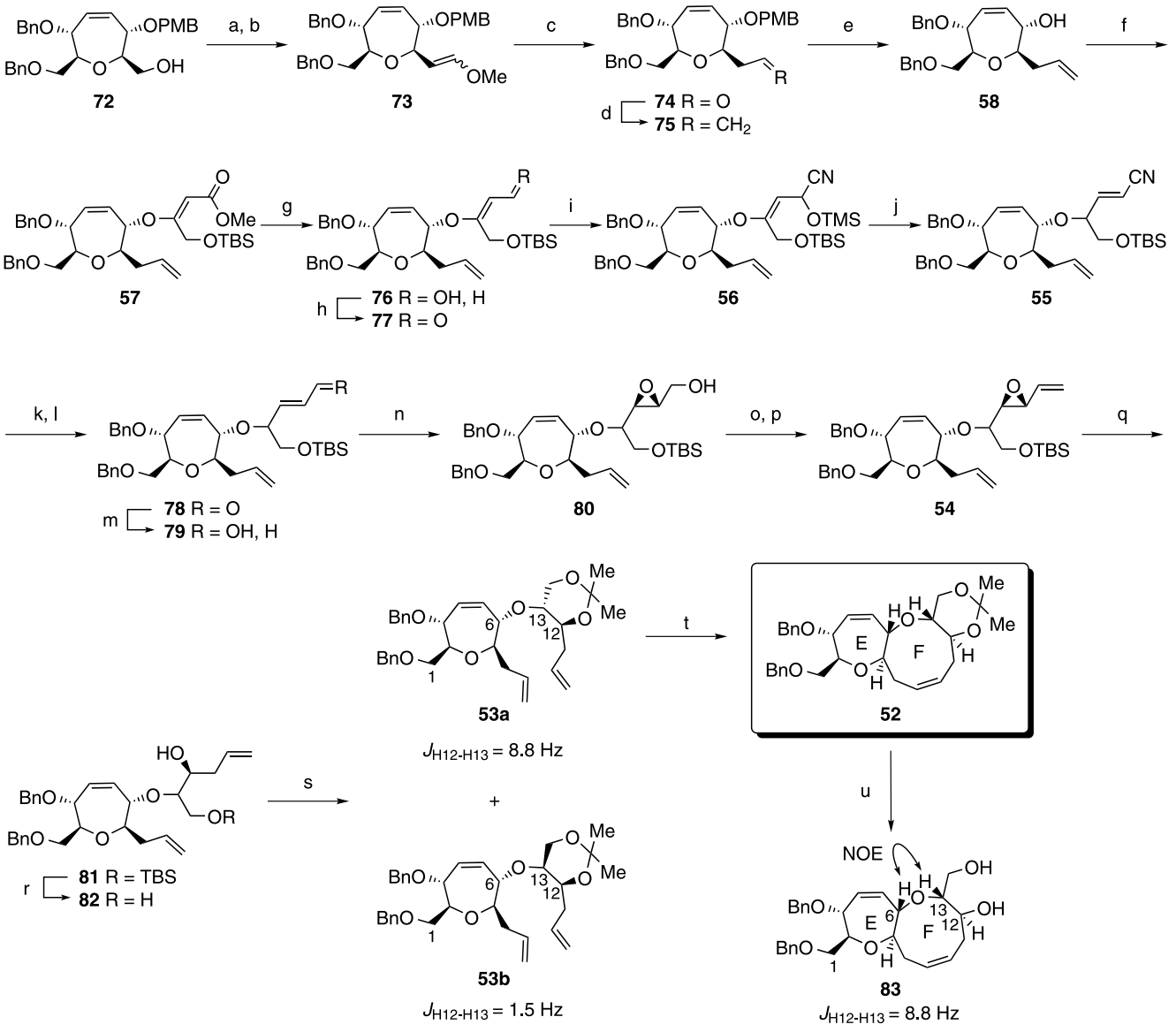
The synthesis of **52** is illustrated in Scheme 10. The Michael donor **58** was prepared from known oxepan alcohol **72**.[32] Swern oxidation of alcohol **72** followed by Wittig reaction gave enol ether **73** (overall yield 70%). Hydrolysis of **73** with $Hg(OAc)_2$ and TBAI in THF–H_2O[33] afforded aldehyde **74** (95%), which was subjected to Wittig reaction and the subsequent removal of the PMB group to provide **58** (overall yield 92%). According to the procedure described in the synthesis of **50**, the alcohol **58** was transformed into α-silyloxy nitrile **56** in four steps ((i) hetero-Michael addition of **58** with **20**;[18] (ii) reduction of ester **57**; (iii) oxidation of the resulting **76** into aldehyde **77**; (iv) addition of TMSCN) (overall yield 57%). The reduction of **56** with Bu_3SnH in CH_2Cl_2 in the presence of $BF_3\cdot OEt_2$ (3.0 equiv) at 0 °C gave nitrile **55** as an inseparable 1:1 mixture of diastereomers in 63% yield with the complete consumption of **56**. The nitrile **55** was reduced to allyl alcohol **79** by a DIBAH reduction–hydrolysis–DIBAH reduction sequence (overall 76%). The allyl alcohol was subjected to Katsuki-Sharpless asymmetric epoxidation[24] using (−)-DET to produce epoxide **80** (76%), which was converted to vinyl epoxide **54** by oxidation with SO_3–pyridine/DMSO[34] and the subsequent Wittig reaction (overall yield 59%). The reduction of vinyl epoxide **54** with $Bu_3SnH/Pd(PPh_3)_4$ under the same conditions described above gave **81** selectively in 88% yield. Removal of the TBS group of **81** followed by protection of the resulting diol afforded acetonides **53a** and **53b**. The diastereomers at C13 were facilely separated by silica gel chromatography at this stage. Stereochemistry of each compound was determined by the J value between H12 and H13. RCM[16,17] of **53a** with Grubbs' first-generation catalyst in CH_2Cl_2 at ambient temperature smoothly produced the desired **52** as the sole product in 97% yield.

Stereochemical confirmation of **52** by ^1H NMR was difficult because the signals of **52** at ambient temperature were extremely broadened due to the slow conformational changes of the F-ring part, as reported for natural CTX1B[5] and other model compounds.[11f,j,o,s,v] Although the spectrum of **52** in pyridine-d_5 at −30 °C exhibited sharp signals of a ca. 1:1 mixture of two conformers, stereochemistry of **52** could not be confirmed due to overlapping signals of both conformers. In order to solve the conformational problem, acetonide **52** was converted to diol **83**. The diol **83** was flexible enough to give a set of sharp and clear signals at ambient temperature. Eventually, the stereochemistry of **52** was proved by the detailed NMR analysis of **83**, which showed the presence of NOE between H6 and H13 as well as the large J value (8.8 Hz) between H12 and H13.

Thus, the F-ring was efficiently cyclized at the final stage whereby the improved synthesis of the EF-ring segment of CTX1B was accomplished.

4. Conclusion

Aiming at the convergent total synthesis of CTX1B, construction of its EF-ring segment has been investigated. During the study, a novel method for the construction of branched ethers based on regioselective reduction of γ-alkoxy β,γ-unsaturated α-silyloxy nitriles with $BF_3\cdot OEt_2$ and R_3SiH or Bu_3SnH has been developed. Combined use of the branched ether synthesis and RCM successfully provided medium-sized cyclic ether **34**, and also contributed to the synthesis of the EF-ring segment **50**. Although a difficulty in the cyclization of the E-ring by RCM arose in the synthesis of **50**, it was solved in a revised synthetic route to the EF-ring part **52**, where the F-ring was cyclized at the final stage. In the course of the synthesis of **52**, efficient site-selective reduction of vinyl epoxides into homoallyl alcohols mediated by Bu_3SnH and $Pd(PPh_3)_4$ was also developed. Thus, a novel route to the EF-ring segment of CTX1B has been established. Further studies toward the



Scheme 10. Synthesis of the EF-ring segment **52**. Reagents and conditions: (a) $(COCl)_2$ (3.0 equiv), DMSO (5.0 equiv), CH_2Cl_2, −78 °C, 15 min then NEt_3 (10 equiv), −18 °C, 10 min; (b) Ph_3PCH_2OMeCl (5.1 equiv), NaHMDS (4.9 equiv), THF, −78 °C, 1.5 h→24 °C, 17.5 h, 70% for two steps; (c) $Hg(OAc)_2$ (3.0 equiv), THF–H_2O (10:1), 24 °C, 1.5 h then TBAI (9.0 equiv), 1 h, 95%; (d) Ph_3PCH_3Br (3.5 equiv), NaHMDS (3.2 equiv), THF, −78 °C, 2 h→22 °C, 18 h, 100%; (e) DDQ (2.5 equiv), CH_2Cl_2—pH 7 buffer (4:1), 0 °C, 3 h, 92%; (f) PMe_3 (1.5 equiv), **20** (3.0 equiv), CH_2Cl_2, 0 °C, →24 °C, 30 min, 98% (only *E*); (g) DIBAH (3.5 equiv), CH_2Cl_2, −78 °C, 1 h, 94%; (h) TPAP (0.2 equiv), NMO (2.0 equiv), MS 4A, CH_2Cl_2, 23 °C, 1.5 h, 84%; (i) Me_3Al (1.1 equiv), TMSCN (2.5 equiv), benzene, 25 °C, 1 h, 74%; (j) $BF_3\cdot OEt_2$ (3.0 equiv), CH_2Cl_2–Bu_3SnH (1:1), 0 °C, 10 min, 63%; (k) DIBAH (4.0 equiv), CH_2Cl_2, −78 °C, 1 h; (l) 0.5 M HCl, CH_2Cl_2, 24 °C, 20 min, 83% for two steps; (m) DIBAH (3.0 equiv), CH_2Cl_2, −78 °C, 30 min, 91%; (n) D-(−)-DET (2.0 equiv), $Ti(O^iPr)_4$ (1.5 equiv), TBHP (20 equiv), MS 4A, CH_2Cl_2, −40 °C, 30 min→−25 °C, 2.5 d, 76%; (o) $SO_3\cdot$pyridine (25 equiv), DMSO–NEt_3–CH_2Cl_2 (1:1.4:3), 0 °C→24 °C, 3 h; (p) Ph_3PCH_3Br (28 equiv), NaHMDS (23 equiv), THF, −78 °C, 2 h→24 °C, 2.5 d, 59% for two steps; (q) $Pd(PPh_3)_4$ (0.1 equiv), Bu_3SnH (1.1 equiv), CH_2Cl_2, 24 °C, 25 min, 88%; (r) TBAF (2.0 equiv), THF, 23 °C, 1.5 h, 100%; (s) 2,2-dimethoxypropane (10 equiv), CSA (0.5 equiv), CH_2Cl_2, 24 °C, 3 h, **53a**: 47%, **53b**: 50%; (t) $(Cy_3P)_2Cl_2Ru{=}CHPh$ (20 mol%), CH_2Cl_2 (3 mM), 23 °C, 4 h, 97%; (u) THF–H_2O–TFA (10:10:1), 23 °C, 3 h, 88%.

total synthesis of CTX1B are currently under way in our laboratory.

5. Experimental

5.1. General methods

All reactions sensitive to air or moisture were carried out under an argon atmosphere in dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Sensitive liquids and solutions were transferred by syringe-septum and cannula techniques. All commercially available reagents were used without further purification with the following exceptions. THF was distilled from sodium-benzophenone ketyl under argon. CH_2Cl_2, benzene were distilled from CaH_2 prior to use. All reactions were monitored by TLC with precoated SiO_2 plates (Merck, silica gel 60 F_{254}). Plates were visualized by ultraviolet light and by treatment with acidic anisaldehyde or phosphomolybdic acid stain followed by heating. Flash chromatography was performed on YMC Silica Gel 60 (230–400 mesh) as a stationary phase. Melting points were measured on YANAGIMOTO micro-melting apparatus without

calibration. Optical rotations were recorded on a JASCO P-1020 digital polarimeter. Infrared spectra (IR) were measured on a JEOL JIR-WINSPEC100 infrared spectrometer in noted states and are reported in wave numbers (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-AL300 (^1H at 300 MHz, ^{13}C at 75 MHz), JNM-α-400 (^1H at 400 MHz), and/or JNM-α-600 (^{13}C at 150 MHz) magnetic resonance spectrometer. ^1H NMR spectra are reported as chemical shifts (δ) in parts-per-million (ppm) based on tetramethylsilane (0 ppm), C_6HD_5 (7.15 ppm), $CHD_2C(=O)CD_3$ (2.04 ppm) or C_5HD_4N (8.71 ppm). The following abbreviations are used to describe spin multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad, dd=double doublets, dt=double triplets, dq=double quartets, and ddd=double double doublets; other combination is derived from those listed. Coupling constants (*J*) are reported in Hertz (Hz). ^{13}C NMR spectra are reported as chemical shifts (δ) in ppm based on $^{13}CDCl_3$ (77.0 ppm) or $^{13}C_6D_6$ (128.0 ppm). Low and high resolution mass spectra were measured on a JEOL JMS-600H mass spectrometer under electron ionization (EI) condition and a JEOL JMS-SX102A mass spectrometer under field desorption (FD) condition.

5.1.1. 4-(3-Phenylpropoxy)but-2-enenitrile (13). To a solution of **10** (50.6 mg, 0.175 mmol) in CH_2Cl_2–Et_3SiH (1:1, v/v, 3.2 ml) was added $BF_3\cdot OEt_2$ (65 μl, 0.524 mmol) at 0 °C and the mixture was stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10) to give **13** (22.6 mg, 64%) as a inseparable mixture of *E* and *Z*-isomers (*E*/*Z*=6.2:1 from ^1H NMR). **13**: a colorless oil; IR (film), ν_{max} 3063, 3026, 2943, 2861, 2795, 2224, 1693, 1602, 1496, 1477, 1454, 1365, 1264, 1180, 1135, 1047, 1029, 952, 913, 748, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.32–7.15 (5H, m), 6.73 (0.86H, dt, *J*=16.3, 3.7 Hz), 6.57 (0.14H, dt, *J*=11.4, 5.9 Hz), 5.64 (0.86H, dt, *J*=16.3, 2.4 Hz), 5.45 (0.14H, dt, *J*=11.4, 1.8 Hz), 4.29 (0.28H, dd, *J*=5.9, 1.8 Hz), 4.08 (1.72H, dd, *J*=3.7, 2.4 Hz), 3.48 (2H, t, *J*=6.4 Hz), 2.70 (2H, t, *J*=7.7 Hz), 1.98–1.88 (2H, m); LR-EIMS, *m*/*z* 201 (43.5%, $[M]^+$), 118 (bp); HR-EIMS, calcd for $C_{13}H_{15}NO$ $[M]^+$: 201.1154, found: 201.1162.

5.1.2. (2*E*)-4-(3-Phenylpropoxy)pent-2-enenitrile (14). To a solution of **11** (27.3 mg, 0.0900 mmol) in CH_2Cl_2–Et_3SiH (1:1, v/v, 1.8 ml) was added $BF_3\cdot OEt_2$ (33 μl, 0.270 mmol) at 0 °C and the mixture was stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20→10) to give **14** (12.1 mg, 62%). **14**: a colorless oil; IR (film), ν_{max} 3062, 3026, 2978, 2932, 2856, 2224, 1636, 1602, 1584, 1496, 1476, 1454, 1370, 1340, 1246, 1178, 1150, 1102, 963, 748, 700 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.32–7.16 (5H, m), 6.64 (1H, dd, *J*=16.3, 5.0 Hz), 5.54 (1H, dd, *J*=16.3, 1.7 Hz), 3.96 (1H, qdd, *J*=6.6, 5.0, 1.7 Hz), 3.41 (2H, t, *J*=6.4 Hz), 2.69 (2H, brt, *J*=7.7 Hz), 1.95–1.86 (2H, m), 1.26 (3H, d, *J*=6.6 Hz); LR-EIMS, *m*/*z* 215 (42.5%, $[M]^+$), 91 (bp); HR-EIMS, calcd for $C_{14}H_{17}NO$ $[M]^+$: 215.1310, found: 215.1336.

5.1.3. (2*E*)-5-(*tert*-Butyldimethylsilyloxy)-4-(3-phenylpropoxy)pent-2-enenitrile (15). To a solution of **12** (82.2 mg, 0.190 mmol) in CH_2Cl_2–Et_3SiH (1:1, v/v, 3.0 ml) was added $BF_3\cdot OEt_2$ (70 μl, 0.570 mmol) at 0 °C and the mixture was stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (7 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20→10) to give **15** (41.8 mg, 64%). **15**: a colorless oil; IR (film), ν_{max} 3085, 3063, 3027, 2953, 2929, 2858, 2739, 2225, 1629, 1603, 1497, 1471, 1462, 1455, 1406, 1389, 1361, 1343, 1306, 1254, 1222, 1111, 1006, 965, 939, 837, 814, 779, 747, 700, 670 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.31–7.16 (5H, m), 6.72 (1H, dd, *J*=16.4, 4.7 Hz), 5.61 (1H, dd, *J*=16.4, 1.7 Hz), 3.91 (1H, dddd, *J*=6.2, 5.9, 4.7, 1.7 Hz), 3.70 (1H, dd, *J*=10.2, 5.9 Hz), 3.55 (1H, dd, *J*=10.2, 6.2 Hz), 3.49 (2H, t, *J*=6.4 Hz), 2.72–2.67 (2H, m), 1.95–1.86 (2H, m), 0.89 (9H, s), 0.06 (6H, s); LR-EIMS, *m*/*z* 288 (21.9%, $[M-t\text{-Bu}]^+$), 91 (bp); HR-EIMS, calcd for $C_{16}H_{22}NO_2Si$ $[M-t\text{-Bu}]^+$: 288.1420, found: 288.1417.

5.1.4. Methyl (2*E*,2′*R*,3′*S*)-3-{(2′-allyloxan-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenoate (21). A solution of butynoate **20** (1.29 g, 5.65 mmol) in CH_2Cl_2 (10 ml) was slowly added dropwise to a solution of **19** (479.0 mg, 3.37 mmol) and PMe_3 (3.7 ml, 1.0 M in THF, 3.71 mmol) in CH_2Cl_2 (24 ml) at 0 °C by means of a syringe. The mixture was warmed to 24 °C and stirred for 1 h. The mixture was cooled to 0 °C and diluted with Et_2O (20 ml) and hexane (20 ml). Then, saturated aqueous NH_4Cl (15 ml) was added and the mixture was extracted with Et_2O (3×15 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20) to give **21** (1.19 g, 95%). **21**: a colorless oil; $[\alpha]_D^{26}$ +58.1 (*c* 0.75, $CHCl_3$); IR (film), ν_{max} 3077, 2951, 2856, 1716, 1626, 1472, 1436, 1434, 1389, 1361, 1342, 1310, 1295, 1252, 1189, 1147, 1098, 1051, 1005, 939, 914, 837, 778, 675 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.92–5.78 (1H, m), 5.11–5.09 (2H, m), 5.05 (1H, s), 4.90 (1H, d, *J*=13.6 Hz), 4.65 (1H, d, *J*=13.6 Hz), 3.98–3.92 (1H, m), 3.91–3.83 (1H, m), 3.68 (3H, s), 3.45–3.33 (2H, m), 2.59–2.50 (1H, m), 2.34–2.30 (1H, m), 2.25–2.15 (1H, m), 1.75–1.66 (2H, m), 1.52–1.37 (1H, m), 0.90 (9H, s), 0.09 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 170.4 (C), 167.3 (C), 134.2 (CH), 117.1 (CH_2), 91.6 (CH), 79.4 (CH), 74.8 (CH), 67.7 (CH_2), 60.0 (CH_2), 50.8 (CH_3), 36.0 (CH_2), 27.8 (CH_2), 25.7 ($CH_3\times3$), 24.9 (CH_2), 18.2 (C), −5.35 (CH_3), −5.38 (CH_3); LR-EIMS, *m*/*z* 313 (82.4%, $[M-t\text{-Bu}]^+$), 189 (bp); HR-EIMS, calcd for $C_{15}H_{25}O_5Si$ $[M-t\text{-Bu}]^+$: 313.1471, found: 313.1466.

5.1.5. (2*E*,2′*R*,3′*S*)-3-{(2′-Allyloxan-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenol (22). To a solution of **21** (115.6 mg, 0.312 mmol) in CH_2Cl_2 (5.0 ml) was added

DIBAH (1.3 ml, 0.95 M in *n*-hexane, 1.25 mmol) at −78 °C and the mixture was stirred for 10 min. Then, saturated aqueous potassium sodium tartrate (5 ml) was added and the mixture was stirred at 24 °C for 3 h. The layers were separated and the aqueous layer was extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 5 → 3 → 1) to give **22** (106.6 mg, ~100%). **22**: a colorless oil; $[\alpha]_D^{26}$ +45.1 (*c* 0.65, $CHCl_3$); IR (film), ν_{max} 3417, 3075, 2930, 2857, 2725, 1661, 1472, 1463, 1436, 1389, 1361, 1340, 1306, 1276, 1252, 1184, 1098, 1004, 913, 837, 814, 777, 674 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.93–5.79 (1H, m), 5.12–5.04 (2H, m), 4.95 (1H, t, J=7.9 Hz), 4.20–4.15 (4H, m), 3.94 (1H, dtd, J=11.0, 3.3, 1.8 Hz), 3.74 (1H, ddd, J=10.3, 9.0, 4.2 Hz), 3.42–3.29 (2H, m), 2.58–2.49 (1H, m), 2.38–2.29 (1H, m), 2.24–2.13 (1H, m), 1.72–1.63 (2H, m), 1.43–1.21 (1H, m), 0.91 (9H, s), 0.10 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 155.9 (C), 134.6 (CH), 116.5 (CH_2), 100.3 (CH), 79.8 (CH), 73.1 (CH), 67.5 (CH_2), 60.6 (CH_2), 57.6 (CH_2), 36.0 (CH_2), 28.0 (CH_2), 25.5 (CH_3×3), 24.8 (CH_2), 17.9 (C), −5.52 (CH_3), −5.56 (CH_3); LR-EIMS, *m/z* 285 (19.1%, $[M-t\text{-Bu}]^+$), 227 (bp); HR-EIMS, calcd for $C_{14}H_{25}O_4Si$ $[M-t\text{-Bu}]^+$: 285.1522, found: 285.1518.

5.1.6. (2*E*,2′*R*,3′*S*)-3-{(2′-Allyloxan-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenal (23). To a mixture of **22** (158.2 mg, 0.462 mmol) and MS 4 Å (158.2 mg, 100 wt%) in CH_2Cl_2 (5.0 ml) was added NMO (108.2 mg, 0.924 mmol) at 24 °C and the mixture was stirred for 10 min. Then, TPAP (16.2 mg, 0.0462 mmol) was added to the reaction mixture at 24 °C and the mixture was stirred for 50 min. The mixture was filtered through Celite and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 10 → 5) to give **23** (139.0 mg, 88%). **23**: a colorless oil; $[\alpha]_D^{25}$ +49.0 (*c* 1.01, $CHCl_3$); IR (film), ν_{max} 3075, 2954, 2930, 2857, 2764, 1665, 1615, 1472, 1463, 1438, 1389, 1361, 1323, 1279, 1254, 1209, 1164, 1099, 1041, 1005, 974, 957, 947, 939, 914, 837, 778, 680 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 9.98 (1H, d, J=7.7 Hz), 5.91–5.77 (1H, m), 5.41 (1H, d, J=7.7 Hz), 5.09–5.04 (2H, m), 4.52 (1H, d, J=13.2 Hz), 4.46 (1H, d, J=13.2 Hz), 3.98–3.86 (2H, m), 3.45–3.35 (2H, m), 2.52–2.44 (1H, m), 2.32–2.16 (1H, m), 1.73–1.65 (2H, m), 1.52–1.37 (1H, m), 0.91 (9H, s), 0.11 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 189.9 (CH), 173.7 (C), 133.7 (CH), 117.1 (CH_2), 105.7 (CH), 78.9 (CH), 75.1 (CH), 67.4 (CH_2), 61.1 (CH_2), 35.9 (CH_2), 27.6 (CH_2), 25.5 (CH_3×3), 24.6 (CH_2), 17.9 (C), −5.58 (CH_3×2); LR-EIMS, *m/z* 283 (73.8%, $[M-t\text{-Bu}]^+$), 159 (bp); HR-EIMS, calcd for $C_{14}H_{23}O_4Si$ $[M-t\text{-Bu}]^+$: 283.1365, found: 283.1362.

5.1.7. (3*E*,2′*R*,3′*S*)-4-{(2′-Allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-(trimethylsilyloxy)pent-3-enenitrile (24). To a solution of **23** (43.9 mg, 0.129 mmol) and TMSCN (43 µl, 0.323 mmol) in benzene (1.3 ml) was added Me_3Al (0.14 ml, 1.03 M in *n*-hexane, 0.142 mmol) at 24 °C and the mixture was stirred for 1 h. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 30 → 10) to give **24** (42.1 mg, 74%) as an inseparable 1:1 mixture of diastereomers. The nitrile **24** was unstable and immediately used for the next reaction. **24**: a colorless oil; ^1H NMR (300 MHz, $CDCl_3$), δ 5.92–5.78 (1H, m), 5.58 (0.5H, d, J=9.1 Hz), 5.57 (0.5H, d, J=9.1 Hz), 5.12–5.04 (2H, m), 4.75–4.70 (1H, m), 4.20–4.08 (2H, m), 3.97–3.87 (1H, m), 3.79–3.65 (1H, m), 3.42–3.28 (2H, m), 2.54–2.43 (1H, m), 2.37–2.25 (1H, m), 2.23–2.12 (1H, m), 1.74–1.65 (2H, m), 1.41–1.30 (1H, m), 0.92 (9H, s), 0.21 (9H, s), 0.11 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 156.8 (C), 134.5 (CH), 119.9 (C×0.5), 117.2 (C×0.5), 117.0 (CH_2), 98.4 (CH), 79.6 (CH), 74.2 (CH), 67.8 (CH_2), 62.2 (CH_2), 57.6 (CH), 36.3 (CH_2), 28.1 (CH_2), 25.8 (CH_3×3), 25.0 (CH_2), 18.3 (C), −0.10 (CH_3×3), −5.38 (CH_3), −5.53 (CH_3).

5.1.8. (2*E*,4*S*,2′*R*,3′*S*)-4-{(2′-Allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-2-enenitrile (25a) and (2*E*,4*R*,2′*R*,3′*S*)-4-{(2′-allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-2-enenitrile (25b). *Silane reduction*. The reduction of **24** with organosilanes in entries 1–4 in Table 2 followed the procedure described in the synthesis of model compounds **13**, **14** and **15**.

Stannane reduction. To a solution of **24** (14.7 mg, 0.0334 mmol) in CH_2Cl_2–Bu_3SnH (1:1, v/v, 0.60 ml) was added $BF_3 \cdot OEt_2$ (12 µl, 0.100 mmol) at −18 °C and the mixture was stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 30 → 10) to give **25** (7.5 mg, 64%) as an inseparable mixture of two diastereomers (**25a**:**25b** = 1.0:1.3). **25**: a colorless oil; IR (film), ν_{max} 3074, 2954, 2930, 2857, 2225, 2211, 1628, 1472, 1463, 1434, 1389, 1361, 1341, 1255, 1222, 1178, 1099, 1005, 965, 913, 837, 779 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 6.78 (0.57H, dd, J=16.3, 4.6 Hz), 6.69 (0.43H, dd, J=16.3, 5.4 Hz), 5.95–5.80 (1H, m), 5.66 (0.57H, dd, J=16.3, 1.8 Hz), 5.63 (0.43H, dd, J=16.3, 1.6 Hz), 5.13–5.07 (2H, m), 4.11–4.02 (1H, m), 3.92–3.87 (1H, m), 3.72–3.63 (1H, m), 3.54–3.47 (1H, m), 3.39–3.08 (3H, m), 2.67–2.61 (0.57H, m), 2.58–2.50 (0.43H, m), 2.26–2.05 (2H, m), 1.67–1.57 (2H, m), 1.29–1.26 (1H, m), 0.89 (9H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) (A 1:2 mixture of **25a** and **25b** was measured), δ 153.6 (CH×0.67), 152.8 (CH×0.33), 135.0 (CH×0.67), 134.7 (CH×0.33), 117.1 (C×0.67), 116.9 (CH_2×0.33), 116.8 (C×0.33), 116.6 (CH_2×0.67), 101.2 (CH×0.33), 100.4 (CH×0.67), 80.2 (CH×0.33), 80.1 (CH×0.67), 77.6 (CH×0.67), 77.4 (CH×0.33), 76.6 (CH×0.33), 76.2 (CH×0.67), 67.6 (CH_2×0.67), 67.5 (CH_2×0.33), 65.3 (CH_2×0.33), 64.6 (CH_2×0.67), 36.41 (CH_2×0.33), 36.37 (CH_2×0.67), 30.2 (CH_2×0.67), 29.7 (CH_2×0.33), 25.8 (CH_3×2), 25.7 (CH_3), 25.23 (CH_2×0.67), 25.19 (CH_2×0.33), 18.14 (C×0.67), 18.09 (C×0.33), −5.45 (CH_3×0.33), −5.53 (CH_3), −5.59 (CH_3×0.67); LR-EIMS, *m/z* 294 (23.7%, $[M-t\text{-Bu}]^+$), 125 (bp);

HR-EIMS, calcd for $C_{15}H_{24}NO_3Si$ $[M-t\text{-}Bu]^+$: 294.1525, found: 294.1523.

5.1.9. (2*E*,4*S*,2′*R*,3′*S*)-4-{(2′-Allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenal (27a) and (2*E*,4*R*,2′*R*,3′*S*)-4-{(2′-allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenal (27b). To a solution of **25** (**25a**:**25b**=1:2, 24.0 mg, 0.0683 mmol) in CH_2Cl_2 (1.0 ml) was added DIBAH (0.18 ml, 0.95 M in *n*-hexane, 0.171 mmol) at −78 °C and the mixture was stirred for 10 min. Then, saturated aqueous potassium sodium tartrate (3 ml) was added and the mixture was stirred at 24 °C for 12 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→5) to give **27** (16.5 mg, 68%) as an inseparable mixture of two diastereomers (**27a**:**27b**=1.0:2.0). **27**: a yellow oil; IR (film), ν_{max} 3074, 2953, 2929, 2857, 2723, 1697, 1641, 1472, 1463, 1437, 1389, 1361, 1339, 1279, 1254, 1214, 1186, 1099, 1005, 978, 939, 912, 838, 814, 778, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 9.58 (1H, d, J=7.7 Hz), 6.84 (0.67H, dd, J=15.8, 5.1 Hz), 6.73 (0.33H, dd, J=15.8, 5.9 Hz), 6.38–6.26 (1H, m), 5.97–5.80 (1H, m), 5.15–5.07 (2H, m), 4.28–4.15 (1H, m), 3.92–3.87 (1H, m), 3.78–3.70 (1H, m), 3.62–3.55 (1H, m), 3.36–3.10 (3H, m), 2.72–2.56 (1H, m), 2.27–2.18 (1H, m), 2.12–2.07 (1H, m), 1.68–1.54 (2H, m), 1.44–1.31 (1H, m), 0.89 (9H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 193.3 (CH×0.67), 193.1 (CH×0.33), 155.7 (CH×0.67), 154.6 (CH×0.33), 135.2 (CH×0.67), 135.0 (CH×0.33), 133.3 (CH×0.33), 132.5 (CH×0.67), 116.7 (CH_2×0.33), 116.6 (CH_2×0.67), 80.30 (CH×0.33), 80.26 (CH×0.67), 78.1 (CH×0.67), 77.5 (CH×0.67), 77.2 (CH×0.33), 75.9 (CH×0.33), 67.6 (CH_2), 65.6 (CH_2×0.33), 64.9 (CH_2×0.67), 36.44 (CH_2×0.67), 36.40 (CH_2×0.33), 30.4 (CH_2×0.67), 29.6 (CH_2×0.33), 25.77 (CH_3×2), 25.75 (CH_3), 25.29 (CH_2×0.67), 25.25 (CH_2×0.33), 18.2 (C), −5.37 (CH_3×0.33), −5.46 (CH_3), −5.52 (CH_3×0.67); LR-EIMS, *m*/*z* 354 (14.0%, $[M]^+$), 125 (bp); HR-EIMS, calcd for $C_{15}H_{25}O_4Si$ $[M-t\text{-}Bu]^+$: 297.1522, found: 297.1540.

5.1.10. (2*E*,4*S*,2′*R*,3′*S*)-4-{(2′-Allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenol (28a) and (2*E*,4*R*,2′*R*,3′*S*)-4-{(2′-allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenol (28b). To a solution of **27** (**27a**:**27b**=1:2, 16.5 mg, 0.0465 mmol) in CH_2Cl_2 (1.0 ml) was added DIBAH (0.15 ml, 0.95 M in *n*-hexane, 0.140 mmol) at −78 °C and the mixture was stirred for 8 min. Then, saturated aqueous potassium sodium tartrate (3 ml) was added and the mixture was stirred at 24 °C for 12 h. The layers were separated and the aqueous layer was extracted with EtOAc (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5) to give **28** (16.6 mg, ~100%) as an inseparable mixture of two diastereomers (**28a**:**28b**=1.0:2.0). **28**: a pale yellow oil; IR (film), ν_{max} 3345, 3076, 2931, 2858, 1642, 1472, 1463, 1443, 1378, 1342, 1321, 1279, 1256, 1213, 1098, 1004, 974, 948, 912, 837, 814, 778, 666 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.96–5.83 (2H, m), 5.68 (0.67H, dt, J=7.0, 1.5 Hz), 5.62 (0.33H, dt, J=6.6, 1.5 Hz), 5.14–5.05 (2H, m), 4.17 (2H, brs), 3.96–3.86 (2H, m), 3.68–3.59 (1H, m), 3.56–3.49 (1H, m), 3.36–3.10 (3H, m), 2.78–2.69 (0.67H, m), 2.66–2.57 (0.33H, m), 2.22 (1H, dt, J=15.0, 7.7 Hz), 2.14–2.07 (1H, m), 1.64–1.57 (2H, m), 1.43–1.25 (1H, m), 0.89 (9H, s), 0.05 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 135.54 (CH×0.67), 135.45 (CH×0.33), 133.1 (CH×0.33), 131.9 (CH×0.67), 130.2 (CH×0.67), 129.3 (CH×0.33), 116.4 (CH_2), 80.8 (CH×0.67), 80.5 (CH×0.33), 80.4 (CH×0.67), 78.1 (CH×0.33), 76.7 (CH×0.67), 74.2 (CH×0.33), 67.71 (CH_2×0.33), 67.68 (CH_2×0.67), 66.4 (CH_2×0.33), 66.2 (CH_2×0.67), 62.83 (CH_2×0.67), 62.81 (CH_2×0.33), 36.4 (CH_2), 31.0 (CH_2×0.67), 29.3 (CH_2×0.33), 25.85 (CH_3×2), 25.83 (CH_3), 25.5 (CH_2×0.67), 25.3 (CH_2×0.33), 18.29 (C×0.67), 18.27 (C×0.33), −5.21 (CH_3×0.33), −5.33 (CH_3), −5.43 (CH_3×0.67); LR-EIMS, *m*/*z* 299 (2.1%, $[M-t\text{-}Bu]^+$), 125 (bp); HR-EIMS, calcd for $C_{15}H_{27}O_4Si$ $[M-t\text{-}Bu]^+$: 299.1678, found: 299.1674.

5.1.11. (2*R*,3*S*,4*R*,2′*R*,3′*S*)-4-{(2′-Allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2,3-epoxypentanol (29a) and (2*R*,3*S*,4*S*,2′*R*,3′*S*)-4-{(2′-allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2,3-epoxypentanol (29b). To a mixture of D-(−)-DET (14.4 μl, 0.0840 mmol) and predried MS 4 Å (49.7 mg, 133 wt%) in CH_2Cl_2 (0.5 ml) was added $Ti(O^iPr)_4$ (21.6 μl, 0.0735 mmol) at −40 °C and the mixture was stirred for 30 min. Then, TBHP (0.14 ml, 3.7 M in toluene, 0.525 mmol) was added and the mixture was stirred at −40 °C for 30 min. To the mixture was added dropwise a solution of **28** (**28a**:**28b**=1:2, 37.5 mg, 0.105 mmol) in CH_2Cl_2 (1.5 ml). The reaction mixture was stirred at −40 °C for 30 min. Then, the reaction mixture was warmed to −25 °C and stirred for 24 h. DMS (38.6 μl, 0.525 mmol) was added at −25 °C and the mixture was stirred for 2 h until unreacted TBHP was consumed. To the mixture was added 10% DL-tartaric acid (43.2 μl) and NaF (18.5 mg) at −25 °C. The suspension was warmed to 26 °C and stirred for 24 h. The mixture was filtered through Celite and concentrated in vacuo. To the resultant residue was added Et_2O (3.0 ml) and 30% aqueous NaOH in brine (1.0 ml) at 0 °C and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5→3) to give **29** (39.2 mg, ~100%) as an inseparable mixture of two diastereomers (**29a**:**29b**=1.0:2.0). **29**: a colorless oil; IR (film), ν_{max} 3468, 3075, 2930, 2857, 1642, 1472, 1463, 1389, 1368, 1257, 1130, 1097, 1027, 939, 910, 837, 815, 778, 669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.96–5.82 (1H, m), 5.15–5.05 (2H, m), 3.96 (1H, brddd, J=12.8, 5.1, 2.2 Hz), 3.92–3.86 (1H, m), 3.73–3.61 (2H, m), 3.60 (1H, dd, J=10.3, 7.3 Hz), 3.36–3.27 (1H, m), 3.24–3.17 (3H, m), 3.12 (1H, dt, J=4.4, 2.2 Hz), 3.03 (1H, dd, J=6.8, 2.2 Hz), 2.67–2.58 (1H, m), 2.33–2.28 (1H, m), 2.25–2.15 (1H, m), 1.71–1.61 (3H, m), 0.90 (3H, s), 0.89 (6H, s), 0.07 (2H, s), 0.06 (4H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 135.33 (CH×0.67), 135.29 (CH×0.33), 116.5 (CH_2×0.33), 116.4 (CH_2×0.67), 80.5 (CH×0.33), 80.4 (CH×0.67), 80.2 (CH), 77.3 (CH×0.67), 76.8 (CH×0.33), 67.7 (CH_2×0.67), 67.6 (CH_2×0.33),

64.3 ($CH_2\times0.33$), 63.0 ($CH_2\times0.67$), 61.4 ($CH_2\times0.33$), 61.2 ($CH_2\times0.67$), 56.9 (CH), 56.3 ($CH\times0.67$), 54.5 ($CH\times0.33$), 36.5 ($CH_2\times0.67$), 36.3 ($CH_2\times0.33$), 30.6 ($CH_2\times0.67$), 29.9 ($CH_2\times0.33$), 25.79 (CH_3), 25.77 ($CH_3\times2$), 25.5 ($CH_2\times0.67$), 25.3 ($CH_2\times0.33$), 18.2 ($C\times0.33$), 18.1 ($C\times0.67$), −5.41 ($CH_3\times0.33$), −5.49 ($CH_3\times0.33$), −5.59 ($CH_3\times1.34$); LR-EIMS, *m/z* 315 (12.4%, $[M-t\text{-Bu}]^+$), 125 (bp); HR-EIMS, calcd for $C_{15}H_{27}O_5Si$ $[M-t\text{-Bu}]^+$: 315.1628, found: 315.1626.

5.1.12. (3*S*,4*R*,2′*R*,3′*S*)-4-{(2′-Allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-1-en-3-ol (30a) and (3*S*,4*S*,2′*R*,3′*S*)-4-{(2′-allyloxan-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-1-en-3-ol (30b). To a solution of **29** (**29a**:**29b**=1:2, 23.0 mg, 0.0617 mmol), PPh_3 (80.9 mg, 0.309 mmol), and imidazole (21.0 mg, 0.309 mmol) in THF (1.0 ml) was added I_2 (62.6 mg, 0.247 mmol) at 25 °C and the mixture was stirred for 45 min. Then, saturated aqueous $Na_2S_2O_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=30→10→5) to give **30** (13.4 mg, 61%) as an inseparable mixture of two diastereomers (**30a**:**30b**=1.0:2.0). **30**: a colorless oil; IR (film), ν_{max} 3457, 3075, 2929, 2857, 1642, 1472, 1463, 1434, 1389, 1361, 1340, 1279, 1257, 1186, 1098, 1030, 995, 919, 837, 814, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.97–5.82 (2H, m), 5.36 (0.67H, dt, J=17.3, 1.5 Hz), 5.35 (0.33H, dt, J=17.3, 1.5 Hz), 5.21 (1H, dt, J=10.3, 1.5 Hz), 5.15–5.06 (2H, m), 4.32–4.17 (1H, m), 3.92–3.87 (1H, m), 3.77–3.69 (1H, m), 3.67–3.61 (1H, m), 3.51–3.41 (1H, m), 3.36–3.26 (1H, m), 3.24–3.13 (2H, m), 2.78–2.65 (2H, m), 2.30–2.13 (2H, m), 1.68–1.53 (2H, m), 1.40–1.23 (1H, m), 0.90 (9H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 137.5 ($CH\times0.67$), 136.9 ($CH\times0.33$), 135.4 ($CH\times0.33$), 135.3 ($CH\times0.67$), 116.5 ($CH_2\times0.33$), 116.44 ($CH_2\times0.67$), 116.36 ($CH_2\times0.33$), 116.26 ($CH_2\times0.67$), 80.34 ($CH\times0.33$), 80.31 ($CH\times0.67$), 79.8 ($CH\times0.33$), 78.7 ($CH\times0.67$), 76.7 ($CH\times0.33$), 76.0 ($CH\times0.67$), 73.7 ($CH\times0.33$), 72.3 ($CH\times0.67$), 67.7 ($CH_2\times0.67$), 67.5 ($CH_2\times0.33$), 63.8 ($CH_2\times0.33$), 62.1 ($CH_2\times0.67$), 36.6 (CH_2), 30.2 ($CH_2\times0.33$), 29.8 ($CH_2\times0.67$), 25.8 ($CH_3\times3$), 25.32 ($CH_2\times0.67$), 25.28 ($CH_2\times0.33$), 18.1 (C), −5.47 ($CH_3\times0.33$), −5.57 ($CH_3\times0.33$), −5.62 ($CH_3\times0.67$), −5.64 ($CH_3\times0.67$); LR-EIMS, *m/z* 299 (24.8%, $[M-t\text{-Bu}]^+$), 125 (bp); HR-EIMS, calcd for $C_{15}H_{27}O_4Si$ $[M-t\text{-Bu}]^+$: 299.1678, found: 299.1692.

5.1.13. (1*S*,3*S*,4*S*,5*Z*,8*R*)-3-(*tert*-Butyldimethylsilyloxymethyl)-2,9-dioxabicyclo[6.4.0]dodec-5-en-4-ol (31). To a solution of **30** (**30a**:**30b**=1:2, 13.4 mg, 0.0376 mmol) in CH_2Cl_2 (6.0 ml) was added a solution of $(H_2IMes)(PCy_3)Cl_2Ru$=CHPh (3.2 mg, 3.76 μmol) in CH_2Cl_2 (6.0 ml). The resultant solution was stirred at 45 °C for 6 h. The mixture was cooled to 25 °C and stirred for 24 h under O_2 atmosphere. The reaction mixture was filtered through Celite-Florisil and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=15→7→3) to give **31** (5.4 mg, 44%). **31**: a yellow oil; $[\alpha]_D^{24}$ −14.3 (*c* 0.27, $CHCl_3$); IR (film), ν_{max} 3610, 3428, 3025, 2928, 2856, 1471, 1462, 1439, 1388, 1376, 1361, 1323, 1256, 1217, 1179, 1146, 1089, 1057, 1026, 993, 972, 956, 939, 838, 777, 664 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 5.88–5.73 (2H, m), 4.53–4.52 (1H, m), 4.10 (1H, dt, J=7.9, 4.6 Hz), 4.04 (1H, dd, J=10.8, 4.6 Hz), 3.97 (1H, dd, J=10.8, 7.9 Hz), 3.90–3.80 (1H, m), 3.71–3.65 (1H, m), 3.28 (1H, ddd, J=8.8, 5.1, 2.4 Hz), 3.06–2.97 (1H, m), 2.68 (1H, ddd, J=13.7, 8.4, 5.1 Hz), 2.42–2.34 (1H, m), 2.21–2.04 (1H, m), 1.77–1.75 (1H, m), 1.54–1.42 (2H, m), 1.32–1.16 (1H, m), 0.95 (9H, s), 0.04 (3H, s), 0.03 (3H, s); ^{13}C NMR (75 MHz, C_6D_6), δ 135.8 (CH), 127.8 (CH), 84.8 (CH), 80.3 (CH), 71.1 (CH), 69.2 (CH), 68.1 (CH_2), 60.7 (CH_2), 32.4 (CH_2), 32.0 (CH_2), 26.6 (CH_2), 26.0 ($CH_3\times3$), 18.4 (C), −5.43 (CH_3), −5.46 (CH_3); LR-EIMS, *m/z* 271 (36.4%, $[M-t\text{-Bu}]^+$), 71 (bp); HR-EIMS, calcd for $C_{13}H_{23}O_4Si$ $[M-t\text{-Bu}]^+$: 271.1365, found: 271.1355.

5.1.14. (2*R*,3*S*,2′*R*,3′*S*)-2-{(2′-Allyloxan-3′-yl)oxy}pent-4-en-1,3-diol (32a) and (2*S*,3*S*,2′*R*,3′*S*)-2-{(2′-allyloxan-3′-yl)oxy}pent-4-en-1,3-diol (32b). To a solution of **30** (**30a**:**30b**=1:2, 48.4 mg, 0.136 mmol) in THF (2.0 ml) was added TBAF (0.27 ml, 1.0 M in THF, 0.272 mmol) at 24 °C and the mixture was stirred for 20.5 h. Then, the solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=3→1/2→1/5) to give **32** (32.7 mg, 99%) as an inseparable mixture of two diastereomers (**32a**:**32b**=1.0:2.0). **32**: a pale yellow oil; IR (film), ν_{max} 3423, 3074, 2938, 2855, 1641, 1479, 1463, 1433, 1375, 1340, 1280, 1213, 1099, 995, 918, 869, 856, 681 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.99–5.84 (2H, m), 5.42–5.36 (1H, m), 5.25 (1H, dt, J=10.6, 1.5 Hz), 5.16–5.07 (2H, m), 4.36 (0.33H, dd, J=5.5, 4.0 Hz), 4.22 (0.67H, t, J=5.5 Hz), 3.90 (1H, ddd, J=11.4, 4.2, 1.8 Hz), 3.79 (1H, dd, J=11.7, 4.4 Hz), 3.71 (0.33H, dd, J=10.6, 5.5 Hz), 3.66 (0.67H, dd, J=11.7, 4.4 Hz), 3.50 (0.33H, dt, J=5.5, 4.0 Hz), 3.43 (0.67H, dt, J=5.5, 4.4 Hz), 3.34 (1H, td, J=11.4, 2.9 Hz), 3.28–3.21 (2H, m), 2.69–2.57 (1H, m), 2.46 (1H, brs) 2.34–2.21 (2H, m), 1.99 (1H, brs), 1.74–1.53 (2H, m), 1.43–1.26 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$), δ 137.0 ($CH\times0.67$), 136.6 ($CH\times0.33$), 135.24 ($CH\times0.33$), 135.19 ($CH\times0.67$), 117.1 (CH_2), 116.7 ($CH_2\times0.67$), 116.6 ($CH_2\times0.33$), 80.1 ($CH\times0.33$), 79.8 ($CH\times0.67$), 79.2 ($CH\times0.33$), 78.7 ($CH\times0.67$), 76.4 ($CH\times0.33$), 75.7 ($CH\times0.67$), 72.9 ($CH\times0.33$), 72.7 ($CH\times0.67$), 67.6 ($CH_2\times0.33$), 67.5 ($CH_2\times0.67$), 62.0 ($CH_2\times0.33$), 61.4 ($CH_2\times0.67$), 36.7 ($CH_2\times0.33$), 36.5 ($CH_2\times0.67$), 30.1 ($CH_2\times0.33$), 29.6 ($CH_2\times0.67$), 25.3 ($CH_2\times0.33$), 25.2 ($CH_2\times0.67$); LR-EIMS, *m/z* 242 (1.6%, $[M]^+$), 185 (35.3%, $[M-C_3H_5O]^+$), 125 (bp); HR-EIMS, calcd for $C_{10}H_{17}O_3$ $[M-C_3H_5O]^+$: 185.1178, found: 185.1200.

5.1.15. (2*R*,3*S*,4′*S*,5′*R*)-2-Allyl-3-{(2′,2′-dimethyl-4′-vinyl-1′,3′-dioxan-5′-yl)oxy}oxane (33a) and (2*R*,3*S*,4′*S*,5′*S*)-2-allyl-3-{(2′,2′-dimethyl-4′-vinyl-1′,3′-dioxan-5′-yl)oxy}oxane (33b). To a solution of **32** (**32a**:**32b**=1:2, 32.7 mg, 0.135 mmol) and 2,2-dimethoxypropane (83 μl, 0.675 mmol) in CH_2Cl_2 (1.5 ml) was added CSA (15.7 mg, 0.0675 mmol) at 24 °C and the mixture was stirred for 3.5 h. Then, to the mixture was added 2,2-dimethoxypropane (83 μl, 0.675 mmol) and stirred at 24 °C for 4 h. Then, saturated aqueous $NaHCO_3$ (3 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined

organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 10 → 5) to give **33a** (9.0 mg, 24%) and **33b** (22.8 mg, 60%). **33a**: a colorless oil; $[\alpha]_D^{23}$ +17.2 (*c* 0.45, $CHCl_3$); IR (film), ν_{max} 3074, 2991, 2939, 2852, 2726, 1641, 1462, 1433, 1409, 1372, 1340, 1261, 1225, 1201, 1167, 1097, 1022, 993, 926, 870, 655 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 6.21–5.99 (2H, m), 5.49 (1H, dd, *J* = 17.3, 1.3 Hz), 5.22 (1H, dd, *J* = 17.3, 1.8 Hz), 5.15 (1H, dd, *J* = 10.6, 1.3 Hz), 5.13–5.09 (1H, m), 4.15 (1H, dd, *J* = 9.2, 5.9 Hz), 3.79 (1H, dd, *J* = 11.4, 5.5 Hz), 3.66–3.64 (1H, m), 3.59 (1H, dd, *J* = 11.4, 9.2 Hz), 3.20 (1H, td, *J* = 9.2, 5.5 Hz), 3.10 (1H, td, *J* = 8.4, 2.9 Hz), 2.98–2.87 (2H, m), 2.82–2.74 (1H, m), 2.36–2.27 (1H, m), 1.64–1.54 (1H, m), 1.49 (3H, s), 1.35–0.89 (3H, m), 1.28 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 135.8 (CH), 135.4 (CH), 118.4 (CH_2), 116.6 (CH_2), 98.5 (C), 80.5 (CH), 77.6 (CH), 74.2 (CH), 73.5 (CH), 67.6 (CH_2), 64.0 (CH_2), 36.5 (CH_2), 31.0 (CH_2), 28.7 (CH_3), 25.2 (CH_2), 19.3 (CH_3); LR-EIMS, *m/z* 267 (17.1%, $[M-CH_3]^+$), 140 (bp); HR-EIMS, calcd for $C_{16}H_{26}O_4$ $[M]^+$: 282.1831, found: 282.1831. **33b**: a colorless oil; $[\alpha]_D^{24}$ +106.4 (*c* 1.14, $CHCl_3$); IR (film), ν_{max} 3076, 2990, 2937, 2854, 2725, 1641, 1453, 1434, 1380, 1371, 1270, 1240, 1199, 1133, 1096, 1049, 1029, 993, 950, 920, 886, 857, 811 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 6.32–6.18 (1H, m), 6.08 (1H, ddd, *J* = 17.3, 10.3, 6.2 Hz), 5.37–5.32 (1H, m), 5.23 (1H, dt, *J* = 17.3, 1.8 Hz), 5.18–5.13 (1H, m), 5.08 (1H, ddd, *J* = 10.3, 1.8, 1.1 Hz), 4.11 (1H, ddt, *J* = 6.2, 1.8, 1.1 Hz), 3.74 (1H, dd, *J* = 12.8, 1.8 Hz), 3.73–3.69 (1H, m), 3.45 (1H, dd, *J* = 12.8, 1.8 Hz), 3.25 (1H, ddd, *J* = 8.8, 7.3, 3.3 Hz), 3.05–2.96 (1H, m), 3.03 (1H, td, *J* = 11.6, 2.5 Hz), 2.87 (1H, ddd, *J* = 10.3, 8.8, 4.4 Hz), 2.64 (1H, q, *J* = 1.8 Hz), 2.56 (1H, dt, *J* = 14.3, 7.3 Hz), 1.75–1.71 (1H, m), 1.50 (3H, s), 1.37–1.01 (3H, m), 1.26 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 135.9 (CH), 135.5 (CH), 117.0 (CH_2), 116.5 (CH_2), 98.6 (C), 80.1 (CH), 74.9 (CH), 73.3 (CH), 70.5 (CH), 67.8 (CH_2), 61.7 (CH_2), 36.4 (CH_2), 29.3 (CH_3), 29.1 (CH_2), 25.4 (CH_2), 19.0 (CH_3); LR-EIMS, *m/z* 282 (1.9%, $[M]^+$), 267 (52.4%, $[M-CH_3]^+$), 125 (bp); HR-EIMS, calcd for $C_{16}H_{26}O_4$ $[M]^+$: 282.1831, found: 282.1824.

5.1.16. (1*S*,3*R*,8*S*,9*Z*,12*R*)-6,6-Dimethyl-2,5,7,13-tetraoxatricyclo[10.4.0.0^{3,8}]hexadeca-9-ene (34). To a solution of **33a** (3.9 mg, 0.0138 mmol) in CH_2Cl_2 (2.5 ml) was added a solution of $(H_2IMes)(PCy_3)Cl_2Ru{=}CHPh$ (1.2 mg, 1.38 μmol) in CH_2Cl_2 (1.0 ml). The resultant solution was stirred at 45 °C for 4.5 h. The mixture was cooled to 24 °C and stirred for 14 h under O_2 atmosphere. The reaction mixture was filtered through Celite-Florisil and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 10 → 5) to give **34** (2.9 mg, 83%). **34**: a colorless oil; $[\alpha]_D^{22}$ −79.9 (*c* 0.15, $CHCl_3$); IR (film), ν_{max} 3032, 2991, 2924, 2851, 2722, 1462, 1439, 1372, 1333, 1305, 1298, 1285, 1265, 1223, 1198, 1163, 1147, 1102, 1088, 1050, 1032, 987, 959, 943, 866, 843, 758, 645 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 5.95 (1H, dd, *J* = 10.6, 4.8 Hz), 5.87–5.77 (1H, m), 4.54–4.49 (1H, m), 3.92 (1H, dd, *J* = 11.2, 5.7 Hz), 3.65 (1H, dd, *J* = 11.2, 10.1 Hz), 3.65–3.60 (1H, m), 3.32–3.22 (2H, m), 3.15 (1H, ddd, *J* = 9.2, 4.8, 2.2 Hz), 2.95 (1H, td, *J* = 11.6, 2.6 Hz), 2.72 (1H, ddd, *J* = 13.6, 8.8, 4.8 Hz), 2.33 (1H, ddd, *J* = 13.6, 7.7, 2.2 Hz), 1.67–1.64 (1H, m), 1.49 (3H, s), 1.35–1.11 (3H, m), 1.13 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 134.4 (CH), 126.7 (CH), 98.2 (C), 83.1 (CH), 77.2 (CH), 75.2 (CH), 72.2 (CH), 68.3 (CH_2), 63.0 (CH_2), 31.24 (CH_2), 31.17 (CH_2), 29.0 (CH_3), 26.3 (CH_2), 18.8 (CH_3); LR-EIMS, *m/z* 254 (4.1%, $[M]^+$), 239 (38.5%, $[M-CH_3]^+$), 196 (bp); HR-EIMS, calcd for $C_{13}H_{19}O_4$ $[M-CH_3]^+$: 239.1283, found: 239.1269.

5.1.17. (1*S*,3*R*,4*S*,5*Z*,8*R*)-3-Hydroxymethyl-2,9-dioxabicyclo[6.4.0]dodec-5-en-4-ol (35). To a solution of **34** (2.9 mg, 0.0114 mmol) in THF–H_2O (1:1, v/v, 0.80 ml) was added TFA (40 μl) at 0 °C. The mixture was warmed to 23 °C and stirred for 11.5 h. After the mixture was diluted with Et_2O (3 ml), saturated aqueous $NaHCO_3$ (5 ml) was added. The mixture was extracted with EtOAc (4×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 1 → 1/5) to give **35** (1.7 mg, 71%). **35**: a colorless oil; $[\alpha]_D^{23}$ −38.5 (*c* 0.085, $CHCl_3$); IR (film), ν_{max} 3413, 3023, 2932, 2856, 1678, 1439, 1377, 1334, 1308, 1281, 1264, 1205, 1144, 1117, 1085, 1033, 979, 959, 918, 909, 855, 844, 760, 699, 647 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N), δ 6.23 (1H, dd, *J* = 11.0, 5.9 Hz), 5.90–5.82 (1H, m), 4.85 (1H, ddd, *J* = 8.8, 5.9, 2.0 Hz), 4.45 (1H, dd, *J* = 11.5, 3.4 Hz), 4.24 (1H, dd, *J* = 11.5, 5.9 Hz), 3.80–3.77 (1H, m), 3.77 (1H, ddd, *J* = 8.8, 5.9, 3.4 Hz), 3.60 (1H, td, *J* = 9.3, 4.4 Hz), 3.31 (1H, ddd, *J* = 9.3, 4.4, 2.0 Hz), 3.17 (1H, td, *J* = 11.2, 2.9 Hz), 2.91 (1H, ddd, *J* = 13.7, 9.8, 4.4 Hz), 2.34 (1H, ddd, *J* = 13.7, 6.8, 2.0 Hz), 2.06–2.04 (1H, m), 1.50–1.39 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$), δ 136.7 (CH), 127.0 (CH), 83.1 (CH), 82.8 (CH), 77.5 (CH), 70.8 (CH), 68.3 (CH_2), 64.2 (CH_2), 31.0 (CH_2), 29.7 (CH_2), 26.2 (CH_2); LR-EIMS, *m/z* 214 (20.0%, $[M]^+$), 71 (bp); HR-EIMS, calcd for $C_{11}H_{18}O_4$ $[M]^+$: 214.1205, found: 214.1232.

5.1.18. (1*R*,3*S*,4*R*,6*Z*,9*S*,11*R*)-4-(*tert*-Butyldimethylsilyloxy)-3-hydroxymethyl-11-phenyl-2,10,12-trioxabicyclo[7.4.0]tridec-6-ene (37). To a solution of **36** (87.0 mg, 0.160 mmol) in THF–H_2O (1:1, v/v, 2.0 ml) was added TFA (100 μl) at 0 °C and the mixture was stirred for 4 h. After the mixture was diluted with Et_2O (5 ml), saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with EtOAc (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 10 → 3) to give **37** (46.5 mg, 68%) along with recovered **36** (21.2 mg, 24%). **37**: a colorless oil; $[\alpha]_D^{21}$ −2.2 (*c* 0.34, $CHCl_3$); IR (film), ν_{max} 3478, 3018, 2955, 2929, 2857, 1471, 1462, 1455, 1390, 1360, 1340, 1294, 1252, 1213, 1139, 1104, 1074, 1041, 1028, 971, 940, 889, 777, 697, 673 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.37–7.22 (5H, m), 5.73–5.62 (2H, m), 5.31 (1H, s), 4.25 (1H, dd, *J* = 9.3, 3.4 Hz), 3.74–3.70 (1H, m), 3.59–3.52 (3H, m), 3.49–3.42 (2H, m), 3.27–3.22 (1H, m), 2.67–2.61 (1H, m), 2.44–2.40 (3H, m), 0.78 (9H, s), 0.11 (3H, s), 0.08 (3H, s); LR-EIMS, *m/z* 363 (53.8%, $[M-t\text{-Bu}]^+$), 75 (bp); HR-EIMS, calcd for $C_{19}H_{27}O_5Si$ $[M-t\text{-Bu}]^+$: 363.1628, found: 363.1650.

5.1.19. (2*R*,3*S*,5*Z*,8*R*,9*S*)-8-(*tert*-Butyldimethylsilyloxy)-2-hydroxymethyl-9-vinyl-2,3,4,7,8,9-hexahydrooxonin-3-ol (38). To oxalyl chloride (157 μl, 1.80 mmol) in CH_2Cl_2 (2.0 ml) was added DMSO (0.21 ml, 3.00 mmol) in CH_2Cl_2 (1.0 ml) dropwise at −78 °C and the mixture was stirred for 10 min. Then, **37** (259.1 mg, 0.599 mmol) in CH_2Cl_2 (0.9 ml) was added dropwise at −78 °C and the mixture was stirred for 15 min. Et_3N (0.83 ml, 5.99 mmol) was added dropwise at −78 °C. The mixture was warmed to −20 °C and stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×8 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant crude aldehyde was used immediately in the next reaction without purification. To a stirred suspension of $Ph_3P^+CH_3Br^-$ (1.07 g, 3.00 mmol) in THF (4.0 ml) was added NaHMDS (2.88 ml, 1.0 M in THF, 2.88 mmol) and the mixture was stirred at 24 °C. After 1 h, the resulting yellow suspension was allowed to stand at −78 °C. To the mixture was added dropwise a solution of the above crude aldehyde in THF (4.0 ml) at −78 °C and the mixture was stirred for 2.5 h. Then, the reaction mixture was warmed to 25 °C and stirred for 2.5 h. After the mixture was diluted with hexane (10 ml) and Et_2O (5 ml), saturated aqueous NH_4Cl (10 ml) was added and the mixture was extracted with Et_2O (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/EtOAc=20) to give a crude olefin compound, and it was used in the next reaction without further purification. To a suspension of the above olefin compound, 1,2-ehtanedithiol (0.79 ml, 9.40 mmol) and $NaHCO_3$ (394.8 mg, 4.70 mmol) in CH_2Cl_2 (7.0 ml) was added $Zn(OTf)_2$ (170.8 mg, 0.470 mmol) at 0 °C and the mixture was stirred for 2.5 h. After the mixture was diluted with Et_2O (5 ml), saturated aqueous $NaHCO_3$ (5 ml) and H_2O (5 ml) were added and the mixture was extracted with Et_2O–EtOAc (1:1, v/v, 3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=3→1) to give **38** (113.5 mg, 58% from **37**). **38**: a colorless oil; $[\alpha]_D^{24}$ −46.9 (*c* 0.21, $CHCl_3$); IR (film), ν_{max} 3584, 3382, 3018, 2955, 2928, 2858, 1472, 1462, 1449, 1256, 1098, 1051, 933, 833, 811, 775, 664 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 5.91–5.78 (3H, m), 5.25 (1H, dt, *J*=17.3, 1.1 Hz), 5.19 (1H, dt, *J*=10.3, 1.1 Hz), 4.02–3.94 (1H, m), 3.74–3.69 (3H, m), 3.52 (1H, brtd, *J*=8.4, 1.1 Hz), 3.23 (1H, dt, *J*=8.8, 4.4 Hz), 2.83–2.73 (2H, m), 2.23–1.99 (4H, m), 0.87 (9H, s), 0.06 (3H, s), 0.01 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 139.5 (CH), 128.6 (CH), 127.2 (CH), 117.2 (CH_2), 89.0 (CH), 86.7 (CH), 74.5 (CH), 71.6 (CH), 63.8 (CH_2), 32.9 (CH_2), 32.5 (CH_2), 25.7 (CH_3×3), 17.9 (C), −4.44 (CH_3), −4.59 (CH_3); LR-EIMS, *m/z* 271 (15.3%, $[M-t\text{-Bu}]^+$), 73 (bp); HR-EIMS, calcd for $C_{13}H_{23}O_4Si$ $[M-t\text{-Bu}]^+$: 271.1365, found: 271.1360.

5.1.20. (2*S*,3*R*,5*Z*,8*S*,9*R*)-8-Benzyloxy-9-benzyloxymethyl-2-vinyl-2,3,4,7,8,9-hexahydrooxonin-3-ol (5). To a solution of **38** (113.5 mg, 0.345 mmol) in THF (4.0 ml) was added NaH (165.7 mg, 60 wt% in oil, 4.14 mmol) at 0 °C and the mixture was stirred for 20 min. Then, to the mixture was added benzyl bromide (0.25 ml, 2.07 mmol) and TBAI (12.7 mg, 0.0345 mmol) at 0 °C. The reaction mixture was warmed to 24 °C and stirred for 15 h. After the mixture was cooled to 0 °C and diluted with Et_2O (5 ml), saturated aqueous NH_4Cl (10 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/EtOAc=20) to give a crude product, and it was used in the next reaction without further purification. To a solution of the above crude product in THF (3.0 ml) was added TBAF (0.69 ml, 1.0 M in THF, 0.690 mmol) at 25 °C and the mixture was stirred for 4 h. Since TLC analysis showed the starting material remained, TBAF (2.41 ml, 1.0 M in THF, 2.41 mmol) was added, and the stirring was continued for further 14 h. The solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5→3) to give **5** (135.8 mg, 100% from **38**) **5**: a colorless oil; $[\alpha]_D^{27}$ +28.3 (*c* 0.33, $CHCl_3$); IR (film), ν_{max} 3439, 3087, 3063, 3027, 2913, 2864, 1495, 1453, 1364, 1338, 1317, 1274, 1257, 1207, 1097, 1070, 1043, 1027, 928, 773, 736, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.37–7.20 (10H, m), 5.96–5.77 (3H, m), 5.28–5.22 (1H, m), 5.19–5.15 (1H, m), 4.59 (1H, d, *J*=11.4 Hz), 4.54 (1H, d, *J*=12.5 Hz), 4.41 (1H, d, *J*=12.5 Hz), 4.29 (1H, d, *J*=11.4 Hz), 3.93 (1H, dt, *J*=8.4, 3.7 Hz), 3.72 (1H, ddd, *J*=8.8, 4.0, 2.9 Hz), 3.53 (1H, dd, *J*=10.3, 2.9 Hz), 3.49 (1H, dd, *J*=10.3, 2.9 Hz), 3.44 (1H, brt, *J*=8.8 Hz), 3.28 (1H, dt, *J*=8.4, 2.9 Hz), 2.95–2.86 (1H, m), 2.74–2.65 (1H, m), 2.35 (1H, brdt, *J*=13.9, 4.0 Hz), 2.21–2.17 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$), δ 139.0 (CH), 138.4 (C), 138.2 (C), 128.7 (CH), 128.3 (CH×2), 128.2 (CH×2), 128.0 (CH×2), 127.8 (CH×2), 127.5 (CH×2), 127.3 (CH), 117.7 (CH_2), 88.7 (CH), 84.3 (CH), 78.1 (CH), 73.2 (CH_2), 72.7 (CH), 71.6 (CH_2), 69.3 (CH_2), 31.3 (CH_2), 27.4 (CH_2); LR-EIMS, *m/z* 394 (11.4%, $[M]^+$), 92 (bp); HR-EIMS, calcd for $C_{25}H_{30}O_4$ $[M]^+$: 394.2144, found: 394.2145.

5.1.21. Methyl (2*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-3-{(8′-benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydro-oxonin-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenoate (39). A solution of butynoate **20** (148.0 mg, 0.648 mmol) in CH_2Cl_2 (2.0 ml) was slowly added dropwise to a solution of **5** (85.4 mg, 0.216 mmol) and PMe_3 (0.32 ml, 1.0 M in THF, 0.324 mmol) in CH_2Cl_2 (3.0 ml) at 0 °C by means of a syringe. The mixture was warmed to 24 °C and stirred for 1 h. The mixture was cooled to 0 °C and diluted with hexane (5 ml) and Et_2O (5 ml). Then, saturated aqueous NH_4Cl (5 ml) was added and the mixture was extracted with Et_2O (3×8 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=30→20→10) to give **39** (121.5 mg, 90%). **39**: a colorless oil; $[\alpha]_D^{23}$ +24.8 (*c* 0.91, $CHCl_3$); IR (film), ν_{max} 3027, 2949, 2928, 2857, 1716, 1626, 1471, 1453, 1434, 1389, 1360, 1297, 1253, 1206, 1138, 1096, 1048, 837, 776, 735, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.34–7.19 (10H, m), 5.94–5.80 (2H, m), 5.70 (1H, td, *J*=10.8, 5.5 Hz), 5.32 (1H, dt, *J*=17.2, 1.4 Hz), 5.07 (1H, dt, *J*=10.5,

1.4 Hz), 4.99 (1H, s), 4.80 (1H, d, $J=13.3$ Hz), 4.68 (1H, d, $J=13.3$ Hz), 4.59 (1H, d, $J=11.2$ Hz), 4.54 (1H, d, $J=12.2$ Hz), 4.40 (1H, d, $J=12.2$ Hz), 4.29 (1H, d, $J=11.2$ Hz), 4.23–4.20 (1H, m), 3.98 (1H, dt, $J=8.8$, 3.0 Hz), 3.80–3.76 (1H, m), 3.66 (3H, s), 3.59 (1H, dd, $J=10.1$, 2.4 Hz), 3.53 (1H, dd, $J=10.1$, 2.4 Hz), 3.28 (1H, brdt, $J=8.8$, 2.4 Hz), 2.86–2.76 (1H, m), 2.74–2.65 (1H, m), 2.39–2.30 (2H, m), 0.89 (9H, s), 0.07 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 170.5 (C), 167.4 (C), 138.3 (C), 138.1 (C), 137.8 (CH), 129.1 (CH), 128.3 (CH×2), 128.2 (CH×2), 128.1 (CH×2), 127.8 (CH×2), 127.6 (CH×2), 126.6 (CH), 116.4 (CH_2), 92.6 (CH), 84.9 (CH), 84.7 (CH), 79.6 (CH), 77.5 (CH), 73.3 (CH_2), 71.7 (CH_2), 68.9 (CH_2), 60.1 (CH_2), 50.9 (CH_3), 27.4 (CH_2), 27.0 (CH_2), 25.8 (CH_3×3), 18.3 (C), −5.26 (CH_3), −5.28 (CH_3); LR-EIMS, *m/z* 565 (2.3%, $[M-t\text{-Bu}]^+$), 91 (bp); HR-EIMS, calcd for $C_{32}H_{41}O_7Si$ $[M-t\text{-Bu}]^+$: 565.2621, found: 565.2660.

5.1 .22. (2*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-3-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenol (40). To a solution of **39** (50.2 mg, 0.0806 mmol) in CH_2Cl_2 (1.5 ml) was added DIBAH (0.34 ml, 0.94 M in *n*-hexane, 0.322 mmol) at −78 °C and the mixture was stirred for 10 min. Then, saturated aqueous potassium sodium tartrate (5 ml) was added and the mixture was stirred at 24 °C for 3 h. The layers were separated and the aqueous layer was extracted with EtOAc (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→5→3) to give **40** (47.4 mg, 99%). **40**: a colorless oil; $[\alpha]_D^{22}$ +14.4 (*c* 0.39, $CHCl_3$); IR (film), ν_{max} 3584, 3416, 3087, 3064, 3027, 2952, 2927, 2857, 1660, 1496, 1471, 1462, 1454, 1389, 1360, 1337, 1319, 1295, 1274, 1253, 1203, 1183, 1145, 1096, 1027, 1004, 960, 929, 836, 815, 776, 735, 697, 673 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.33–7.20 (10H, m), 5.94–5.77 (2H, m), 5.69 (1H, td, $J=10.6$, 5.4 Hz), 5.28 (1H, dt, $J=17.3$, 1.7 Hz), 5.05 (1H, ddd, $J=10.5$, 1.7, 1.1 Hz), 4.88 (1H, t, $J=8.1$ Hz), 4.59 (1H, d, $J=11.2$ Hz), 4.54 (1H, d, $J=12.3$ Hz), 4.40 (1H, d, $J=12.3$ Hz), 4.29 (1H, d, $J=11.2$ Hz), 4.19 (1H, d, $J=12.3$ Hz), 4.16–4.09 (2H, m), 4.11 (1H, d, $J=12.3$ Hz), 4.11–4.08 (1H, m), 3.99–3.94 (1H, m), 3.70–3.65 (1H, m), 3.59 (1H, dd, $J=10.1$, 2.8 Hz), 3.53 (1H, dd, $J=10.1$, 2.2 Hz), 3.30–3.26 (1H, m), 2.78–2.66 (1H, m), 2.40–2.31 (2H, m), 0.90 (9H, s), 0.09 (3H, s), 0.08 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 157.4 (C), 138.4 (CH), 138.3 (C), 138.2 (C), 128.5 (CH), 128.3 (CH×2), 128.2 (CH×2), 128.1 (CH×2), 127.8 (CH×2), 127.6 (CH×2), 127.4 (CH), 115.9 (CH_2), 101.0 (CH), 85.5 (CH), 84.5 (CH), 78.0 (CH), 77.6 (CH), 73.3 (CH_2), 71.6 (CH_2), 69.0 (CH_2), 61.5 (CH_2), 58.2 (CH_2), 27.4 (CH_2), 26.8 (CH_2), 25.8 (CH_3×3), 18.2 (C), −5.30 (CH_3), −5.33 (CH_3); LR-EIMS, *m/z* 537 (3.0%, $[M-t\text{-Bu}]^+$), 91 (bp); HR-EIMS, calcd for $C_{31}H_{41}O_6Si$ $[M-t\text{-Bu}]^+$: 537.2672, found: 537.2698.

5.1.23. (2*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-3-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenal (41). To a mixture of **40** (42.8 mg, 0.0720 mmol) and MS 4 Å (42.8 mg, 100 wt%) in CH_2Cl_2 (1.2 ml) was added NMO (16.7 mg, 0.144 mmol) at 24 °C and the mixture was stirred for 10 min. Then, TPAP (5.1 mg, 0.0144 mmol) was added to the reaction mixture at 24 °C and the mixture was stirred for 1.5 h. The mixture was filtered through Celite and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→5) to give **41** (32.5 mg, 76%). **41**: a colorless oil; $[\alpha]_D^{26}$ +33.2 (*c* 0.52, $CHCl_3$); IR (film), ν_{max} 3087, 3064, 3027, 2953, 2928, 2885, 2857, 2766, 1663, 1615, 1496, 1471, 1462, 1389, 1361, 1321, 1295, 1255, 1207, 1144, 1097, 1037, 1006, 921, 837, 777, 736, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), δ 9.98 (1H, d, $J=7.3$ Hz), 7.35–7.20 (10H, m), 5.91–5.79 (2H, m), 5.66 (1H, td, $J=10.7$, 5.9 Hz), 5.35 (1H, d, $J=7.3$ Hz), 5.28 (1H, dt, $J=17.3$, 1.3 Hz), 5.08 (1H, dt, $J=10.3$, 1.3 Hz), 4.60 (1H, d, $J=11.0$ Hz), 4.53 (1H, d, $J=12.1$ Hz), 4.51 (1H, d, $J=13.2$ Hz), 4.41 (1H, d, $J=12.1$ Hz), 4.39 (1H, d, $J=13.2$ Hz), 4.29 (1H, d, $J=11.0$ Hz), 4.25–4.20 (1H, m), 4.00–3.94 (1H, m), 3.79–3.74 (1H, m), 3.58 (1H, dd, $J=9.9$, 2.8 Hz), 3.52 (1H, dd, $J=9.9$, 2.4 Hz), 3.29 (1H, brdt, $J=8.8$, 2.8 Hz), 2.88–2.79 (1H, m), 2.73–2.63 (1H, m), 2.40–2.26 (2H, m), 0.90 (9H, s), 0.09 (3H, s), 0.08 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 190.5 (CH), 174.2 (C), 138.2 (C), 138.1 (C), 137.5 (CH), 129.6 (CH), 128.32 (CH×2), 128.26 (CH×2), 128.1 (CH×2), 127.8 (CH×2), 127.6 (CH×2), 126.0 (CH), 117.1 (CH_2), 106.6 (CH), 84.8 (CH), 84.6 (CH), 79.9 (CH), 77.6 (CH), 73.3 (CH_2), 71.7 (CH_2), 68.9 (CH_2), 61.6 (CH_2), 27.4 (CH_2), 27.0 (CH_2), 25.7 (CH_3×3), 18.2 (C), −5.33 (CH_3), −5.36 (CH_3); LR-EIMS, *m/z* 535 (29.5%, $[M-t\text{-Bu}]^+$), 50 (bp); HR-EIMS, calcd for $C_{31}H_{39}O_6Si$ $[M-t\text{-Bu}]^+$: 535.2516, found: 535.2514.

5.1.24. (3*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-4-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-(trimethylsilyloxy)pent-3-enenitrile (42). To a solution of **41** (5.2 mg, 8.77 μmol) and TMSCN (5.8 μl, 0.0439 mmol) in benzene (0.40 ml) was added Me_3Al (9.6 μl, 1.01 M in *n*-hexane, 9.65 μmol) at 24 °C and the mixture was stirred for 1 h. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20→10) to give **42** (4.8 mg, 79%) as an inseparable 1:1 mixture of diastereomers. The nitrile **42** was unstable and immediately used for the next reaction. **42**: a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.31–7.20 (10H, m), 5.91–5.78 (2H, m), 5.72–5.62 (1H, m), 5.60 (0.5H, d, $J=9.0$ Hz), 5.58 (0.5H, d, $J=9.0$ Hz), 5.29–5.23 (1H, m), 5.10–5.03 (1H, m), 4.68–4.65 (1H, m), 4.60 (1H, d, $J=11.2$ Hz), 4.56–4.52 (1H, m), 4.41 (0.5H, d, $J=12.3$ Hz), 4.40 (0.5H, d, $J=12.3$ Hz), 4.30 (0.5H, d, $J=11.2$ Hz), 4.29 (0.5H, d, $J=11.2$ Hz), 4.19–4.04 (3H, m), 4.00–3.94 (1H, m), 3.68–3.60 (1H, m), 3.57–3.49 (2H, m), 3.29–3.26 (1H, m), 2.82–2.64 (1H, m), 2.39–2.27 (2H, m), 0.91 (4.5H, s), 0.90 (4.5H, s), 0.20 (4.5H, s), 0.18 (4.5H, s), 0.10 (1.5H, s), 0.09 (1.5H, s), 0.08 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 157.3 (C×0.5), 156.9 (C×0.5), 138.3 (C), 138.18 (CH×0.5), 138.16 (C), 138.0 (CH×0.5), 129.0 (CH×0.5), 128.9 (CH×0.5), 128.33 (CH×2), 128.26 (CH×2), 128.1 (CH×2), 127.9 (CH×2), 127.6 (CH×2), 126.9 (CH×0.5), 126.8 (CH×0.5), 120.0 (C×0.5), 119.9

(C×0.5), 116.6 (CH_2×0.5), 116.3 (CH_2×0.5), 99.3 (CH×0.5), 99.1 (CH×0.5), 85.4 (CH×0.5), 85.3 (CH×0.5), 84.6 (CH×0.5), 84.5 (CH×0.5), 78.7 (CH×0.5), 78.5 (CH×0.5), 77.6 (CH), 73.3 (CH_2), 71.6 (CH_2×0.5), 71.3 (CH_2×0.5), 69.0 (CH_2), 62.4 (CH_2×0.5), 62.2 (CH_2×0.5), 57.7 (CH×0.5), 57.6 (CH×0.5), 27.4 (CH_2), 26.9 (CH_2), 25.8 (CH_3×3), 18.3 (C), 0.02 (CH_3×1.5), −0.05 (CH_3×1.5), −5.38 (CH_3×0.5), −5.40 (CH_3×0.5), −5.51 (CH_3×0.5), −5.54 (CH_3×0.5).

5.1.25. (2*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-4-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-2-enenitrile (43). To a solution of **42** (58.8 mg, 0.0850 mmol) in CH_2Cl_2–Bu_3SnH (1:1, v/v, 1.0 ml) was added $BF_3\cdot OEt_2$ (31 μl, 0.255 mmol) at −18 °C and the mixture was stirred for 15 min. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with EtOAc (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=50→10→5) to give **43** (28.3 mg, 55%) as an inseparable 1:1 mixture of diastereomers at C2[35] along with recovered **42** (10.7 mg, 18%). **43**: a colorless oil; IR (film), ν_{max} 3064, 3027, 2954, 2927, 2858, 2225, 1496, 1471, 1462, 1454, 1388, 1361, 1331, 1294, 1254, 1204, 1098, 1027, 1005, 967, 928, 837, 814, 776, 736, 698, 670 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.39–7.20 (10H, m), 6.81 (0.5H, dd, J=16.5, 4.4 Hz), 6.62 (0.5H, dd, J=16.5, 4.8 Hz), 5.99–5.70 (3H, m), 5.68 (0.5H, dd, J=16.5, 1.8 Hz), 5.65 (0.5H, dd, J=16.5, 1.5 Hz), 5.32–5.21 (1H, m), 5.12 (0.5H, dd, J=10.3, 1.8 Hz), 5.08 (0.5H, dd, J=10.3, 1.8 Hz), 4.59 (1H, d, J=11.4 Hz), 4.53 (0.5H, d, J=12.1 Hz), 4.52 (0.5H, d, J=12.5 Hz), 4.40 (0.5H, d, J=12.5 Hz), 4.38 (0.5H, d, J=12.1 Hz), 4.28 (1H, d, J=11.4 Hz), 4.08–3.93 (2H, m), 3.69–3.46 (5H, m), 3.38 (1H, dd, J=9.9, 7.3 Hz), 3.24–3.20 (1H, m), 2.82–2.74 (1H, m), 2.67 (1H, ddd, J=13.9, 10.6, 3.3 Hz), 2.36–2.29 (1H, m), 2.21–2.17 (0.5H, m), 2.08–2.02 (0.5H, m), 0.88 (4.5H, s), 0.87 (4.5H, s), 0.05 (1.5H, s), 0.04 (1.5H, s), 0.03 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 153.7 (CH×0.5), 152.7 (CH×0.5), 138.9 (CH×0.5), 138.8 (CH×0.5), 138.4 (C), 138.3 (C), 128.8 (CH×0.5), 128.5 (CH×0.5), 128.32 (CH×2), 128.26 (CH×2), 128.1 (CH×2), 127.8 (CH×2), 127.6 (CH×2), 127.4 (CH×0.5), 127.1 (CH×0.5), 117.4 (CH_2×0.5), 117.2 (C×0.5), 117.1 (C×0.5), 116.1 (CH_2×0.5), 101.1 (CH×0.5), 100.2 (CH×0.5), 86.4 (CH×0.5), 85.9 (CH×0.5), 84.6 (CH×0.5), 84.5 (CH×0.5), 82.7 (CH×0.5), 81.6 (CH×0.5), 78.4 (CH×0.5), 78.2 (CH×0.5), 77.7 (CH×0.5), 77.4 (CH×0.5), 73.3 (CH_2), 71.6 (CH_2), 68.8 (CH_2), 65.2 (CH_2×0.5), 64.2 (CH_2×0.5), 28.9 (CH_2), 28.7 (CH_2), 25.8 (CH_3×3), 18.23 (C×0.5), 18.19 (C×0.5), −5.41 (CH_3×0.5), −5.50 (CH_3×0.5), −5.52 (CH_3×0.5), −5.63 (CH_3×0.5); LR-EIMS, *m/z* 546 (6.0%, $[M-t\text{-Bu}]^+$), 91 (bp); HR-EIMS, calcd for $C_{32}H_{40}NO_5Si$ $[M-t\text{-Bu}]^+$: 546.2675, found: 546.2678.

5.1.26. (2*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-4-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenal (44). To a solution of **43** (39.4 mg, 0.0652 mmol) in CH_2Cl_2 (1.0 ml) was added DIBAH (0.17 ml, 0.94 M in *n*-hexane, 0.163 mmol) at −78 °C and the mixture was stirred for 10 min. Then, saturated aqueous potassium sodium tartrate (5 ml) was added and the mixture was stirred at 24 °C for 10 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20→10→5) to give **44** (39.6 mg, ~100%) as an inseparable 1:1 mixture of diastereomers at C2.[35] **44**: a colorless oil; IR (film), ν_{max} 3064, 3027, 2953, 2927, 2858, 2736, 1694, 1496, 1471, 1462, 1454, 1377, 1361, 1329, 1294, 1254, 1206, 1099, 1069, 1027, 1005, 981, 928, 837, 814, 776, 736, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 9.58 (0.5H, d, J=8.1 Hz), 9.52 (0.5H, d, J=8.1 Hz), 7.31–7.20 (10H, m), 6.88 (0.5H, dd, J=15.8, 4.8 Hz), 6.66 (0.5H, dd, J=15.8, 5.5 Hz), 6.35 (0.5H, ddd, J=15.8, 8.1, 1.5 Hz), 6.29 (0.5H, ddd, J=15.8, 8.1, 1.5 Hz), 6.03–5.69 (3H, m), 5.33–5.28 (1H, m), 5.11–5.06 (1H, m), 4.58 (1H, d, J=11.4 Hz), 4.53 (0.5H, d, J=12.1 Hz), 4.52 (0.5H, d, J=12.1 Hz), 4.39 (1H, d, J=12.1 Hz), 4.28 (0.5H, d, J=11.4 Hz), 4.27 (0.5H, d, J=11.4 Hz), 4.19–4.18 (1H, m), 3.96 (1H, brdt, J=8.6, 3.1 Hz), 3.76–3.44 (6H, m), 3.24–3.21 (1H, m), 2.82–2.62 (2H, m), 2.36–2.29 (1H, m), 2.25–2.19 (0.5H, m), 2.12–2.10 (0.5H, m), 0.88 (4.5H, s), 0.87 (4.5H, s), 0.06 (1.5H, s), 0.05 (1.5H, s), 0.04 (3H, s); LR-EIMS, *m/z* 606 (12.9%, $[M]^+$), 213 (bp); HR-EIMS, calcd for $C_{32}H_{41}O_6Si$ $[M-t\text{-Bu}]^+$: 549.2672, found: 549.2669.

5.1.27. (2*E*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-4-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenol (45). To a solution of **44** (39.6 mg, 0.0652 mmol) in CH_2Cl_2 (1.5 ml) was added DIBAH (0.21 ml, 0.94 M in *n*-hexane, 0.196 mmol) at −78 °C and the mixture was stirred for 20 min. Then, saturated aqueous potassium sodium tartrate (5 ml) was added and the mixture was stirred at 24 °C for 2 h. The layers were separated and the aqueous layer was extracted with EtOAc (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→3) to give **45** (28.5 mg, 72% from **43**) as an inseparable 1:1 mixture of diastereomers at C2.[35] **45**: a colorless oil; IR (film), ν_{max} 3584, 3443, 3087, 3064, 3027, 2952, 2927, 2857, 1496, 1471, 1454, 1388, 1360, 1336, 1294, 1256, 1206, 1098, 1068, 1027, 1005, 928, 836, 815, 775, 736, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.32–7.19 (10H, m), 6.09–5.76 (4H, m), 5.74–5.66 (0.5H, m), 5.46 (0.5H, ddt, J=15.4, 7.3, 1.5 Hz), 5.36–5.27 (1H, m), 5.05 (1H, ddd, J=10.5, 5.0, 1.7 Hz), 4.58 (1H, d, J=11.0 Hz), 4.54 (0.5H, d, J=12.5 Hz), 4.53 (0.5H, d, J=12.5 Hz), 4.38 (1H, d, J=12.5 Hz), 4.27 (1H, d, J=11.0 Hz), 4.17–4.10 (2H, m), 3.98–3.92 (1H, m), 3.91–3.81 (1H, m), 3.64 (1H, dd, J=13.2, 5.9 Hz), 3.62–3.50 (4H, m), 3.45 (1H, dd, J=10.3, 5.9 Hz), 3.24–3.20 (1H, m), 2.78–2.65 (2H, m), 2.34–2.29 (1H, m), 2.25–2.21 (0.5H, m), 2.17–2.12 (0.5H, m), 0.88 (4.5H, s), 0.87 (4.5H, s), 0.05 (1.5H, s), 0.04 (1.5H, s), 0.03 (3H, s); LR-EIMS, *m/z* 551 (5.0%, $[M-t\text{-Bu}]^+$), 215 (bp); HR-EIMS, calcd for $C_{32}H_{43}O_6Si$ $[M-t\text{-Bu}]^+$: 551.2829, found: 551.2819.

5.1.28. (2*S*,3*R*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-4-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2,3-epoxypentanol (46). To a mixture of L-(+)-DET (12 μl, 0.0702 mmol) and pre-dried MS 4 Å (28.5 mg, 100 wt%) in CH_2Cl_2 (0.5 ml) was added $Ti(O^iPr)_4$ (18 μl, 0.0608 mmol) at −40 °C and the mixture was stirred for 30 min. Then, TBHP (0.13 ml, 3.7 M in toluene, 0.468 mmol) was added and the mixture was stirred at −40 °C for 30 min. To the mixture was added dropwise a solution of **45** (28.5 mg, 0.0468 mmol) in CH_2Cl_2 (1.0 ml). The reaction mixture was stirred at −40 °C for 30 min. Then, the reaction mixture was warmed to −25 °C and stirred for 26 h. DMS (34 μl, 0.463 mmol) was added at −25 °C and the mixture was stirred for 2 h until unreacted TBHP was consumed. To the mixture was added 10% DL-tartaric acid (36 μl) and NaF (15.3 mg) at −25 °C. The suspension was warmed to 24 °C and stirred for 24 h. The mixture was filtered through Celite and concentrated in vacuo. To the resultant residue was added Et_2O (4.0 ml) and 30% aqueous NaOH in brine (2.0 ml) at 0 °C and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5→3→1) to give **46** (29.2 mg, ~100%) as an inseparable 1:1 mixture of diastereomers at C2.[35] **46**: a colorless oil; IR (film), ν_{max} 3454, 3064, 3027, 2927, 2858, 1496, 1471, 1454, 1389, 1361, 1256, 1096, 1027, 930, 901, 837, 776, 737, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.31–7.19 (10H, m), 6.03–5.89 (1H, m), 5.84–5.74 (2H, m), 5.31–5.26 (1H, m), 5.06 (1H, ddd, *J*=10.3, 4.8, 1.8 Hz), 4.60–4.51 (3H, m), 4.41–4.25 (3H, m), 3.98–3.90 (2H, m), 3.66–3.43 (6H, m), 3.29–3.20 (1H, m), 3.18–3.05 (2H, m), 2.84–2.64 (2H, m), 2.34–2.20 (2H, m), 0.89 (4.5H, s), 0.87 (4.5H, s), 0.06 (3H, s), 0.03 (3H, s); LR-EIMS, *m/z* 624 (14.6%, $[M]^+$), 92 (bp); HR-EIMS, calcd for $C_{36}H_{52}O_7Si$ $[M]^+$: 624.3482, found: 624.3487.

5.1.29. (3*R*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-4-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-1-en-3-ol (47). To a solution of **46** (29.2 mg, 0.0468 mmol), PPh_3 (59.8 mg, 0.228 mmol), and imidazole (15.5 mg, 0.228 mmol) in THF (1.0 ml) was added I_2 (46.3 mg, 0.182 mmol) at 25 °C and the mixture was stirred for 35 min. Then, saturated aqueous $Na_2S_2O_3$ (8 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was roughly purified by column chromatography (silica gel, hexane/EtOAc=10→5→3) to give the corresponding crude epoxy iodide, and it was used in the next reaction without further purification. To a solution of the above crude epoxy iodide in EtOH (0.80 ml) was added Zn (17.8 mg, 0.272 mmol) and saturated aqueous NH_4Cl (20 μl) at 25 °C and the mixture was stirred for 2.5 h. The mixture was filtered through Celite-anhydrous $MgSO_4$ and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20→10→5) to give **47** (19.3 mg, 68% from **45**) as an inseparable 1:1 mixture of diastereomers at C2.[35] **47**: a colorless oil; IR (film), ν_{max} 3488, 3064, 3027, 2953, 2927, 2857, 1496, 1471, 1462, 1454, 1388, 1361, 1337, 1295, 1256, 1206, 1098, 1027, 1004, 925, 837, 814, 776, 735, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.32–7.19 (10H, m), 6.05–5.85 (2H, m), 5.83–5.71 (2H, m), 5.42–5.05 (4H, m), 4.59 (0.5H, d, *J*=11.4 Hz), 4.58 (0.5H, d, *J*=11.4 Hz), 4.53 (0.5H, d, *J*=12.5 Hz), 4.52 (0.5H, *J*=12.5 Hz), 4.40 (0.5H, d, *J*=12.5 Hz), 4.38 (0.5H, d, *J*=12.5 Hz), 4.28 (0.5H, d, *J*=11.4 Hz), 4.27 (0.5H, d, *J*=11.4 Hz), 4.26–4.19 (1H, m), 3.99–3.93 (1H, m), 3.70–3.38 (7H, m), 3.26–3.19 (1H, m), 2.80–2.64 (2H, m), 2.66 (0.5H, d, *J*=7.0 Hz), 2.61 (0.5H, d, *J*=5.5 Hz), 2.35–2.17 (2H, m), 0.88 (9H, s), 0.05 (3H, s), 0.04 (3H, s); LR-FDMS, *m/z* 608 (bp, $[M]^+$); HR-FDMS, calcd for $C_{36}H_{52}O_6Si$ $[M]^+$: 608.3533, found: 608.3512.

5.1.30. (3*R*,2′*S*,3′*R*,5′*Z*,8′*S*,9′*R*)-2-{(8′-Benzyloxy-9′-benzyloxymethyl-2′-vinyl-2′,3′,4′,7′,8′,9′-hexahydrooxonin-3′-yl)oxy}pent-4-en-1,3-diol (48). To a solution of **47** (19.3 mg, 0.0317 mmol) in THF (1.0 ml) was added TBAF (48 μl, 1.0 M in THF, 0.0476 mmol) at 25 °C and the mixture was stirred for 11.5 h. Then, the solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=3→1/2→EtOAc) to give **48** (10.7 mg, 68%) as an inseparable 1:1 mixture of diastereomers at C2.[35] **48**: a colorless oil; IR (film), ν_{max} 3357, 3087, 3063, 3028, 2911, 1498, 1453, 1425, 1422, 1402, 1361, 1337, 1294, 1257, 1207, 1146, 1096, 1070, 1027, 994, 930, 774, 735, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.34–7.20 (10H, m), 6.03–5.87 (1H, m), 5.86–5.69 (3H, m), 5.44–5.17 (4H, m), 4.59 (1H, d, *J*=11.4 Hz), 4.52 (1H, d, *J*=12.5 Hz), 4.41 (0.5H, d, *J*=12.5 Hz), 4.41 (0.5H, *J*=12.5 Hz), 4.33–4.23 (1H, m), 4.28 (1H, d, *J*=11.4 Hz), 3.95 (1H, brdqn, *J*=8.9, 3.0 Hz), 3.73–3.63 (2H, m), 3.60–3.46 (5H, m), 3.28–3.25 (1H, m), 2.85–2.75 (1H, m), 2.73–2.64 (1H, m), 2.54 (0.5H, d, *J*=3.3 Hz), 2.46 (0.5H, d, *J*=2.9 Hz), 2.31–2.22 (2H, m), 1.99–1.90 (1H, m); LR-EIMS, *m/z* 494 (7.7%, $[M]^+$), 91 (bp); HR-EIMS, calcd for $C_{30}H_{38}O_6$ $[M]^+$: 494.2668, found: 494.2666.

5.1.31. (2*R*,3*S*,5*Z*,8*R*,9*S*,4′*R*,5′*S*)-3-Benzyloxy-2-benzyloxymethyl-8-{(2′,2′-dimethyl-4′-vinyl-1′,3′-dioxan-5′-yl)oxy}-9-vinyl-2,3,4,7,8,9-hexahydrooxonin (49a) and (2*R*,3*S*,5*Z*,8*R*,9*S*,4′*R*,5′*R*)-3-benzyloxy-2-benzyloxymethyl-8-{(2′,2′-dimethyl-4′-vinyl-1′,3′-dioxan-5′-yl)-oxy}-9-vinyl-2,3,4,7,8,9-hexahydrooxonin (49b). To a solution of **48** (10.7 mg, 0.0216 mmol) and 2,2-dimethoxypropane (11 μl, 0.0864 mmol) in CH_2Cl_2 (0.5 ml) was added CSA (2.5 mg, 0.0108 mmol) at 24 °C and the mixture was stirred for 3 h. Then, to the mixture was added acetone (7.9 μl, 0.108 mmol) and stirred at 24 °C for 11.5 h. Then, saturated aqueous $NaHCO_3$ (3 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→5) to give **49a** (5.8 mg, 50%) and **49b** (5.3 mg, 46%). **49a**: a colorless oil; $[\alpha]_D^{19}$ +39.4 (*c* 0.27, $CHCl_3$); IR (film), ν_{max} 3065, 3026, 2992, 2914, 2865, 1453, 1372, 1262, 1201, 1146, 1099, 1027, 696 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 7.29–7.15 (10H, m), 6.14–5.90 (2H, m), 5.89–5.81 (1H, m), 5.79–5.74 (1H, m), 5.52 (1H, dt, *J*=17.3, 1.7 Hz), 5.42 (1H, ddd, *J*=

17.3, 2.0, 1.1 Hz), 5.15 (1H, dt, J=10.6, 1.7 Hz), 5.06 (1H, ddd, J=10.3, 2.6, 1.1 Hz), 4.43 (1H, d, J=11.7 Hz), 4.37 (1H, d, J=11.7 Hz), 4.27 (1H, d, J=11.7 Hz), 4.22 (1H, d, J=11.7 Hz), 4.19–4.16 (1H, m), 3.99 (1H, dt, J=7.7, 3.1 Hz), 3.89 (1H, dd, J=11.2, 5.5 Hz), 3.68–3.63 (1H, m), 3.65 (1H, dd, J=11.2, 9.5 Hz), 3.58 (2H, d, J=2.9 Hz), 3.43–3.36 (2H, m), 3.23 (1H, td, J=9.5, 5.5 Hz), 2.77–2.71 (1H, m), 2.69–2.63 (1H, m), 2.29–2.22 (1H, m), 1.93–1.85 (1H, m), 1.49 (3H, s), 1.27 (3H, s); LR-FDMS, *m/z* 534 (bp, $[M]^+$); HR-FDMS, calcd for $C_{33}H_{42}O_6$ $[M]^+$: 534.2981, found: 534.2957. **49b**: a colorless oil; $[\alpha]_D^{19}$ −15.5 (*c* 0.27, $CHCl_3$); IR (film), ν_{max} 3065, 3012, 2921, 2866, 2803, 1453, 1373, 1365, 1335, 1272, 1241, 1198, 1183, 1152, 1120, 1095, 1063, 1044, 1028, 989, 978, 935, 915, 857, 773, 752, 697 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 7.28–7.15 (10H, m), 6.48 (1H, ddd, J=17.3, 10.6, 4.4 Hz), 6.12 (1H, ddd, J=17.3, 10.6, 6.2 Hz), 6.03–5.97 (2H, m), 5.75 (1H, ddd, J=17.3, 2.2, 1.5 Hz), 5.25 (1H, dt, J=17.3, 1.5 Hz), 5.18 (1H, ddd, J=10.6, 2.2, 1.5 Hz), 5.09 (1H, dt, J=10.6, 1.5 Hz), 4.44 (1H, d, J=11.7 Hz), 4.37 (1H, d, J=11.7 Hz), 4.23 (2H, d, J=11.7 Hz), 4.11–4.05 (2H, m), 3.80–3.76 (1H, m), 3.70 (1H, dd, J=12.8, 1.8 Hz), 3.61 (2H, d, J=2.6 Hz), 3.40 (1H, dd, J=12.8, 1.8 Hz), 3.35 (1H, dt, J=8.4, 2.6 Hz), 3.26 (1H, dt, J=8.8, 2.9 Hz), 2.86–2.70 (2H, m), 2.55 (1H, brqn, J=1.8 Hz), 2.36–2.28 (1H, m), 2.07–2.00 (1H, m), 1.52 (3H, s), 1.24 (3H, s); LR-FDMS, *m/z* 534 (bp, $[M]^+$), 519 (22.0, $[M-Me]^+$); HR-FDMS, calcd for $C_{33}H_{42}O_6$ $[M]^+$: 534.2981, found: 534.2964.

5.1.32. (1*R*,3*S*,8*R*,9*Z*,11*S*,13*R*,14*S*,16*Z*)-14-Benzyloxy-13-benzyloxymethyl-6,6-dimethyl-2,5,7,12-tetraoxatricyclo[9.7.0.0^{3,8}]octadeca-9,16-diene (50) and (1*S*,3*R*,5*Z*,7*R*,2′*S*,3′*Z*,6′*S*,7′*R*)-3-(6′-benzyloxy-7′-benzyloxymethyl-2′,5′,6′,7′-tetrahydrooxepin-2′-yl)-9,9-dimethyl-2,8,10-trioxabicyclo[5.4.0]undec-5-ene (51). To a solution of **49a** (5.8 mg, 0.0108 mmol) in degassed CH_2Cl_2 (3.0 ml) was added a solution of $(Cy_3P)_2Cl_2Ru$=CHPh (0.9 mg, 1.08 μmol) in degassed CH_2Cl_2 (1.0 ml). The resultant solution was stirred at 24 °C for 3 h. Then, extra $(Cy_3P)_2Cl_2Ru$=CHPh (1.3 mg, 1.62 μmol) in degassed CH_2Cl_2 (2.0 ml) was added, and the stirring was continued for further 12 h. After that, the mixture was stirred for 2 h under O_2 atmosphere, and the solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/EtOAc=40→10) to give the 1:1:0.5 mixture of **49a**, **50** and **51**. In order to consume **49a** completely, the process was repeated as follows. To a solution of the above mixture in degassed CH_2Cl_2 (2.0 ml) was added a solution of $(Cy_3P)_2Cl_2Ru$=CHPh (0.60 ml, 1.64mM in degassed CH_2Cl_2, 0.984 μmol). The resultant solution was stirred at 26 °C for 8 h. Then, extra $(Cy_3P)_2Cl_2Ru$=CHPh (0.80 ml, 1.21 mM in degassed CH_2Cl_2, 0.968 μmol) was added, and the stirring was continued for further 16 h. After that, the mixture was stirred for 2 h under O_2 atmosphere, and the solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/EtOAc=50→40→10) to give **50** (3.7 mg, 67%, after 2 cycles) and **51** (1.3 mg, 24%, after 2 cycles). **50**: a colorless oil; $[\alpha]_D^{23}$ +13.0 (*c* 0.20, $CHCl_3$); IR (film), ν_{max} 3087, 3063, 3027, 2991, 2922, 2855, 1496, 1454, 1372, 1336, 1311, 1292, 1267, 1221, 1200, 1100, 1027, 988, 945, 893, 866, 779, 767, 735, 697, 680 cm^{-1}; ^1H NMR (300 MHz, C_5D_5N), δ 7.46–7.27 (10H, m), 6.29–6.23 (1H, m), 6.01–5.86 (2H, m), 5.77–5.63 (1H, m), 4.69 (1H, d, J=11.5 Hz), 4.53–4.49 (3H, m), 4.42 (1H, d, J=11.5 Hz), 4.10–4.07 (1H, m), 3.96 (1H, dd, J=11.0, 5.5 Hz), 3.84–3.68 (4H, m), 3.67–3.61 (1H, m), 3.58–3.52 (1H, m), 3.40 (1H, td, J=9.5, 5.5 Hz), 3.00–2.90 (1H, m), 2.76–2.67 (1H, m), 2.44–2.36 (1H, m), 2.19–2.09 (1H, m), 1.47 (3H, s), 1.44 (3H, s); ^1H NMR (300 MHz, $(CD_3)_2C$=O), δ 7.34–7.24 (10H, m), 6.00 (1H, dt, J=12.4, 2.6 Hz), 5.78–5.67 (2H, m), 5.40 (1H, dt, J=12.4, 2.6 Hz), 4.68 (1H, d, J=11.6 Hz), 4.49 (2H, s), 4.41 (1H, d, J=11.6 Hz), 4.30 (1H, brdqn, J=9.4, 2.6 Hz), 3.88–3.83 (1H, m), 3.72 (1H, dd, J=11.3, 5.7 Hz), 3.70–3.61 (3H, m), 3.58–3.50 (1H, m), 3.55 (1H, dd, J=11.3, 9.4 Hz), 3.29 (1H, ddd, J=8.5, 5.7, 2.6 Hz), 3.18 (1H, td, J=9.4, 5.7 Hz), 2.89–2.78 (1H, m), 2.66–2.58 (1H, m), 2.39–2.32 (1H, m), 2.07–1.98 (1H, m), 1.42 (3H, s), 1.28 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$), δ 138.4 (C), 138.1 (C), 136.5 (CH), 131.2 (CH), 128.44 (CH×2), 128.42 (CH×2), 128.3 (CH×2), 128.2 (CH×2), 127.93 (CH), 127.91 (CH), 127.8 (CH), 127.7 (CH), 98.3 (C), 85.8 (CH), 85.5 (CH), 83.3 (CH), 77.7 (CH), 75.0 (CH), 73.4 (CH_2), 73.3 (CH), 71.6 (CH_2), 69.6 (CH_2), 62.9 (CH_2), 31.8 (CH_2), 29.8 (CH_2), 29.0 (CH_3), 18.9 (CH_3); LR-FDMS, *m/z* 506 (22.4%, $[M]^+$), 491 (47.9%, $[M-Me]^+$), 91 (bp); HR-FDMS, calcd for $C_{31}H_{38}O_6$ $[M]^+$: 506.2669, found: 506.2650. **51**: a colorless oil; $[\alpha]_D^{24}$ +3.5 (*c* 0.065, $CHCl_3$); IR (film), ν_{max} 3063, 3029, 2991, 2922, 2854, 1496, 1495, 1454, 1434, 1371, 1301, 1267, 1199, 1161, 1102, 1028, 737, 697 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 7.25–7.05 (10H, m), 5.95 (1H, ddd, J=11.4, 2.8, 1.7 Hz), 5.79–5.74 (1H, m), 5.71–5.61 (2H, m), 4.54–4.47 (2H, m), 4.34 (1H, d, J=11.7 Hz), 4.26 (1H, d, J=11.7 Hz), 4.22 (1H, d, J=12.1 Hz), 4.12–3.99 (4H, m), 3.96 (1H, dd, J=11.4, 5.5 Hz), 3.70 (1H, dd, J=11.4, 9.4 Hz), 3.62–3.51 (3H, m), 2.76–2.67 (1H, m), 2.52–2.41 (3H, m), 1.48 (3H, s), 1.26 (3H, s); LR-FDMS, *m/z* 507 (64.2%, $[M+H]^+$), 506 (bp, $[M]^+$); HR-FDMS, calcd for $C_{31}H_{38}O_6$ $[M]^+$: 506.2669, found: 506.2657.

5.1.33. 1-(4-Methoxybenzyloxy)-2-propyne (60). To a solution of propargyl alcohol **59** (1.50 ml, 25.8 mmol) in THF (70 ml) was added NaH (1.86 g, 60 wt% in oil, 46.4 mmol) at 0 °C and the mixture was stirred for 15 min. Then, *p*-methoxy benzyl chloride (PMBCl) (5.20 ml, 38.7 mmol) and TBAI (1.24 g, 3.35 mmol) was added at 0 °C. The reaction mixture was warmed to 24 °C and stirred for 16 h. After the mixture was cooled to 0 °C and diluted with Et_2O (30 ml), saturated aqueous NH_4Cl (50 ml) was added and the mixture was extracted with Et_2O (3×30 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/Et_2O=15→10→5) to give **60** (4.50 g, 99%). **60**: a colorless oil; IR (film), ν_{max} 3289, 3001, 2937, 2907, 2837, 2754, 2115, 1612, 1586, 1513, 1464, 1441, 1422, 1387, 1352, 1302, 1249, 1174, 1078, 1034, 927, 848, 819, 759, 647 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.30–7.26 (2H, m), 6.90–6.87 (2H, m), 4.54 (2H, s), 4.14 (2H, d, J=2.4 Hz), 3.81 (3H, s), 2.45 (1H, t, J=2.4 Hz); LR-EIMS, *m/z* 176 (61.6, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{11}H_{12}O_2$ $[M]^+$: 176.0837, found: 176.0822.

5.1.34. Ethyl 4-(4-methoxybenzyloxy)-2-butynoate (61). To a solution of **60** (2.85 g, 16.2 mmol) in THF (40 ml) was

added *n*-BuLi (15.4 ml, 1.58 M in *n*-hexane, 24.3 mmol) at −78 °C and the mixture was stirred for 10 min. Then, ethylchloroformate (2.30 ml, 24.3 mmol) was added at −78 °C and the mixture was stirred for 1 h. The reaction mixture was warmed to 0 °C and stirred for 15 min. After the mixture was diluted with Et_2O (20 ml), H_2O (30 ml) was added and the mixture was extracted with Et_2O (3×30 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/Et_2O=20→15→10→5→3) to give **61** (3.18 g, 79%). **61**: a pale yellow oil; IR (film), ν_{max} 2983, 2939, 2907, 2869, 2838, 2235, 1713, 1613, 1586, 1513, 1465, 1443, 1388, 1366, 1302, 1248, 1174, 1091, 1055, 1034, 820, 751, 626 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.29–7.26 (2H, m), 6.90–6.87 (2H, m), 4.54 (2H, s), 4.25 (2H, q, J=7.2 Hz), 4.25 (2H, s), 3.81 (3H, s), 1.32 (3H, t, J=7.2 Hz); LR-EIMS, *m/z* 248 (37.4, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{14}H_{16}O_4$ $[M]^+$: 248.1048, found: 248.1051.

5.1.35. 4-(4-Methoxybenzyloxy)-2-butynal (62) and 4-(4-methoxybenzyloxy)-2-butynol (63). To a solution of **61** (2.96 g, 11.9 mmol) in CH_2Cl_2 (35 ml) was added DIBAH (25.6 ml, 0.93 M in *n*-hexane, 23.8 mmol) at −78 °C and the mixture was stirred for 30 min. Then, extra DIBAH (6.40 ml, 0.93 M in *n*-hexane, 5.95 mmol) was added at −78 °C and the mixture was stirred for 45 min. Then, saturated aqueous potassium sodium tartrate (50 ml) was added and the mixture was stirred at 23 °C for 14 h. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3×30 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=3→1→1/2) to give **63** (635.5 mg, 26%) along with **62** (1.80 g, 74%). **62**: a yellow oil; IR (film), ν_{max} 3004, 2973, 2838, 2248, 2182, 1728, 1671, 1612, 1586, 1578, 1513, 1465, 1442, 1428, 1388, 1349, 1302, 1250, 1175, 1160, 1112, 1075, 1032, 817 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 9.25 (1H, d, J=0.55 Hz), 7.29–7.26 (2H, m), 6.92–6.88 (2H, m), 4.56 (2H, s), 4.32 (2H, d, J=0.55 Hz), 3.81 (3H, s); LR-EIMS, *m/z* 204 (75.5%, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{12}H_{12}O_3$ $[M]^+$: 204.0786 found: 204.0747. **63**: a yellow oil; IR (film), ν_{max} 3629, 3394, 3000, 2935, 2859, 1612, 1586, 1513, 1464, 1442, 1422, 1386, 1351, 1302, 1248, 1175, 1122, 1072, 1031, 942, 920, 819, 756, 709, 603 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.30–7.26 (2H, m), 6.91–6.87 (2H, m), 4.53 (2H, s), 4.33 (2H, dt, J=6.2, 1.8 Hz), 4.18 (2H, t, J=1.8 Hz), 3.81 (3H, s), 1.62 (1H, t, J=6.2 Hz); LR-EIMS, *m/z* 206 (21.5%, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{12}H_{14}O_3$ $[M]^+$: 206.0943 found: 206.0904.

5.1.36. 4-(4-Methoxybenzyloxy)-2-butynol (63). To a solution of **62** (1.80 g, 8.81 mmol) in CH_2Cl_2 (25 ml) was added DIBAH (23.5 ml, 0.93 M in *n*-hexane, 21.9 mmol) at −78 °C and the mixture was stirred for 10 min. Then, saturated aqueous potassium sodium tartrate (50 ml) was added and the mixture was stirred at 24 °C for 10 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×20 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=2→1/2) to give **63** (1.83 g, ~100%).

5.1.37. (2*E*)-4-(4-Methoxybenzyloxy)-2-butenol (64). To a mixture of LAH (2.05 g, 43.3 mmol) in THF (25 ml) was added dropwise a solution of **63** (2.23 g, 10.8 mmol) in THF (15 ml) at −78 °C and the mixture was stirred for 13 min. Then, the reaction mixture was warmed to −20 °C and stirred for 26 h. The mixture was warmed to 0 °C and diluted with Et_2O (30 ml). Then, H_2O (2.1 ml), 4N aqueous NaOH (2.1 ml) and H_2O (6.3 ml) were added in turn. The resultant suspension was filtered with Celite-anhydrous $MgSO_4$ and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=3→2→1/2) to give **64** (1.43 g, 64%) along with **63** (0.54 g, 24%). **64**: a colorless oil; IR (film), ν_{max} 3583, 3377, 3003, 2933, 2854, 1612, 1586, 1513, 1463, 1442, 1421, 1404, 1386, 1360, 1302, 1247, 1174, 1093, 1033, 1009, 972, 847, 819, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.28–7.25 (2H, m), 6.89–6.86 (2H, m), 5.96–5.79 (2H, m), 4.46 (2H, s), 4.22–4.16 (2H, m), 4.01 (2H, d, J=5.1 Hz), 3.81 (3H, s); LR-EIMS, *m/z* 208 (21.2%, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{12}H_{16}O_3$ $[M]^+$: 208.1099 found: 208.1106.

5.1.38. (2*R*,3*R*)-2,3-Epoxy-4-(4-methoxybenzyloxy)-1-butanol (65). To a mixture of D-(−)-DET (0.22 ml, 1.29 mmol) and pre-dried MS 4 Å (1.35 g, 100 wt%) in CH_2Cl_2 (15 ml) was added $Ti(O^iPr)_4$ (0.29 ml, 0.97 mmol) at −40 °C and the mixture was stirred for 30 min. Then, TBHP (4.6 ml, 3.5 M in toluene, 16.2 mmol) was added and the mixture was stirred at −40 °C for 30 min. To the mixture was added dropwise a solution of **64** (1.35 g, 6.47 mmol) in CH_2Cl_2 (15 ml). The reaction mixture was stirred at −40 °C for 30 min, warmed to −20 °C and stirred for 2.5 d. Then, DMS (1.2 ml, 16.2 mmol) was added at −20 °C and the mixture was stirred for 2 h until unreacted TBHP was consumed. To the mixture was added 10% DL-tartaric acid (0.58 ml) and NaF (244.4 mg) at −25 °C. The suspension was warmed to 23 °C and stirred for 24 h. The mixture was filtered through Celite and concentrated in vacuo. To the resultant residue was added Et_2O (30 ml) and 30% aqueous NaOH in brine (10 ml) at 0 °C and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×20 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=3→2→1→1/2→1/4) to give **65** (1.03 g, 71%, 93% ee). **65**: a colorless solid (EtOAc), mp 62.0–63.0 °C; $[\alpha]_D^{25}$ +13.9 (*c* 1.01, $CHCl_3$); IR (KBr), ν_{max} 3442, 3020, 2967, 2937, 2869, 1613, 1585, 1514, 1480, 1459, 1444, 1424, 1369, 1325, 1303, 1250, 1212, 1177, 1145, 1090, 1061, 1030, 1005, 987, 974, 932, 866, 850, 816, 759, 728, 709, 642, 636 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.29–7.24 (2H, m), 6.91–6.86 (2H, m), 4.53 (1H, d, J=11.6 Hz), 4.48 (1H, d, J=11.6 Hz), 3.94 (1H, ddd, J=12.7, 5.3, 2.6 Hz), 3.81 (3H, s), 3.73 (1H, dt, J=11.5, 2.6 Hz), 3.65 (1H, ddd, J=12.7, 7.5, 4.0 Hz), 3.50 (1H, dt, J=11.5, 5.4 Hz), 3.22 (1H, dt, J=5.4, 2.6 Hz), 3.09 (1H, dt, J=4.0, 2.6 Hz), 1.71 (1H, brdd, J=7.5, 5.3 Hz); LR-EIMS, *m/z* 224 (20.6%, $[M]^+$), 121 (bp);

HR-EIMS, calcd for $C_{12}H_{16}O_4$ $[M]^+$: 224.1048, found: 224.1044.

5.1.39. (2*R*,3*R*)-2,3-Epoxy-1-(4-methoxybenzyloxy)-4-pentene (66). To a solution of **65** (78.0 mg, 0.348 mmol) in CH_2Cl_2–NEt_3–DMSO (6:1.4:1, v/v/v, 3.36 ml) was added SO_3·pyridine (553.9 mg, 3.48 mmol) at 0 °C. The mixture was warmed to 24 °C and stirred for 1 h. After the mixture was diluted with Et_2O (5 ml), the mixture was washed with H_2O (2×10 ml). The layers were separated and the aqueous layer was extracted with Et_2O (3×8 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant crude aldehyde was used immediately in the next reaction without purification. To a stirred suspension of $Ph_3P^+CH_3Br^-$ (621.6 mg, 1.74 mmol) in THF (4.0 ml) was added NaHMDS (1.64 ml, 1.0 M in THF, 1.64 mmol) and the mixture was stirred at 24 °C. After 1 h, the resulting yellow suspension was allowed to stand at −78 °C. To the mixture was added dropwise a solution of the above crude aldehyde in THF (2.0 ml) at −78 °C and the mixture was stirred for 2 h. Then, the reaction mixture was warmed to 24 °C and stirred for 17 h. After the mixture was diluted with hexane (10 ml) and Et_2O (5 ml), saturated aqueous NH_4Cl (10 ml) was added and the mixture was extracted with Et_2O (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20→10) to give **66** (59.8 mg, 78% from **65**). **66**: a colorless oil; $[\alpha]_D^{24}$ +10.0 (*c* 0.68, $CHCl_3$); IR (film), ν_{max} 3086, 3062, 2995, 2934, 2909, 2855, 2836, 1642, 1612, 1586, 1512, 1464, 1442, 1422, 1405, 1387, 1359, 1302, 1247, 1209, 1173, 1099, 1058, 1033, 987, 928, 877, 849, 820, 757, 681 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.27 (2H, d, J=8.8 Hz), 6.88 (2H, d, J=8.8 Hz), 5.59 (1H, ddd, J=17.3, 9.8, 7.2 Hz), 5.48 (1H, dd, J=17.3, 2.0 Hz), 5.29 (1H, dd, J=9.8, 2.0 Hz), 4.53 (1H, d, J=11.6 Hz), 4.48 (1H, d, J=11.6 Hz), 3.80 (3H, s), 3.72 (1H, dd, J=11.6, 3.3 Hz), 3.49 (1H, dd, J=11.6, 5.3 Hz), 3.26 (1H, dd, J=7.2, 2.2 Hz), 3.09 (1H, ddd, J=5.3, 3.3, 2.2 Hz); LR-EIMS, *m*/*z* 220 (4.2%, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{13}H_{16}O_3$ $[M]^+$: 220.1099, found: 220.1082.

5.1.40. (2*S*)-1-(4-Methoxybenzyloxy)pent-4-en-2-ol (67). To a solution of $Pd(PPh_3)_4$ (6.5 mg, 5.63 μmol) in CH_2Cl_2 (0.5 ml) was added Bu_3SnH (16.5 μl, 0.0619 mmol) at 24 °C. To the mixture was added dropwise a solution of **66** (12.4 mg, 0.0563 mmol) in CH_2Cl_2 (1.0 ml) at 24 °C and the mixture was stirred for 20 min. The solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→3) to give **67** (11.9 mg, 95%). **67**: a colorless oil; $[\alpha]_D^{26}$ +2.8 (*c* 0.76, $CHCl_3$); IR (film), ν_{max} 3447, 3074, 3001, 2910, 2859, 1641, 1612, 1586, 1513, 1465, 1442, 1419, 1363, 1302, 1249, 1209, 1173, 1101, 1035, 997, 916, 820 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.26 (2H, d, J=8.7 Hz), 6.88 (2H, d, J=8.7 Hz), 5.82 (1H, ddt, J=17.2, 10.1, 7.1 Hz), 5.15–5.07 (2H, m), 4.49 (2H, s), 3.90–3.82 (1H, m), 3.81 (3H, s), 3.49 (1H, dd, J=9.5, 3.5 Hz), 3.34 (1H, dd, J=9.5, 7.3 Hz), 2.25 (2H, t, J=7.1 Hz); LR-EIMS, *m*/*z* 222 (7.0%, $[M]^+$), 121 (bp); HR-EIMS, calcd for $C_{13}H_{18}O_3$ $[M]^+$: 222.1256, found: 222.1250.

5.1.41. (2*S*,3*R*,4*Z*,6*S*,7*R*,1′*E*)-3-Benzyloxy-2-benzyloxymethyl-6-(4-methoxybenzyloxy)-7-(2′-methoxyvinyl)-2,3,6,7-tetrahydrooxepin and (2*S*,3*R*,4*Z*,6*S*,7*R*,1′*Z*)-3-benzyloxy-2-benzyloxymethyl-6-(4-methoxybenzyloxy)-7-(2′-methoxyvinyl)-2,3,6,7-tetrahydrooxepin (73). To oxalyl chloride (0.19 ml, 2.18 mmol) in CH_2Cl_2 (6.0 ml) was added DMSO (0.28 ml, 3.91 mmol) in CH_2Cl_2 (3.0 ml) dropwise at −78 °C and the mixture was stirred for 10 min. Then, **72** (383.4 mg, 0.782 mmol) in CH_2Cl_2 (6.0 ml) was added dropwise at −78 °C and the mixture was stirred for 15 min. Et_3N (1.10 ml, 7.82 mmol) was added dropwise at −78 °C. The mixture was warmed to −18 °C and stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (10 ml) was added and the mixture was extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant crude aldehyde was used in the next reaction without purification. To a stirred suspension of $Ph_3P^+CH_2OMeCl^-$ (1.37 g, 3.99 mmol) in THF (8.0 ml) was added NaHMDS (3.8 ml, 1.0 M in THF, 3.83 mmol) and the mixture was stirred at 0 °C. After 30 min, the resulting red solution was allowed to stand at −78 °C. To the mixture was added dropwise a solution of the above crude aldehyde in THF (5.0 ml) at −78 °C and the mixture was stirred for 1.5 h. Then, the reaction mixture was warmed to 24 °C and stirred for 17.5 h. Et_2O (10 ml) and brine (10 ml) were added and the mixture was extracted with Et_2O (3×10 ml). The combined organic layers were washed with H_2O, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=15→10→5→3) to give **73** (282.6 mg, 70% from **72**, *E*/*Z*=2:1 from ^1H NMR). **73**: a colorless oil; IR (film), ν_{max} 3062, 3029, 2862, 1657, 1612, 1586, 1513, 1496, 1454, 1365, 1301, 1247, 1210, 1172, 1089, 1029, 938, 820, 736, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.36–7.22 (12H, m), 6.90–6.79 (2H, m), 6.61 (0.67H, d, J=12.8 Hz), 6.04 (0.33H, dd, J=6.2, 0.7 Hz), 5.87 (2H, s), 4.84 (0.67H, dd, J=12.8, 7.3 Hz), 4.71–4.47 (4.33H, m), 4.59 (1H, d, J=11.2 Hz), 4.43 (1H, d, J=11.2 Hz), 4.22–4.17 (1H, m), 4.08–4.03 (1H, m), 3.99–3.91 (1H, m), 3.87 (1H, ddd, J=8.8, 5.1, 3.7 Hz), 3.79 (3H, s), 3.68–3.59 (2H, m), 3.58 (1H, s), 3.53 (2H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 159.3 (C×0.67), 159.1 (C×0.33), 150.3 (CH×0.67), 148.8 (CH×0.33), 138.6 (C×0.33), 138.4 (C×0.67), 138.2 (C×0.33), 138.0 (C×0.67), 132.0 (CH×0.33), 131.9 (CH×0.67), 131.7 (CH×0.33), 131.6 (CH×0.67), 130.5 (C×0.33), 130.1 (C×0.67), 129.6 (CH×0.66), 129.5 (CH×1.34), 128.33 (CH×1.34), 128.29 (CH×0.66), 128.27 (CH×1.34), 128.21 (CH×0.66), 127.82 (CH×0.66), 127.78 (CH×1.34), 127.65 (CH×1.34), 127.55 (CH×0.66), 127.48 (CH×1.34), 127.37 (CH×0.66), 113.8 (CH×1.34), 113.6 (CH×0.66), 106.2 (CH×0.33), 102.5 (CH×0.67), 81.7 (CH×0.67), 81.6 (CH×0.33), 81.4 (CH_3×0.67), 80.3 (CH×0.67), 79.6 (CH×0.33), 77.1 (CH), 76.7 (CH), 76.5 (CH_3×0.33), 73.3 (CH_2×0.67), 73.2 (CH_2×0.33), 71.3 (CH_2), 71.1 (CH_2×0.33), 71.0 (CH_2×0.67), 70.8 (CH_2×0.67), 70.5 (CH_2×0.33), 55.8 (CH_3×0.33), 55.2 (CH_3×0.67); LR-EIMS, *m*/*z* 516 (6.0%, $[M]^+$), 395

(bp); HR-EIMS, calcd for $C_{32}H_{36}O_6$ $[M]^+$: 516.2511, found: 516.2504.

5.1.42. (2*R*,3*S*,4*Z*,6*R*,7*S*)-{6-Benzyloxy-7-benzyloxymethyl-3-(4-methoxybenzyloxy)-2,3,6,7-tetrahydrooxepin-2-yl}ethanal (74). To a solution of **73** (282.6 mg, 0.547 mmol) in THF–H_2O (10:1, v/v, 11.0 ml) was added $Hg(OAc)_2$ (523.0 mg, 1.64 mmol) at 24 °C and the mixture was stirred for 1.5 h. Then, TBAI (1.82 g, 4.92 mmol) was added at 24 °C and the mixture was stirred for 1 h. Then, saturated aqueous NH_4Cl (20 ml) was added and the mixture was extracted with Et_2O (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5→3) to give **74** (262.1 mg, 95%). **74**: a colorless oil; $[\alpha]_D^{26}$ +22.5 (*c* 1.35, $CHCl_3$); IR (film), ν_{max} 3063, 3030, 2862, 2735, 1725, 1612, 1586, 1513, 1496, 1454, 1372, 1301, 1248, 1174, 1087, 1029, 821, 737, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 9.73 (1H, t, *J*=2.0 Hz), 7.36–6.85 (14H, m), 5.92 (1H, d, *J*=14.5 Hz), 5.86 (1H, d, *J*=14.5 Hz), 4.63–4.34 (6H, m), 4.21 (1H, d, *J*=8.8 Hz), 4.11 (1H, td, *J*=8.8, 4.0 Hz), 3.92 (1H, d, *J*=8.8 Hz), 3.80 (3H, s), 3.74 (1H, dt, *J*=8.8, 3.5 Hz), 3.61 (2H, d, *J*=3.5 Hz), 2.79 (1H, ddd, *J*=16.5, 4.0, 2.0 Hz), 2.54 (1H, ddd, *J*=16.5, 8.8, 2.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ 200.6 (CH), 159.3 (C), 138.0 (C), 137.7 (C), 132.3 (CH), 131.1 (CH), 129.6 (CH×2), 129.2 (C), 128.2 (CH×2), 128.1 (CH×2), 127.6 (CH×2), 127.5 (CH×2), 127.4 (CH×2), 113.7 (CH×2), 82.8 (CH), 79.0 (CH), 78.6 (CH), 76.7 (CH), 73.2 (CH_2), 71.4 (CH_2), 70.7 (CH_2), 70.3 (CH_2), 55.0 (CH_3), 47.4 (CH_2); LR-EIMS, *m*/*z* 502 (6.2%, $[M]^+$), 258 (bp); HR-EIMS, calcd for $C_{31}H_{34}O_6$ $[M]^+$: 502.2355, found: 502.2353.

5.1.43. (2*R*,3*S*,4*Z*,6*R*,7*S*)-2-Allyl-6-benzyloxy-7-benzyloxymethyl-3-(4-methoxybenzyloxy)-2,3,6,7-tetrahydrooxepin (75). To a stirred suspension of $Ph_3P^+CH_3Br^-$ (51.8 mg, 0.145 mmol) in THF (0.5 ml) was added NaHMDS (0.13 ml, 1.0 M in THF, 0.132 mmol) and the mixture was stirred at 20 °C. After 1 h, the resulting yellow suspension was allowed to stand at −78 °C. To the mixture was added dropwise a solution of **74** (20.8 mg, 0.0414 mmol) in THF (1.5 ml) at −78 °C and the mixture was stirred for 2 h. Then, the reaction mixture was warmed to 22 °C and stirred for 18 h. After the mixture was diluted with hexane (5 ml) and Et_2O (3 ml), saturated aqueous NH_4Cl (5 ml) was added. The aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10) to give **75** (20.6 mg, 100%). **75**: a colorless oil; $[\alpha]_D^{25}$ +12.7 (*c* 1.0, $CHCl_3$); IR (film), ν_{max} 3064, 3029, 2864, 1641, 1612, 1586, 1513, 1496, 1454, 1382, 1300, 1248, 1209, 1173, 1074, 1035, 914, 821, 735, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.34–6.85 (14H, m), 5.93 (1H, ddt, *J*=17.1, 10.3, 6.8 Hz), 5.86 (2H, s), 5.07 (1H, d, *J*=17.1 Hz), 5.02 (1H, d, *J*=10.3 Hz), 4.62–4.38 (6H, m), 4.19 (1H, d, *J*=8.8 Hz), 3.91 (1H, d, *J*=8.4 Hz), 3.80 (3H, s), 3.71 (1H, ddd, *J*=8.8, 4.6, 2.0 Hz), 3.64 (2H, m), 3.60 (1H, td, *J*=8.4, 3.1 Hz), 2.54 (1H, ddd, *J*=14.7, 6.8, 3.1 Hz), 2.21 (1H, dt, *J*=14.7, 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ 159.2 (C), 138.3 (C), 137.9 (C), 135.2 (CH), 131.6 (CH), 131.5 (CH), 129.9 (C), 129.4 (CH×2), 128.2 (CH×2), 128.1 (CH×2), 127.7 (CH×2), 127.5 (CH×2), 127.3 (CH×2), 116.6 (CH_2), 113.7 (CH×2), 83.1 (CH), 82.3 (CH), 79.4 (CH), 76.9 (CH), 73.2 (CH_2), 71.2 (CH_2), 70.7 (CH_2), 70.5 (CH_2), 55.1 (CH_3), 37.4 (CH_2); LR-EIMS, *m*/*z* 500 (5.0%, $[M]^+$), 122 (bp); HR-EIMS, calcd for $C_{32}H_{36}O_5$ $[M]^+$: 500.2562, found: 500.2572.

5.1.44. (2*R*,3*S*,4*Z*,6*R*,7*S*)-2-Allyl-6-benzyloxy-7-benzyloxymethyl-2,3,6,7-tetrahydrooxepin-3-ol (58). To a solution of **75** (69.6 mg, 0.139 mmol) in CH_2Cl_2—pH 7 buffer (4:1, v/v, 2.0 ml) was added DDQ (47.3 mg, 0.208 mmol) at 0 °C and the mixture was stirred for 2 h. Then, to the mixture was added DDQ (31.6 mg, 0.139 mmol) at 0 °C and the stirring was continued for further 1 h. After the mixture was diluted with Et_2O (3 ml), saturated aqueous $NaHCO_3$ (10 ml) was added. The mixture was extracted with Et_2O (2×5 ml) and EtOAc (5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5) to give **58** (48.5 mg, 92%). **58**: a colorless oil; $[\alpha]_D^{25}$ −33.7 (*c* 0.90, $CHCl_3$); IR (film), ν_{max} 3426, 3065, 3029, 2866, 1818, 1769, 1641, 1496, 1454, 1365, 1208, 1097, 913, 736, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.35–7.24 (10H, m), 5.98 (1H, ddt, *J*=17.1, 10.1, 7.0 Hz), 5.81 (1H, dt, *J*=12.8, 2.2 Hz), 5.74 (1H, dt, *J*=12.8, 2.0 Hz), 5.16–5.09 (1H, m), 5.08–5.04 (1H, m), 4.61 (1H, d, *J*=11.4 Hz), 4.60 (1H, d, *J*=12.3 Hz), 4.55 (1H, d, *J*=12.3 Hz), 4.46 (1H, d, *J*=11.4 Hz), 4.18 (1H, ddd, *J*=8.0, 4.2, 2.0 Hz), 4.15–4.09 (1H, m), 3.74–3.68 (1H, m), 3.62 (2H, d, *J*=3.3 Hz), 3.49 (1H, td, *J*=8.5, 3.5 Hz), 2.60–2.51 (1H, m), 2.37–2.26 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.2 (C), 137.7 (C), 135.1 (CH), 134.1 (CH), 130.0 (CH), 128.3 (CH×2), 128.2 (CH×2), 127.8 (CH×2), 127.6 (CH), 127.5 (CH×2), 127.4 (CH), 116.7 (CH_2), 84.2 (CH), 82.7 (CH), 76.8 (CH), 73.3 (CH), 73.2 (CH_2), 71.4 (CH_2), 70.4 (CH_2), 37.7 (CH_2); LR-FDMS, *m*/*z* 381 (bp, $[M+H]^+$), 380 (21.8%, $[M]^+$); HR-FDMS, calcd for $C_{24}H_{29}O_4$ $[M+H]^+$: 381.2066, found: 381.2081.

5.1.45. Methyl (2*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-3-{(2′-allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenoate (57). A solution of butynoate **20** (462.4 mg, 2.03 mmol) in CH_2Cl_2 (6.0 ml) was slowly added dropwise to a solution of **58** (256.8 mg, 0.675 mmol) and PMe_3 (1.0 ml, 1.0 M in THF, 1.01 mmol) in CH_2Cl_2 (7.0 ml) at 0 °C by means of a syringe. The mixture was warmed to 24 °C and stirred for 30 min. The mixture was cooled to 0 °C and diluted with hexane (10 ml) and Et_2O (10 ml). Then, saturated aqueous NH_4Cl (10 ml) was added and the mixture was extracted with Et_2O (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=30→20→10) to give **57** (401.6 mg, 98%). **57**: a colorless oil; $[\alpha]_D^{21}$ +17.3 (*c* 0.90, $CHCl_3$); IR (film), ν_{max} 3065, 3030, 2953, 2929, 2885, 2857, 1716, 1629, 1497, 1472, 1462, 1454, 1435, 1389, 1361, 1346, 1313, 1288, 1253, 1207, 1188, 1141, 1051, 1005, 939, 916, 837, 779, 735, 697, 674 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ

7.36–7.23 (10H, m), 5.99–5.85 (1H, m), 5.91 (1H, dt, J= 13.0, 2.4 Hz), 5.59 (1H, dt, J=13.0, 2.4 Hz), 5.11–5.02 (2H, m), 4.97 (1H, d, J=13.6 Hz), 4.95 (1H, s), 4.62 (1H, d, J= 11.2 Hz), 4.61 (1H, d, J=13.6 Hz), 4.60 (1H, d, J= 12.5 Hz), 4.56 (1H, d, J=12.5 Hz), 4.54–4.51 (1H, m), 4.46 (1H, d, J=11.2 Hz), 4.28–4.23 (1H, m), 3.75–3.65 (5H, m), 3.67 (3H, s), 2.58–2.50 (1H, m), 2.28–2.19 (1H, m), 0.90 (9H, s), 0.08 (3H, s), 0.07 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 170.0 (C), 167.1 (C), 138.3 (C), 137.6 (C), 134.1 (CH), 133.6 (CH), 128.9 (CH), 128.3 (CH×2), 128.2 (CH×2), 127.7 (CH×2), 127.6 (CH), 127.5 (CH×2), 127.3 (CH), 117.2 (CH_2), 92.7 (CH), 83.2 (CH), 82.9 (CH), 77.6 (CH), 76.8 (CH), 73.3 (CH_2), 71.6 (CH_2), 70.4 (CH_2), 60.1 (CH_2), 50.9 (CH_3), 36.9 (CH_2), 25.8 (CH_3×3), 18.2 (C), −5.36 (CH_3×2); LR-FDMS, m/z 609 (55.5%, $[M+H]^+$), 608 (34.0%, $[M]^+$), 551 (bp, $[M-t\text{-Bu}]^+$); HR-FDMS, calcd for $C_{35}H_{49}O_7Si$ $[M+H]^+$: 609.3247, found: 609.3228.

5.1.46. (2*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-3-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenol (76). To a solution of **57** (401.6 mg, 0.660 mmol) in CH_2Cl_2 (7.0 ml) was added DIBAH (2.50 ml, 0.94 M in *n*-hexane, 2.31 mmol) at −78 °C and the mixture was stirred for 1 h. Then, saturated aqueous potassium sodium tartrate (10 ml) was added and the mixture was stirred at 23 °C for 12 h. The layers were separated and the aqueous layer was extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→3→1) to give **76** (361.4 mg, 94%). **76**: a colorless oil; $[\alpha]_D^{25}$ +11.7 (c 0.78, $CHCl_3$); IR (film), ν_{max} 3423, 3065, 3030, 2953, 2928, 2857, 1659, 1497, 1471, 1462, 1454, 1389, 1361, 1294, 1274, 1252, 1187, 1094, 1028, 1004, 914, 837, 778, 735, 697 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.36–7.24 (10H, m), 6.00–5.86 (1H, m), 5.85 (1H, dt, J=13.2, 2.2 Hz), 5.66 (1H, dt, J=13.2, 2.2 Hz), 5.11–5.00 (2H, m), 4.83 (1H, t, J=7.9 Hz), 4.61 (1H, d, J=11.2 Hz), 4.59 (2H, s), 4.45 (1H, d, J=11.2 Hz), 4.42–4.39 (1H, m), 4.26–4.22 (1H, m), 4.20 (2H, s), 4.17–4.15 (2H, m), 3.73–3.60 (4H, m), 2.54–2.46 (1H, m), 2.27–2.17 (1H, m), 1.86–1.84 (1H, m), 0.90 (9H, s), 0.10 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 156.4 (C), 138.3 (C), 137.7 (C), 134.7 (CH), 132.3 (CH), 130.3 (CH), 128.2 (CH×2), 128.1 (CH×2), 127.7 (CH×2), 127.5 (CH), 127.4 (CH), 127.2 (CH×2), 116.7 (CH_2), 101.3 (CH), 83.2 (CH), 83.1 (CH), 76.9 (CH), 76.3 (CH), 73.2 (CH_2), 71.5 (CH_2), 70.4 (CH_2), 61.0 (CH_2), 57.8 (CH_2), 37.1 (CH_2), 25.7 (CH_3×3), 18.1 (C), −5.38 (CH_3), −5.43 (CH_3); LR-FDMS, m/z 581 (44.9%, $[M+H]^+$), 580 (bp, $[M]^+$); HR-FDMS, calcd for $C_{34}H_{48}O_6Si$ $[M]^+$: 580.3220, found: 580.3203.

5.1.47. (2*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-3-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-4-(*tert*-butyldimethylsilyloxy)-2-butenal (77). To a mixture of **76** (185.2 mg, 0.319 mmol) and MS 4 Å (185.2 mg, 100 wt%) in CH_2Cl_2 (4.0 ml) was added NMO (74.7 mg, 0.638 mmol) at 23 °C and the mixture was stirred for 10 min. Then, TPAP (22.4 mg, 0.0638 mmol) was added to the reaction mixture at 23 °C and the mixture was stirred for 1.5 h. The mixture was filtered through Celite and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→5) to give **77** (155.3 mg, 84%). **77**: a colorless oil; $[\alpha]_D^{20}$ +22.2 (c 0.90, $CHCl_3$); IR (film), ν_{max} 3065, 3030, 2954, 2928, 2885, 2857, 2766, 1666, 1615, 1497, 1471, 1462, 1454, 1389, 1361, 1328, 1292, 1255, 1206, 1100, 1043, 1005, 916, 837, 779, 735, 697 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$), δ 9.98 (1H, d, J=7.7 Hz), 7.36–7.24 (10H, m), 5.98–5.84 (1H, m), 5.92 (1H, dt, J=13.2, 2.6 Hz), 5.55 (1H, dt, J=13.2, 2.6 Hz), 5.32 (1H, d, J=7.7 Hz), 5.10–5.02 (2H, m), 4.63–4.43 (7H, m), 4.22 (1H, dqn, J=8.4, 2.2 Hz), 3.78–3.61 (4H, m), 2.51–2.43 (1H, m), 2.29–2.19 (1H, m), 0.90 (9H, s), 0.10 (3H, s), 0.09 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 189.9 (CH), 173.3 (C), 138.2 (C), 137.5 (C), 134.0 (CH), 133.9 (CH), 128.3 (CH×2), 128.2 (CH×2), 128.1 (CH), 127.8 (CH×2), 127.7 (CH), 127.5 (CH×2), 127.4 (CH), 117.4 (CH_2), 106.7 (CH), 83.1 (CH), 82.3 (CH), 78.0 (CH), 76.7 (CH), 73.3 (CH_2), 71.7 (CH_2), 70.3 (CH_2), 61.2 (CH_2), 37.0 (CH_2), 25.6 (CH_3×3), 18.1 (C), −5.43 (CH_3), −5.46 (CH_3); LR-FDMS, m/z 579 (51.5%, $[M+H]^+$), 578 (47.2, $[M]^+$), 363 (bp); HR-FDMS, calcd for $C_{34}H_{47}O_6Si$ $[M+H]^+$: 579.3142, found: 579.3120.

5.1.48. (3*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-4-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-(trimethylsilyloxy)pent-3-enenitrile (56). To a solution of **77** (155.3 mg, 0.268 mmol) and TMSCN (89 μl, 0.670 mmol) in benzene (5.5 ml) was added Me_3Al (0.29 ml, 1.01 M in *n*-hexane, 0.295 mmol) at 25 °C and the mixture was stirred for 1 h. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×8 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=30→10) to give **56** (134.6 mg, 74%) as an inseparable 1:1 mixture of diastereomers. The nitrile **56** was unstable and immediately used for the next reaction. **56**: a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.36–7.24 (10H, m), 5.99–5.84 (2H, m), 5.64 (1H, dt, J=12.8, 2.4 Hz), 5.58 (0.5H, d, J= 8.8 Hz), 5.56 (0.5H, d, J=8.8 Hz), 5.11–5.00 (2H, m), 4.67–4.54 (4H, m), 4.46 (1H, d, J=11.4 Hz), 4.41–4.36 (1H, m), 4.25–4.21 (1H, m), 4.18 (2H, m), 3.72–3.60 (4H, m), 2.48–2.42 (1H, m), 2.28–2.15 (1H, m), 0.92 (4.5H, s), 0.91 (4.5H, s), 0.21 (4.5H, s), 0.20 (4.5H, s), 0.11 (3H, s), 0.10 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 156.6 (C×0.5), 156.4 (C×0.5), 138.4 (C×0.5), 138.3 (C×0.5), 137.75 (C×0.5), 137.70 (C×0.5), 134.4 (CH×0.5), 134.3 (CH×0.5), 133.2 (CH×0.5), 133.1 (CH×0.5), 129.6 (CH×0.5), 129.5 (CH×0.5), 128.31 (CH), 128.30 (CH), 128.2 (CH×2), 127.83 (CH), 127.79 (CH), 127.69 (CH×0.5), 127.60 (CH×0.5), 127.5 (CH×2), 127.4 (CH), 119.8 (C×0.5), 119.6 (C×0.5), 117.2 (CH_2×0.5), 117.0 (CH_2×0.5), 99.55 (CH×0.5), 99.52 (CH×0.5), 83.2 (CH×0.5), 83.1 (CH×0.5), 82.8 (CH), 77.0 (CH×0.5), 76.93 (CH×0.5), 76.92 (CH×0.5), 76.85 (CH×0.5), 73.34 (CH_2×0.5), 73.32 (CH_2×0.5), 71.7 (CH_2), 70.51 (CH_2×0.5), 70.46 (CH_2×0.5), 62.0 (CH_2×0.5), 61.9 (CH_2×0.5), 57.6 (CH×0.5), 57.5 (CH×0.5), 37.23 (CH_2×0.5), 37.15 (CH_2×0.5), 25.8 (CH_3×1.5), 25.7 (CH_3×1.5), 18.2 (C), −0.08 (CH_3×1.5), −0.10 (CH_3×1.5), −5.44 (CH_3×0.5), −5.47 (CH_3×0.5), −5.53 (CH_3×0.5), −5.61 (CH_3×0.5).

5.1.49. (2*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-4-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)pent-2-enenitrile (55). To a solution of **56** (220.0 mg, 0.324 mmol) in CH_2Cl_2–Bu_3SnH (1:1, v/v, 3.4 ml) was added $BF_3\cdot OEt_2$ (0.12 ml, 0.972 mmol) at 0 °C and the mixture was stirred for 10 min. Then, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=50→20→10→5) to give **55** (119.8 mg, 63%) as an inseparable 1:1 mixture of diastereomers at C13.[36] **55**: a colorless oil; IR (film), ν_{max} 3065, 3030, 2954, 2928, 2858, 2225, 1641, 1496, 1471, 1462, 1454, 1388, 1361, 1294, 1253, 1207, 1103, 1028, 1005, 967, 939, 914, 837, 778, 735, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.36–7.24 (10H, m), 6.75 (0.5H, dd, J=16.3, 4.6 Hz), 6.66 (0.5H, dd, J=16.3, 5.7 Hz), 6.00–5.84 (2H, m), 5.75 (0.5H, dt, J=12.8, 2.2 Hz), 5.67–5.59 (1.5H, m), 5.12–5.04 (2H, m), 4.60 (1H, J=11.8 Hz), 4.56 (2H, s), 4.45 (1H, d, J=11.8 Hz), 4.20–4.15 (1H, m), 4.12–4.04 (1H, m), 3.98–3.89 (1H, m), 3.75–3.47 (6H, m), 2.56–2.41 (1H, m), 2.27–2.15 (1H, m), 0.89 (4.5H, s), 0.88 (4.5H, s), 0.05 (3H, s), 0.04 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 152.9 (CH×0.5), 152.3 (CH×0.5), 138.3 (C), 137.9 (C×0.5), 137.8 (C×0.5), 134.9 (CH×0.5), 134.8 (CH×0.5), 132.5 (CH×0.5), 132.2 (CH×0.5), 131.5 (CH×0.5), 130.8 (CH×0.5), 128.4 (CH), 128.3 (CH), 128.2 (CH×2), 127.85 (CH), 127.79 (CH), 127.72 (CH×0.5), 127.69 (CH×0.5), 127.6 (CH×2), 127.4 (CH), 117.03 (C×0.5), 116.96 (C×0.5), 116.8 (CH_2×0.5), 116.7 (CH_2×0.5), 101.7 (CH×0.5), 101.0 (CH×0.5), 82.9 (CH×0.5), 82.5 (CH×0.5), 82.4 (CH×0.5), 82.1 (CH×0.5), 80.3 (CH×0.5), 79.5 (CH×0.5), 78.4 (CH×0.5), 78.2 (CH×0.5), 76.76 (CH×0.5), 76.66 (CH×0.5), 73.4 (CH_2×0.5), 73.3 (CH_2×0.5), 71.59 (CH_2×0.5), 71.57 (CH_2×0.5), 70.4 (CH_2), 64.9 (CH_2×0.5), 64.1 (CH_2×0.5), 37.5 (CH_2×0.5), 37.4 (CH_2×0.5), 25.8 (CH_3×3), 18.2 (C), −5.43 (CH_3×0.5), −5.49 (CH_3×0.5), −5.52 (CH_3×0.5), −5.53 (CH_3×0.5); LR-EIMS, *m*/*z* 589 (5.5%, $[M]^+$), 91 (bp); HR-EIMS, calcd for $C_{31}H_{38}NO_5Si$ $[M-t\text{-Bu}]^+$: 532.2519, found: 532.2510.

5.1.50. (2*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-4-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenal (78). To a solution of **55** (119.8 mg, 0.203 mmol) in CH_2Cl_2 (3.5 ml) was added DIBAH (0.86 ml, 0.94 M in *n*-hexane, 0.812 mmol) at −78 °C and the mixture was stirred for 1 h. Then, saturated aqueous potassium sodium tartrate (5 ml) was added and the mixture was stirred at 24 °C for 10 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. To a mixture of the resultant imine in CH_2Cl_2 (3.0 ml) was added 0.5 M aqueous HCl (1.5 ml) at 24 °C and the mixture was stirred for 20 min. The mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→5) to give **78** (100.2 mg, 83% from **55**) as an inseparable 1:1 mixture of diastereomers at C13.[36] **78**: a colorless oil; IR (film), ν_{max} 3066, 3030, 2954, 2928, 2857, 1694, 1641, 1497, 1471, 1462, 1454, 1389, 1361, 1294, 1253, 1207, 1110, 1028, 1005, 979, 939, 913, 837, 778, 735, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 9.57 (0.5H, d, J=7.7 Hz), 9.55 (0.5H, d, J=7.7 Hz), 7.35–7.24 (10H, m), 6.81 (0.5H, dd, J=15.8, 5.5 Hz), 6.71 (0.5H, dd, J=15.8, 6.2 Hz), 6.31 (0.5H, ddd, J=15.8, 7.7, 1.5 Hz), 6.30 (0.5H, ddd, J=15.8, 7.7, 1.3 Hz), 6.01–5.78 (2.5H, m), 5.68 (0.5H, dt, J=12.9, 2.4 Hz), 5.13–5.01 (2H, m), 4.60 (1H, d, J=11.2 Hz), 4.59 (1H, d, J=12.5 Hz), 4.54 (1H, d, J=12.5 Hz), 4.45 (1H, d, J=11.2 Hz), 4.26–4.15 (2H, m), 3.98 (0.5H, brdqn, J=8.4, 2.4 Hz), 3.92 (0.5H, brdqn, J=8.4, 2.4 Hz), 3.80–3.52 (6H, m), 2.62–2.48 (1H, m), 2.27–2.15 (1H, m), 0.89 (4.5H, s), 0.88 (4.5H, s), 0.06 (1.5H, s), 0.05 (1.5H, s), 0.04 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 193.1 (CH×0.5), 192.9 (CH×0.5), 154.8 (CH×0.5), 153.8 (CH×0.5), 138.4 (C), 137.9 (C), 135.1 (CH×0.5), 135.0 (CH×0.5), 133.7 (CH×0.5), 132.9 (CH×0.5), 132.4 (CH×0.5), 132.0 (CH×0.5), 131.7 (CH×0.5), 131.5 (CH×0.5), 128.3 (CH×2), 128.2 (CH×2), 127.80 (CH), 127.78 (CH), 127.6 (CH), 127.58 (CH×2), 127.4 (CH), 116.8 (CH_2×0.5), 116.7 (CH_2×0.5), 83.2 (CH×0.5), 82.9 (CH×0.5), 82.4 (CH×0.5), 82.3 (CH×0.5), 80.3 (CH×0.5), 79.2 (CH×0.5), 78.6 (CH×0.5), 78.4 (CH×0.5), 76.8 (CH×0.5), 76.7 (CH×0.5), 73.3 (CH_2), 71.53 (CH_2×0.5), 71.46 (CH_2×0.5), 70.4 (CH_2), 65.2 (CH_2×0.5), 64.5 (CH_2×0.5), 37.42 (CH_2×0.5), 37.37 (CH_2×0.5), 25.8 (CH_3×3), 18.2 (C), −5.36 (CH_3×0.5), −5.44 (CH_3×0.5), −5.47 (CH_3×0.5), −5.49 (CH_3×0.5); LR-FDMS, *m*/*z* 592 (bp, $[M]^+$), 535 (43.7, $[M-t\text{-Bu}]^+$); HR-FDMS, calcd for $C_{35}H_{48}O_6Si$ $[M-t\text{-Bu}]^+$: 592.3220, found: 592.3230.

5.1.51. (2*E*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-4-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2-pentenol (79). To a solution of **78** (100.2 mg, 0.169 mmol) in CH_2Cl_2 (3.0 ml) was added DIBAH (0.54 ml, 0.94 M in *n*-hexane, 0.507 mmol) at −78 °C and the mixture was stirred for 30 min. Then, saturated aqueous potassium sodium tartrate (5 ml) was added and the mixture was stirred at 24 °C for 15 h. The layers were separated and the aqueous layer was extracted with EtOAc (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=5→3) to give **79** (91.2 mg, 91%) as an inseparable 1:1 mixture of diastereomers at C13.[36] **79**: a colorless oil; IR (film), ν_{max} 3584, 3451, 3065, 3029, 2953, 2927, 2857, 1496, 1471, 1462, 1454, 1388, 1361, 1295, 1252, 1207, 1099, 1028, 1005, 913, 836, 815, 777, 734, 697 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 7.33–7.01 (10H, m), 6.30–6.12 (1H, m), 6.03–5.88 (1.5H, m), 5.78 (0.5H, dt, J=13.0, 2.6 Hz), 5.66–5.47 (2H, m), 5.30–5.09 (2H, m), 4.50–4.35 (3H, m), 4.31 (1H, d, J=11.7 Hz), 4.30–4.23 (1H, m), 4.10–4.05 (1H, m), 4.00–3.94 (1H, m), 3.89–3.83 (2H, m), 3.81–3.63 (5H, m), 3.55 (1H, ddd, J=10.6, 9.0, 4.6 Hz), 2.92–2.74 (1H, m), 2.51–2.34 (1H, m), 1.00 (4.5H, s), 0.98 (4.5H, s), 0.10 (1H, s), 0.08 (2.5H, s), 0.07 (2.5H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.4 (C), 138.00 (C×0.5), 137.98 (C×0.5), 135.5 (CH), 133.9 (CH×0.5), 133.2

(CH×0.5), 132.8 (CH×0.5), 132.5 (CH×0.5), 131.5 (CH×0.5), 130.6 (CH×0.5), 129.47 (CH×0.5), 129.46 (CH×0.5), 128.3 (CH×2), 128.2 (CH×2), 127.80 (CH), 127.79 (CH), 127.6 (CH×3), 127.4 (CH), 116.43 (CH_2×0.5), 116.40 (CH_2×0.5), 83.8 (CH×0.5), 83.6 (CH×0.5), 82.7 (CH×0.5), 82.5 (CH×0.5), 81.0 (CH×0.5), 79.7 (CH×0.5), 79.5 (CH×0.5), 77.6 (CH×0.5), 77.0 (CH×0.5), 76.9 (CH×0.5), 73.3 (CH_2), 71.43 (CH_2×0.5), 71.35 (CH_2×0.5), 70.5 (CH_2), 66.1 (CH_2×0.5), 65.9 (CH_2×0.5), 62.8 (CH_2×0.5), 62.7 (CH_2×0.5), 37.31 (CH_2×0.5), 37.26 (CH_2×0.5), 25.86 (CH_3×1.5), 25.84 (CH_3×1.5), 18.29 (C×0.5), 18.27 (C×0.5), −5.19 (CH_3×0.5), −5.32 (CH_3×0.5), −5.36 (CH_3×0.5), −5.41 (CH_3×0.5); LR-FDMS, *m/z* 595 (bp, $[M+H]^+$), 594 (25.1%, $[M]^+$); HR-FDMS, calcd for $C_{35}H_{51}O_6Si$ $[M+H]^+$: 595.3455 found: 595.3467.

5.1.52. (2*R*,3*S*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-4-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-5-(*tert*-butyldimethylsilyloxy)-2,3-epoxypentanol (80). To a mixture of D-(−)-DET (12 μl, 0.0696 mmol) and pre-dried MS 4 Å (20.7 mg, 100 wt%) in CH_2Cl_2 (0.3 ml) was added $Ti(O^iPr)_4$ (15.4 μl, 0.0522 mmol) at −40 °C and the mixture was stirred for 30 min. Then, TBHP (0.14 ml, 4.9 M in toluene, 0.696 mmol) was added and the mixture was stirred at −40 °C for 30 min. To the mixture was added dropwise a solution of **79** (20.7 mg, 0.0348 mmol) in CH_2Cl_2 (1.2 ml). The reaction mixture was stirred at −40 °C for 30 min. Then, the reaction mixture was warmed to −25 °C and stirred for 2.5 d. Then, DMS (52 μl, 0.708 mmol) was added at −25 °C and the mixture was stirred for 2 h until unreacted TBHP was consumed. To the mixture was added 10% DL-tartaric acid (31 μl) and NaF (13.2 mg) at −25 °C. The suspension was warmed to 24 °C and stirred for 24 h. The mixture was filtered through Celite and concentrated in vacuo. To the resultant residue was added Et_2O (1.0 ml) and 30% aqueous NaOH in brine (1.0 ml) at 0 °C and the mixture was stirred for 1 h. The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=10→7→5→3) to give **80** (16.2 mg, 76%) as an inseparable 1:1 mixture of diastereomers at C13.[36] **80**: a colorless oil; IR (film), ν_{max} 3458, 3065, 3029, 2953, 2927, 2857, 1496, 1471, 1462, 1454, 1388, 1361, 1295, 1253, 1208, 1096, 1028, 1005, 911, 837, 814, 778, 735, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.35–7.24 (10H, m), 6.02–5.78 (3H, m), 5.13–5.01 (2H, m), 4.60 (1H, d, *J*=11.4 Hz), 4.59 (1H, d, *J*=12.5 Hz), 4.54 (1H, d, *J*=12.5 Hz), 4.45 (1H, d, *J*=11.4 Hz), 4.21–4.15 (1H, m), 4.02–3.91 (2H, m), 3.74–3.42 (8H, m), 3.24–3.15 (1H, m), 3.04 (1H, ddd, *J*=10.8, 5.5, 2.2 Hz), 2.60–2.52 (1H, m), 2.27–2.15 (1H, m), 0.90 (4.5H, s), 0.88 (4.5H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.4 (C×0.5), 138.3 (C×0.5), 138.0 (C×0.5), 137.9 (C×0.5), 135.3 (CH×0.5), 135.2 (CH×0.5), 132.5 (CH×0.5), 132.3 (CH×0.5), 131.5 (CH×0.5), 131.1 (CH×0.5), 128.23 (CH×2), 128.16 (CH×2), 127.8 (CH×2), 127.5 (CH×3), 127.34 (CH×0.5), 127.32 (CH×0.5), 116.6 (CH_2×0.5), 116.5 (CH_2×0.5), 83.4 (CH×0.5), 83.2 (CH×0.5), 82.6 (CH×0.5), 82.5 (CH×0.5), 80.8 (CH×0.5), 80.5 (CH×0.5), 80.0 (CH×0.5), 77.9 (CH×0.5), 76.9 (CH), 73.3 (CH_2), 71.4 (CH_2×0.5), 71.3 (CH_2×0.5), 70.5 (CH_2), 63.9 (CH_2×0.5), 62.7 (CH_2×0.5), 61.3 (CH_2×0.5), 61.1 (CH_2×0.5), 56.7 (CH×0.5), 56.6 (CH×0.5), 56.4 (CH×0.5), 53.9 (CH×0.5), 37.3 (CH_2×0.5), 37.2 (CH_2×0.5), 25.8 (CH_3×1.5), 25.7 (CH_3×1.5), 18.2 (C×0.5), 18.1 (C×0.5), −5.43 (CH_3×0.5), −5.54 (CH_3×0.5), −5.61 (CH_3×0.5), −5.63 (CH_3×0.5); LR-FDMS, *m/z* 611 (bp, $[M+H]^+$), 610 (51.7, $[M]^+$); HR-FDMS, calcd for $C_{35}H_{51}O_7Si$ $[M+H]^+$: 611.3404, found: 611.3384.

5.1.53. (2*R*,3*S*,4*Z*,6*R*,7*S*,3′*S*,4′*R*)-2-Allyl-6-benzyloxy-7-benzyloxymethyl-3-{1′-(*tert*-butyldimethylsilyloxy)-3′,4′-epoxyhex-5′-en-2′-yl}oxy-2,3,6,7-tetrahydrooxepin (54). To a solution of **80** (3.4 mg, 5.57 μmol) in CH_2Cl_2–NEt_3–DMSO (3:1.4:1, v/v/v, 0.27 ml) was added SO_3·pyridine (22.2 mg, 0.139 mmol) at 0 °C. The mixture was warmed to 24 °C and stirred for 3 h. After the mixture was diluted with Et_2O (5 ml), the mixture was washed with H_2O (2×5 ml). The layers were separated and the aqueous layer was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant crude aldehyde was used in the next reaction without purification. To a stirred suspension of $Ph_3P^+CH_3Br^-$ (55.7 mg, 0.156 mmol) in THF (0.90 ml) was added NaHMDS (0.13 ml, 1.0 M in THF, 0.128 mmol) and the mixture was stirred at 24 °C. After 1 h, the resulting yellow suspension was allowed to stand at −78 °C. To the mixture was added dropwise a solution of the above crude aldehyde in THF (0.60 ml) at −78 °C and the mixture was stirred for 2 h. Then, the reaction mixture was warmed to 24 °C and stirred for 2.5 d. After the mixture was diluted with hexane (5 ml) and Et_2O (3 ml), saturated aqueous NH_4Cl (5 ml) was added and the mixture was extracted with Et_2O (3×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc=20) to give **54** (2.0 mg, 59% from **80**) as an inseparable 1:1 mixture of diastereomers at C13.[36] **54**: a colorless oil; IR (film), ν_{max} 3065, 2926, 2856, 1473, 1462, 1454, 1361, 1293, 1252, 1097, 1028, 837, 777, 733, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.35–7.21 (10H, m), 6.03–5.81 (3H, m), 5.78–5.42 (2H, m), 5.29 (1H, dt, *J*=9.5, 2.2 Hz), 5.13–5.00 (2H, m), 4.61 (1H, d, *J*=11.2 Hz), 4.60 (1H, d, *J*=13.2 Hz), 4.54 (1H, d, *J*=13.2 Hz), 4.44 (1H, d, *J*=11.2 Hz), 4.23–4.16 (1H, m), 4.03–3.93 (1H, m), 3.75–3.58 (5H, m), 3.50 (1H, ddd, *J*=17.5, 8.9, 2.9 Hz), 3.41–3.34 (0.5H, m), 3.33 (0.5H, dd, *J*=6.8, 1.8 Hz), 3.27 (0.5H, dd, *J*=7.3, 1.8 Hz), 3.21–3.14 (0.5H, m), 2.93 (0.5H, dd, *J*=7.2, 1.8 Hz), 2.88 (0.5H, dd, *J*=5.7, 1.8 Hz), 2.61–2.53 (1H, m), 2.32–2.27 (1H, m), 0.90 (4.5H, s), 0.88 (4.5H, s), 0.06 (3H, s), 0.05 (1.5H, s), 0.04 (1.5H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.5 (C×0.5), 138.4 (C×0.5), 138.1 (C×0.5), 138.0 (C×0.5), 135.4 (CH), 134.9 (CH), 132.7 (CH×0.5), 132.3 (CH×0.5), 131.5 (CH×0.5), 131.1 (CH×0.5), 128.33 (CH), 128.31 (CH), 128.2 (CH×2), 127.8 (CH×2), 127.6 (CH×3), 127.41 (CH×0.5), 127.40 (CH×0.5), 119.81 (CH_2×0.5), 119.77 (CH_2×0.5), 116.6 (CH_2), 83.6 (CH×0.5), 83.2 (CH×0.5), 82.7 (CH), 81.2 (CH×0.5), 80.6 (CH×0.5), 80.3 (CH×0.5), 78.6 (CH×0.5), 76.98 (CH×0.5), 76.96 (CH×0.5), 73.4 (CH_2), 71.5 (CH_2×0.5), 71.4 (CH_2×0.5),

70.6 ($CH_2\times0.5$), 70.5 ($CH_2\times0.5$), 64.0 ($CH_2\times0.5$), 62.8 ($CH_2\times0.5$), 61.2 ($CH\times0.5$), 58.5 ($CH\times0.5$), 57.0 ($CH\times0.5$), 56.6 ($CH\times0.5$), 37.42 ($CH_2\times0.5$), 37.38 ($CH_2\times0.5$), 25.9 ($CH_3\times3$), 18.3 ($C\times0.5$), 18.2 ($C\times0.5$), −5.33 ($CH_3\times0.5$), −5.45 ($CH_3\times0.5$), −5.50 ($CH_3\times0.5$), −5.58 ($CH_3\times0.5$); LR-FDMS, *m/z* 606 (bp, $[M]^+$), 549 (29.9, $[M-t\text{-Bu}]^+$); HR-FDMS, calcd for $C_{36}H_{50}O_6Si$ $[M]^+$: 606.3377, found: 606.3369.

5.1.54. (3*S*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-2-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}-1-(*tert*-butyldimethylsilyloxy)hex-5-en-3-ol (81). To a solution of $Pd(PPh_3)_4$ (11.8 mg, 0.0102 mmol) in CH_2Cl_2 (0.5 ml) was added Bu_3SnH (30 μl, 0.112 mmol) at 24 °C. To the mixture was added dropwise a solution of **54** (62.2 mg, 0.102 mmol) in CH_2Cl_2 (1.5 ml) at 24 °C and the mixture was stirred for 25 min. The solution was passed through a short silica gel column, and the filtrate was concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 30 → 20 → 10) to give **81** (54.6 mg, 88%) as an inseparable 1:1 mixture of diastereomers at C13.[36] **81**: a colorless oil; IR (film), ν_{max} 3583, 3491, 3067, 3030, 2927, 2856, 1462, 1454, 1257, 1101, 1028, 1005, 914, 837, 776, 734, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.38–7.24 (10H, m), 6.03–5.78 (4H, m), 5.17–5.00 (4H, m), 4.60 (1H, d, *J* = 11.4 Hz), 4.58–4.52 (2H, m), 4.45 (1H, d, *J* = 11.4 Hz), 4.20–4.14 (1H, m), 4.03–3.98 (1H, m), 3.86–3.53 (7H, m), 3.46–3.35 (1H, m), 2.61–2.51 (1H, m), 2.37–2.16 (3H, m), 0.90 (4.5H, s), 0.89 (4.5H, s), 0.07 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.44 ($C\times0.5$), 138.41 ($C\times0.5$), 138.0 ($C\times0.5$), 137.9 ($C\times0.5$), 135.31 ($CH\times0.5$), 135.29 ($CH\times0.5$), 135.0 ($CH\times0.5$), 134.8 ($CH\times0.5$), 132.8 ($CH\times0.5$), 132.0 ($CH\times0.5$), 131.4 ($CH\times0.5$), 131.0 ($CH\times0.5$), 128.3 ($CH\times2$), 128.2 ($CH\times2$), 127.9 (CH), 127.8 (CH), 127.7 ($CH\times0.5$), 127.64 ($CH\times2$), 127.61 ($CH\times0.5$), 127.42 ($CH\times0.5$), 127.41 ($CH\times0.5$), 117.7 ($CH_2\times0.5$), 117.1 ($CH_2\times0.5$), 116.64 ($CH_2\times0.5$), 116.59 ($CH_2\times0.5$), 83.4 ($CH\times0.5$), 83.2 ($CH\times0.5$), 82.4 ($CH\times0.5$), 82.1 ($CH\times0.5$), 81.0 ($CH\times0.5$), 79.8 ($CH\times0.5$), 79.0 ($CH\times0.5$), 78.5 ($CH\times0.5$), 76.9 ($CH\times0.5$), 76.7 ($CH\times0.5$), 73.3 (CH_2), 71.6 ($CH_2\times0.5$), 71.54 ($CH\times0.5$), 71.45 ($CH_2\times0.5$), 70.8 ($CH\times0.5$), 70.6 ($CH_2\times0.5$), 70.5 ($CH_2\times0.5$), 62.9 ($CH_2\times0.5$), 61.5 ($CH_2\times0.5$), 37.62 (CH_2), 37.58 (CH_2), 25.83 ($CH_3\times1.5$), 25.80 ($CH_3\times1.5$), 18.2 ($C\times0.5$), 18.1 ($C\times0.5$), −5.43 ($CH_3\times0.5$), −5.56 (CH_3), −5.61 ($CH_3\times0.5$); LR-FDMS, *m/z* 609 (bp, $[M+H]^+$), 608 (36.3%, $[M]^+$), 551 (45.2%, $[M-t\text{-Bu}]^+$); HR-FDMS, calcd for $C_{36}H_{53}O_6Si$ $[M+H]^+$: 609.3611, found: 609.3585.

5.1.55. (3*S*,2′*R*,3′*S*,4′*Z*,6′*R*,7′*S*)-2-{(2′-Allyl-6′-benzyloxy-7′-benzyloxymethyl-2′,3′,6′,7′-tetrahydrooxepin-3′-yl)oxy}hex-5-en-1,3-diol (82). To a solution of **81** (2.5 mg, 4.11 μmol) in THF (0.4 ml) was added TBAF (8.2 μl, 1.0 M in THF, 8.22 μmol) at 23 °C and the mixture was stirred for 1.5 h. The solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 1 → 1/2 → EtOAc) to give **82** (2.0 mg, 100%) as an inseparable 1:1 mixture of diastereomers at C13.[36] **82**: a colorless oil; IR (film), ν_{max} 3583, 3420, 3065, 3028, 2924, 2855, 1641, 1454, 1377, 1294, 1207, 1073, 1028, 914, 735, 697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$), δ 7.34–7.24 (10H, m), 6.01–5.75 (4H, m), 5.19–5.01 (4H, m), 4.61 (1H, d, *J* = 11.6 Hz), 4.59 (1H, d, *J* = 12.7 Hz), 4.53 (1H, d, *J* = 12.7 Hz), 4.45 (1H, d, *J* = 11.6 Hz), 4.16–3.98 (2H, m), 3.89–3.79 (2H, m), 3.77–3.60 (4H, m), 3.54 (1H, dd, *J* = 10.5, 4.6 Hz), 3.46–3.42 (1H, m), 2.54–2.14 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.4 (C), 137.8 ($C\times0.5$), 137.7 ($C\times0.5$), 135.1 ($CH\times0.5$), 134.9 ($CH\times0.5$), 134.5 ($CH\times0.5$), 134.3 ($CH\times0.5$), 132.6 ($CH\times0.5$), 132.4 ($CH\times0.5$), 130.65 ($CH\times0.5$), 130.60 ($CH\times0.5$), 128.4 (CH), 128.35 (CH), 128.26 ($CH\times2$), 128.1 (CH), 128.0 (CH), 127.9 ($CH\times0.5$), 127.7 ($CH\times0.5$), 127.67 (CH), 127.65 (CH), 127.5 (CH), 118.2 ($CH_2\times0.5$), 117.6 ($CH_2\times0.5$), 117.0 ($CH_2\times0.5$), 116.8 ($CH_2\times0.5$), 82.6 ($CH\times0.5$), 82.3 ($CH\times0.5$), 81.6 ($CH\times0.5$), 81.1 ($CH\times0.5$), 80.0 ($CH\times0.5$), 79.0 ($CH\times0.5$), 78.6 ($CH\times0.5$), 78.2 ($CH\times0.5$), 76.6 ($CH\times0.5$), 76.2 ($CH\times0.5$), 73.3 (CH_2), 71.9 ($CH_2\times0.5$), 71.7 ($CH_2\times0.5$), 70.9 ($CH\times0.5$), 70.7 ($CH_2\times0.5$), 70.60 ($CH_2\times0.5$), 70.56 ($CH\times0.5$), 61.2 ($CH_2\times0.5$), 61.0 ($CH_2\times0.5$), 37.9 ($CH_2\times0.5$), 37.7 ($CH_2\times0.5$), 37.6 ($CH_2\times0.5$), 37.5 ($CH_2\times0.5$); LR-FDMS, *m/z* 495 (80.0%, $[M+H]^+$), 494 (11.2%, $[M]^+$), 91 (bp); HR-FDMS, calcd for $C_{30}H_{39}O_6$ $[M+H]^+$: 495.2747, found: 495.2754.

5.1.56. (2*R*,3*S*,4*Z*,6*R*,7*S*,4′*S*,5′*R*)-2-Allyl-3-{(4′-allyl-2′,2′-dimethyl-1′,3′-dioxan-5′-yl)oxy}-6-benzyloxy-7-benzyloxymethyl-2,3,6,7-tetrahydrooxepin (53a) and (2*R*,3*S*,4*Z*,6*R*,7*S*,4′*S*,5′*S*)-2-allyl-3-{(4′-allyl-2′,2′-dimethyl-1′,3′-dioxan-5′-yl)oxy}-6-benzyloxy-7-benzyloxymethyl-2,3,6,7-tetrahydrooxepin (53b). To a solution of **82** (44.4 mg, 0.0897 mmol) and 2,2-dimethoxypropane (55 μl, 0.449 mmol) in CH_2Cl_2 (1.5 ml) was added CSA (10.5 mg, 0.0449 mmol) at 24 °C and the mixture was stirred for 2 h. Then, 2,2-dimethoxypropane (55 μl, 0.449 mmol) was added, and the stirring was continued for further 1 h. After that, saturated aqueous $NaHCO_3$ (5 ml) was added and the mixture was extracted with Et_2O (3× 5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 15 → 5 → 3) to give **53a** (22.4 mg, 47%) and **53b** (23.7 mg, 50%). **53a**: a colorless oil; $[\alpha]_D^{20}$ −17.1 (*c* 1.12, $CHCl_3$) IR (film), ν_{max} 2924, 2855, 1466, 1461, 1457, 1453, 1446, 1434, 1378, 1372, 1362, 1360, 1261, 1199, 1165, 1092, 697 cm^{-1}; ^1H NMR (300 MHz, C_6D_6), δ 7.41–7.05 (10H, m), 6.16–5.99 (2H, m), 5.77–5.70 (1H, m), 5.59–5.55 (1H, m), 5.19–5.05 (4H, m), 4.46 (1H, d, *J* = 11.4 Hz), 4.44 (1H, d, *J* = 12.3 Hz), 4.38 (1H, d, *J* = 12.3 Hz), 4.30 (1H, d, *J* = 11.4 Hz), 4.18–4.15 (1H, m), 3.99–3.91 (1H, m), 3.92 (1H, dd, *J* = 11.2, 5.1 Hz), 3.84–3.73 (2H, m), 3.70–3.59 (3H, m), 3.63 (1H, dd, *J* = 11.2, 8.8 Hz), 3.31 (1H, td, *J* = 8.8, 5.1 Hz), 2.68–2.53 (2H, m), 2.41–2.24 (2H, m), 1.45 (3H, s), 1.26 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.4 (C), 138.0 (C), 135.0 (CH), 134.4 (CH), 131.9 (CH), 131.8 (CH), 128.4 ($CH\times2$), 128.3 ($CH\times2$), 127.9 ($CH\times2$), 127.69 (CH), 127.65 ($CH\times2$), 127.5 (CH), 116.94 (CH_2), 116.92 (CH_2), 98.6 (C), 83.0 (CH), 81.8 (CH), 80.1 (CH), 76.7 (CH), 74.4 (CH), 73.4 (CH_2), 72.4 (CH), 71.5 (CH_2), 70.5 (CH_2), 63.6 (CH_2), 37.6 (CH_2), 36.4 (CH_2), 28.4 (CH_3), 19.5 (CH_3); LR-FDMS, *m/z* 535 (bp, $[M+H]^+$), 534 (50.1, $[M]^+$); HR-FDMS, calcd for $C_{33}H_{43}O_6$ $[M+H]^+$; 535.3059, found: 535.3038. **53b**: a colorless oil; $[\alpha]_D^{22}$ +38.0 (*c* 1.19, $CHCl_3$) IR (film), ν_{max} 3065, 2925, 2855, 1496, 1455, 1419, 1377, 1295, 1278, 1245, 1230, 1198,

1092, 1074, 1028, 995, 984, 912, 733, 697 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2C{=}O$), δ 7.38–7.22 (10H, m), 6.12–5.72 (4H, m), 5.11–4.94 (4H, m), 4.66 (1H, d, $J=11.7$ Hz), 4.59 (1H, d, $J=12.5$ Hz), 4.53 (1H, d, $J=12.5$ Hz), 4.49 (1H, d, $J=11.7$ Hz), 4.14–4.09 (1H, m), 4.03–4.00 (1H, m), 4.01 (1H, dd, $J=12.8$, 1.5 Hz), 3.91–3.87 (1H, m), 3.88 (1H, dd, $J=12.8$, 1.5 Hz), 3.75–3.60 (3H, m), 3.57–3.48 (1H, m), 3.29 (1H, brqn, $J=1.5$ Hz), 2.87–2.68 (2H, m), 2.35–2.13 (2H, m), 1.41 (3H, s), 1.28 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.5 (C), 138.0 (C), 135.6 (CH), 134.3 (CH), 131.8 (CH), 131.3 (CH), 128.34 (CH×2), 128.26 (CH×2), 127.8 (CH×2), 127.3 (CH×3), 127.4 (CH), 117.3 (CH_2), 116.5 (CH_2), 98.5 (C), 83.8 (CH), 82.9 (CH), 77.2 (CH), 77.0 (CH), 73.4 (CH_2), 71.6 (CH_2), 71.2 (CH), 70.5 (CH_2), 69.0 (CH), 60.8 (CH_2), 37.3 (CH_2), 35.6 (CH_2), 29.3 (CH_3), 18.9 (CH_3); LR-FDMS, *m/z* 535 (bp, $[M+H]^+$), 534 (58.2%, $[M]^+$); HR-FDMS, calcd for $C_{33}H_{43}O_6$ $[M+H]^+$: 535.3059, found: 535.3066.

5.1.57. (1*S*,3*R*,8*S*,10*Z*,13*R*,15*S*,16*R*,17*Z*)-16-Benzyloxy-15-benzyloxymethyl-6,6-dimethyl-2,5,7,14-tetraoxatricyclo[11.5.0.0^{3,8}]octadeca-10,17-diene (52). To a solution of **53a** (22.4 mg, 0.0419 mmol) in degassed CH_2Cl_2 (12 ml) was added a solution of $(Cy_3P)_2Cl_2Ru{=}CHPh$ (6.9 mg, 8.38 μmol) in degassed CH_2Cl_2 (2 ml). The resultant solution was stirred at 23 °C for 4 h. The mixture was stirred for 15.5 h under O_2 atmosphere, and the solvent was removed in vacuo. The resultant residue was purified by column chromatography (silica gel, benzene/EtOAc = 50 → 10) to give **52** (20.5 mg, 97%). **52**: a colorless oil; $[\alpha]_D^{23}$ −22.3 (*c* 0.050, $CHCl_3$); IR (film), ν_{max} 2924, 2854, 1496, 1457, 1454, 1434, 1378, 1267, 1206, 1097, 1028, 732, 697 cm^{-1}; ^1H NMR (400 MHz, C_5D_5N, −30 °C, a 1:1 mixture of conformers), δ 7.52–7.22 (10H, m), 5.99–5.92 (0.5H, m), 5.89–5.63 (2.5H, m), 5.60–5.49 (1H, m) 4.71 (0.5H, d, $J=11.2$ Hz), 4.67 (0.5H, d, $J=11.2$ Hz), 4.61–4.51 (2.5H, m), 4.47 (0.5H, d, $J=11.2$ Hz), 4.36–4.31 (1H, m), 4.28–4.23 (0.5H, m), 3.99–3.92 (1H, m), 3.90–3.81 (2.5H, m), 3.90–3.47 (1H, m), 3.77–3.61 (2.5H, m), 3.56–3.47 (1H, m), 3.34 (0.5H, td, $J=9.6$, 5.9 Hz), 3.01–3.05 (0.5H, m), 2.92–2.78 (1.5H, m), 2.38–2.32 (0.5H, m), 2.19–2.11 (1H, m), 2.04–1.99 (0.5H, m), 1.46 (1.5H, s), 1.39 (1.5H, s), 1.14 (1.5H, s), 1.13 (1.5H, s); ^{13}C NMR data of **52** is not shown because the spectrum of **52** exhibited extremely broadened signals that gave only unclear chemical shifts.; LR-FDMS, *m/z* 507 (64.5%, $[M+H]^+$), 506 (bp, $[M]^+$), 491 (22.9%, $[M-Me]^+$); HR-FDMS, calcd for $C_{31}H_{38}O_6$ $[M]^+$: 506.2668, found: 506.2648.

5.1.58. (1*S*,3*R*,4*S*,6*Z*,9*R*,11*S*,12*R*,13*Z*)-12-Benzyloxy-11-benzyloxymethyl-3-hydroxymethyl-2,10-dioxabicyclo-[7.5.0]tetradeca-6,13-dien-4-ol (83). To a solution of **52** (20.5 mg, 0.0405 mmol) in THF–H_2O (1:1, v/v, 0.80 ml) was added TFA (40 μl) at 0 °C. The mixture was warmed to 23 °C and stirred for 3 h. After the mixture was diluted with Et_2O (3 ml), saturated aqueous $NaHCO_3$ (5 ml) was added. The mixture was extracted with EtOAc (5×5 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The resultant residue was purified by column chromatography (silica gel, hexane/EtOAc = 2 → 1/3) to give **83** (16.6 mg, 88%). **83**: a colorless oil; $[\alpha]_D^{23}$ −34.9 (*c* 0.045, $CHCl_3$); IR (film), ν_{max} 3586, 3411, 2953, 2923, 2853, 1456, 1419, 1377, 1292, 1231, 1214, 1092, 1071, 1027, 934, 730, 695 cm^{-1}; ^1H NMR (400 MHz, C_6D_6), δ 7.36–7.05 (10H, m), 5.99 (1H, td, $J=10.7$, 5.9 Hz), 5.85–5.81 (1H, m), 5.80 (1H, dt, $J=12.7$, 2.4 Hz), 5.70 (1H, dt, $J=12.7$, 2.4 Hz), 4.50 (1H, d, $J=11.7$ Hz), 4.48 (1H, d, $J=11.7$ Hz), 4.45 (1H, d, $J=11.7$ Hz), 4.33 (1H, d, $J=11.7$ Hz), 4.20 (1H, dqn, $J=8.8$, 2.4 Hz), 4.20 (1H, dqn, $J=8.8$, 2.4 Hz), 3.79–3.70 (3H, m), 3.63 (1H, dd, $J=11.0$, 3.7 Hz), 3.59 (1H, ddd, $J=8.8$, 4.4, 2.4 Hz), 3.55–3.51 (1H, m), 3.53 (1H, dd, $J=11.0$, 5.2 Hz), 2.99 (1H, ddd, $J=8.8$, 5.2, 3.7 Hz), 2.78 (1H, brddd, $J=12.8$, 10.7, 3.8 Hz), 2.67–2.59 (1H, m), 2.26–2.21 (1H, m), 1.99 (1H, brddd, $J=13.9$, 5.4, 3.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.5 (C), 137.8 (C), 136.2 (CH), 131.8 (CH), 128.8 (CH), 128.4 (CH×2), 128.3 (CH×2), 127.8 (CH×2), 127.7 (CH), 127.6 (CH×2), 127.5 (CH), 127.2 (CH), 87.1 (CH), 85.7 (CH), 84.0 (CH), 82.9 (CH), 77.6 (CH), 73.4 (CH_2), 71.7 (CH_2), 71.3 (CH), 70.9 (CH_2), 63.5 (CH_2), 32.5 (CH_2), 32.3 (CH_2); LR-FDMS, *m/z* 467 (66.6%, $[M+H]^+$), 466 (44.3%, $[M]^+$), 91 (bp); HR-FDMS, calcd for $C_{28}H_{35}O_6$ $[M+H]^+$: 467.2434, found: 467.2455.

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21. Several attempts to reduce selectively the α,β-unsaturated ester part of **21** were unsuccessful.
22. Hetero-Michael addition of **19** to butynoate **20** with a catalytic amount of Me_3P according to Paintner's procedure[18] often stopped before completion. The incomplete reaction was due to the oligomerization of **20**. A stoichiometric amount of Me_3P

and excess **20** (1.7 equiv) were required to accelerate the reaction rate and to improve the yield.
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35. The position number is corresponding to that of Scheme 7.
36. The position number is corresponding to that of Scheme 10.

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Chiral amidophosphane–copper-catalyzed asymmetric conjugate addition of dialkylzinc reagents to nitroalkenes

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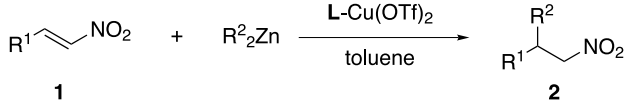
Abstract—The copper–amidophosphane-catalyzed asymmetric addition reaction of dialkylzinc reagents with β-aryl and β-alkylnitroalkenes afforded the corresponding nitroalkanes with moderate to good enantioselectivities (54–80% ee). The performance was highly dependent on the reaction procedure where the addition of nitroalkene to the mixture of copper–amidophosphane and dialkylzinc gave higher ee than the addition of dialkylzinc to a mixture of copper–amidophosphane and nitroalkene.

1. Introduction

The external chiral ligand-controlled asymmetric conjugate addition reaction of organometallics with activated olefins has been a challenge in carbon–carbon bond forming chemistry.[1,2] The dialkylzinc species as a source of nucleophiles is a key to success of the recently reported copper-catalyzed conjugate addition to enones.[3] Among good acceptors for conjugate addition, nitroalkenes are an interesting class of compounds due to the strongly electron-withdrawing nitro group,[4,5] which can be transformed into a wide range of functionalities.[6] Enantioselective addition of dialkylzinc reagents to nitroalkenes has been scarcely studied and different types of catalytic copper–phosphorus ligand were used. The structure of these ligands has an important impact on the selectivity. It is possible to use TADDOL,[7] phosphoramidites,[8] sulfonamides,[9] binaphthol-based thioethers,[10] and dipeptide phosphanes[11] to achieve high performance. We have been involved in the development of chiral amidophosphane ligands[12] and their use in the copper-catalyzed asymmetric reactions. The copper-catalyzed asymmetric alkylation of *N*-sulfonylimines,[13] β-aryl-α,β-unsaturated *N*-sulfonylimines[14] and enones[15] with dialkylzinc is the recent example of our success. In our continuing effort to broaden the scope of the copper–chiral amidophosphane catalyst, we turned our interest to the asymmetric conjugate addition of dialkylzinc reagents to nitroalkenes.[16,17] We report herein, the copper–amidophosphane-catalyzed conjugate addition of dialkylzinc reagents to nitroalkenes (Scheme 1). Moderate to good enantioselectivities were obtained for β-aryl- and β-alkylnitroalkenes.

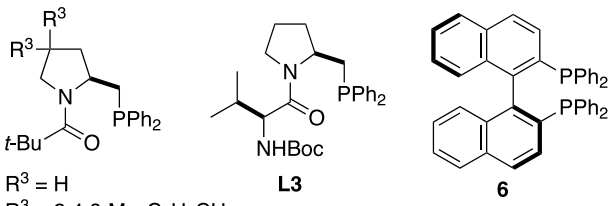


Scheme 1. The chiral amidophosphane **L**-copper-catalyzed asymmetric conjugate addition of dialkylzinc reagents to nitroalkenes **1**.

2. Results and discussion

2.1. Chiral phosphane–copper(II) triflate catalyzed asymmetric conjugate addition of diethylzinc to β-nitrostyrene 1a

In the initial experiments, three different amidophosphanes **L1**, **L2**[13c,17b] and **L3**,[18] and (*S*)-BINAP **6** were tested in the reaction of *trans*-β-nitrostyrene **1a** (R^1 = Ph) with diethylzinc (Fig. 1). A mixture of 5 mol% of copper(II) triflate and



L1 R^3 = H
L2 R^3 = 2,4,6-$Me_3C_6H_2CH_2$
L3
6

Figure 1. Chiral amidophosphanes **L1**–**L3** and (*S*)-BINAP **6**.

Keywords: Organocopper; Asymmetric reaction; Conjugate addition; Nitroolefin.
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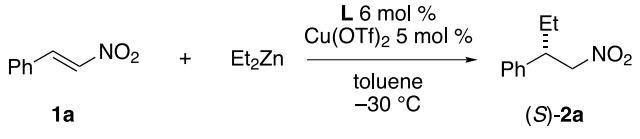
6 mol% of **L** in toluene was stirred at rt for 1 h and then treated with **1a** for additional 15 min. Then, a toluene solution of 3 equiv of diethylzinc was added to the mixture above at −30 °C and the whole was stirred at −30 °C until consumption of **1a**. Workup and column chromatography (hexane–AcOEt=9/1) gave **2a** in 44–67% yield (Table 1).

Table 1. Asymmetric reaction of β-nitrostyrene **1a** with diethylzinc



Entry	Phosphane	Time (h)	Yield (%)	ee (%)
1	**L1**	1	65	54
2	**L2**	3	67	26
3	**L3**	3	56	7
4	**6**	3	44	0

The structure of the chiral amidophosphane **L** was critical for the enantioselectivity. The phosphanes **L2** and **L3** bearing bulky substituents on the pyrrolidine ring or on the nitrogen atom of pyrrolidine were detrimental to the rate of conversion and to the enantioselectivity, giving (*S*)-**2a** with 26% and 7% ee (entries 2 and 3). BINAP **6** gave miserable selectivity (entry 4). The best phosphane was **L1** and afforded (*S*)-**2a** with 54% ee in 65% yield (entry 1).

The use of other copper salts, $(CuOTf)_2 \cdot C_6H_6$, CuCN–2LiCl, $Cu(MeCN)_4BF_4$, $Cu(OAc)_2$–H_2O, gave poorer selectivity than $Cu(OTf)_2$. Other solvents such as THF or ether were detrimental to the ee. Even the use of $Cu(OAc)_2 \cdot 2H_2O$ in ether, which was very efficient with Alexakis' ligand,[19] did not improve our results. Moreover, running the reaction at higher temperature decreased the enantioselectivity, whereas lower temperature did not improve it.

2.2. Reaction procedure dependency of enantioselectivity

The main surprise came from the influence of reaction procedure. Indeed, it was essential for the selectivity. Two procedures A and B of the reaction were tested (Table 2).

Procedure A. A solution of diethylzinc was added to a toluene solution of copper–amidophosphane **L** and nitroalkene **1** at −30 °C.

Procedure B. Nitroalkene **1** was added to a solution of copper–amidophosphane **L**-diethylzinc at −30 °C.

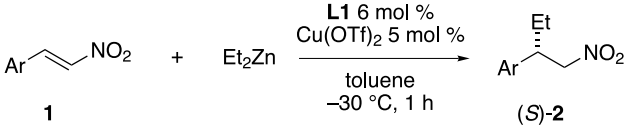
The reaction of a variety of β-arylnitroalkenes **1a**–**1d** has been examined under both procedures using **L1**. The enantioselectivity was quite enhanced by the procedure B. The reactions performed smoothly to afford **2** with higher ee than the procedure A. An electron withdrawing such as fluoro group (**1b**) (60% ee, entry 4) and an electron donating as a methoxy group (**1c**) (60% ee, entry 6) at the *para* position of the phenyl moiety of *trans*-β-styrene **1a** (68% ee, entry 2) did not improve the enantioselectivity. A steric effect was observed in the case of naphthylnitroethene **1d** which afforded **2d** with only 10% ee under procedure A and 54% ee under procedure B (entries 7 and 8).

2.3. Chiral phosphane–copper(II) triflate catalyzed asymmetric conjugate addition of dialkylzinc to β-alkylnitroethene 1

In order to explore the breadth of the copper–amidophosphane **L1** application, the reaction of β-alkylnitroalkenes **1e**–**1g** were examined under the procedure B (Table 3). 2-Isopropylnitroethene **1e** afforded the corresponding alkane **2e** with 80% ee in 50% yield (entry 2). It is also possible to use diisopropylzinc in the reaction of 2-ethylnitroethene **1f** to give *ent*-**2e** with 62% ee in 50% yield (entry 4).

However, with the use of less reactive trisubstituted nitroalkene **1g**, the reaction was slow, after 12 h, to give **2g** with 74% ee as a cis-major 85:15 mixture in 52% yield (entry 6). Isomerization of cis-major mixture with DBU in ether at rt for 2 h gave a trans-major 95:5 mixture of **2g** without loss of enantioselectivity. It is important to note that hydrolysis should be done, in this case, with acetic acid at

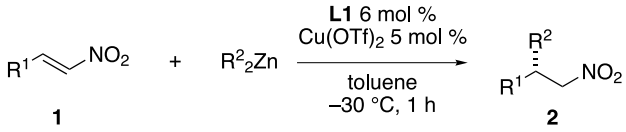
Table 2. Asymmetric reaction of diethylzinc with β-arylnitroalkenes[a]

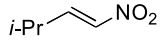
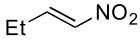
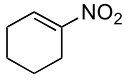


Entry	1	Ar	A/B	2	Yield (%)	ee (%)
1	**1a**	Ph	A	**2a**	65	54
2	**1a**	Ph	B	**2a**	67	68
3	**1b**	4-FC_6H_4	A	**2b**	46	54
4	**1b**	4-FC_6H_4	B	**2b**	50	60
5	**1c**	4-$MeOC_6H_4$	A	**2c**	65	46
6	**1c**	4-$MeOC_6H_4$	B	**2c**	60	60
7	**1d**	1-Naphthyl	A	**2d**	57	10
8	**1d**	1-Naphthyl	B	**2d**	54	54

[a] Nitroalkene **1** was consumed after 1 h.

Table 3. Asymmetric reaction of diethylzinc with β-alkylnitroalkenes[a]



Entry		1	R^2	A/B	2	Yield (%)	ee (%)
1	**1e**		Et	A	**2e**	60	74
2	**1e**		Et	B	**2e**	50	80
3	**1f**		*i*-Pr	A	*ent*-**2e**	45	48
4	**1f**		*i*-Pr	B	*ent*-**2e**	50	62
5	**1g**		Et	A	**2g**[b]	64	58
6	**1g**		Et	B	**2g**[b]	52	74

[a] Nitroalkene **1** was consumed after 1 h except for entries 5 and 6 where the reaction required 12 h for completion.
[b] *cis*-Major 85:15 mixture was obtained as an initial product which was then isomerized to a *trans*-major 95:5 mixture. ee was determined for *trans* **2g** after isomerization.

−30 °C during 1 h to avoid the Nef reaction. However, about 10% of ketone was obtained.

3. Conclusion

In conclusion, the catalytic asymmetric conjugate addition of dialkylzinc reagent to different type of nitroalkenes was achieved with moderate to good enantioselectivities and reasonably good yields using copper–amidophosphane **L1** catalyst.

4. Experimental

4.1. General

All reactions were carried out under argon atmosphere. Column chromatography: Silicycle silica gel 230–400 mesh, TLC: Merck silica gel 60 F_{254}. ^1H and ^{13}C NMR spectra were recorded on a GEOL Unity 500 (500 and 125 MHz respectively) using $CDCl_3$ as a solvent. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Coupling constants *J* values are presented in Hz. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Diastereoisomeric excess was determined by ^1H NMR. MS (EI) is presented in *m*/*z*. Amidophosphane ligands **L1**, **L2**,[13c,17b] and **L3**[18] were prepared according to the laboratory procedure. Nitroalkenes **1a**, **1c** and **1g** are commercially available (Aldrich) and **1b**, **1d**[5a] and **1e**, **1f**[5b] were synthesized by the reported procedure. Diethylzinc in toluene and diisopropylzinc in toluene were purchased from Aldrich. Enantiomer separation of compounds **2a–f** was performed on a Hitachi G-3900 gas chromatograph equipped with a Chirasil-Dex CB column (25 m×0.25 mm) or β-Dex 225 column (30 m×0.25 mm), helium and nitrogen as the carrier gas: 33 mL/min, detector temperature: 200 °C, injection temperature: 200 °C, FID detector. Absolute configurations were obtained by comparison of chiral stationary phase HPLC data (OD-H column, hexane/2-propanol=20:1, 0.5 or 1 mL/min) with literature data.

4.2. Typical procedure for the synthesis of racemic 2

CuCN (2.6 mmol, 230 mg) and LiCl (5.2 mmol, 220 mg) was dissolved in 4 mL of THF and stirred for 15 min. To the mixture 1.3 equiv of 1 M toluene solution of dialkylzinc was added at −10 °C, and the mixture was stirred for additional 10 min. Then, 2 mmol of nitroalkene in 2 mL of THF was added at −78 °C, and the mixture was allowed to warm to rt. After 2 h, the reaction was quenched with saturated NH_4Cl solution. The organic layers were washed with brine, and then concentrated to afford nitroalkanes **2a–g** with moderate to good yields after column chromatography.

4.3. Procedures A and B for the asymmetric conjugate addition of dialkylzinc

Procedure A. Copper(II) triflate (10 mg, 5 mol%) was dried under heat and vacuum. After cooling down, **L1** (12 mg, 6 mol%) and 10 mL of toluene were added. The solution was stirred at rt for 1 h. Then, nitroalkene **1** (0.5 mmol) was added and the reaction mixture was stirred for additional 15 min. The solution was then cooled to −30 °C and diethylzinc in toluene (1.5 mL, 3 equiv) was added over 1 min. After that, the same procedure with B.

Procedure B. To copper(II) triflate (10 mg, 5 mol%) were added **L1** (6 mol%, 12 mg) and 10 mL of toluene. The solution was stirred at rt for 1 h. To the solution was added a toluene solution of diethylzinc (0.7 mL, 1.4 equiv) over 5 min at −35 °C. The mixture was stirred for 15 additional min. Then, nitroalkene **1** (0.5 mmol) was added. The reaction was monitored by TLC and GC. After completion of the reaction, 5 mL of saturated NH_4Cl solution was added and the reaction mixture was allowed to warm to rt. The mixture was extracted with 10 mL of AcOEt and the organic layers were washed with brine, and dried over Na_2SO_4. Concentration and silica gel column chromatography (hexane–AcOEt=9/1) gave **2** and less than 5% of an alkene product arising from the substitution of the nitro group by the alkyl group.

4.3.1. (−)-(*S*)-1-(1-Nitrobutan-2-yl)benzene (2a).[20] Table 2, entry 2: a light yellow oil of $[\alpha]_D^{21}$ −31.7 (*c* 1.25, $CHCl_3$) ($[\alpha]_D^{23}$ +38.2 (*c* 1.1, $CHCl_3$) for (*R*)-**2a** with 97%

ee). 68% ee by Chirasil DEX at 120 °C: 15.5 and 16.2 min for major (*S*) and minor (*R*), and HPLC (0.5 mL/min): 20.5 and 29.7 min for minor (*R*) and major (*S*). R_f 0.5 (hexane–AcOEt=9/1). ^{1}H: 1.60 (t, 3H, J=7.4 Hz), 1.75 (m, 2H), 3.30 (m, 1H), 4.55 (m, 2H), 7.05–7.40 (m, 6H). ^{13}C: 11.4, 26.0, 46.0, 80.7, 127.6, 128.9, 139.3. *m/z*: 179 (M^+).

4.3.2. (−)-(*S*)-1-Fluoro-4-(1-nitrobutan-2-yl)benzene (2b).[8a] Table 2, entry 4: a light yellow oil of $[\alpha]_D^{21}$ −22.9 (*c* 1.00, $CHCl_3$). 60% ee by Chirasil DEX at 110 °C: 36.8 and 39.0 min for major (*S*) and minor (*R*), and HPLC (1 mL/min): 18.6 and 20.5 min for minor (*R*) and major (*S*). R_f 0.4 (hexane/AcOEt =9:1). ^{1}H: 0.80 (t, 3H, J=7.4 Hz), 1.65 (m, 2H), 3.60 (m, 1H), 4.50 (m, 2H), 7.00–7.60 (m, 4H). ^{13}C: 11.4, 26.2, 45.3, 80.7, 115.8 (d, J=21.7 Hz), 129.1 (d, J=8.3 Hz), 135.0, 163.1 (d, J=247.3 Hz). *m/z*: 197 (M^+).

4.3.3. (−)-(*S*)-1-Methoxy-4-(1-nitrobutan-2-yl)benzene (2b).[21] Table 2, entry 6: a light yellow oil of $[\alpha]_D^{21}$ −25.7 (*c* 1.00, $CHCl_3$). 60% ee by Chirasil DEX at 110 °C: 47.2 and 48.7 min for major (*S*) and minor (*R*), and HPLC (1 mL/min): 23.5 and 36.8 min for minor (*R*) and major (*S*). R_f 0.55 (hexane/AcOEt =9:1). ^{1}H: 0.80 (t, 3H, J=7.4 Hz), 1.65 (m, 2H), 3.25 (m, 1H), 3.75 (s, 3H), 4.50 (m, 2H), 6.80 (m, 2H), 7.20 (m, 2H). ^{13}C: 11.5, 26.1, 45.3, 55.2, 81.0, 114.3, 128.6, 131.2, 159.0. *m/z*: 209 (M^+).

4.3.4. (−)-1-(1-Nitrobutan-2-yl)naphthalene (2d). Table 2, entry 8: a yellow oil of $[\alpha]_D^{21}$ −12.0 (*c* 1.30, benzene). 54% ee by Chirasil DEX at 160 °C: 21.2 and 21.7 min for major (*S*) and minor (*R*), and HPLC (1 mL/min): 19.5 and 39.4 min for minor (*R*) and major (*S*). R_f 0.5 (hexane–AcOEt=9/1). ^{1}H: 0.70 (t, 3H, J=7.4 Hz), 1.90 (m, 2H), 4.30 (m, 1H), 4.70 (m, 2H), 7.30–8.20 (m, 7H). ^{13}C: 11.3, 25.9, 80.3, 122.5, 125.4, 125.9, 126.7, 128.1, 129.2, 131.9, 134.2, 135.2. *m/z*: 229 (M^+). HRMS: calcd for $C_{14}H_{15}NO_2$, 229.1103; found, 229.1100.

4.3.5. (−)-2-Methyl-3-(nitromethyl)pentane (2e).[22] Table 3, entry 2: a light yellow oil of $[\alpha]_D^{21}$ −8.30 (*c* 1.00, $CHCl_3$). 80% ee by β-dex 225 at 80 °C: 29.2 and 33.7 min for major and minor. R_f 0.55 (hexane–AcOEt=9/1). ^{1}H: 0.80 (m, 9H), 1.30 (m, 1H), 1.45 (m, 1H), 1.65 (m, 1H), 2.05 (m, 1H), 4.30 (m, 2H). ^{13}C: 11.2, 18.7, 18.9, 21.2, 28.0, 44.9, 78.2. *m/z*: 145 (M^+).

4.3.6. (+)-*trans*-1-Ethyl-2-nitrocyclohexane (2g).[7] Table 3, entry 6: a light yellow oil of $[\alpha]_D^{21}$ +2.0 (*c* 1.30, benzene) as a *trans* major 95:5 mixture that obtained by isomerization of *cis* major 85:15 initial mixture with 1 equiv of DBU in ether at rt for 2 h. 74% ee by Chirasil DEX at 110 °C: 8.5 and 9.0 min for major and minor. R_f 0.55 (hexane–AcOEt=9/1). ^{1}H: 0.94 (m, 3H), 1.20–2.00 (m, 10H), 2.11–2.21 (m, 1H), 4.21 (td, 1H, J=11.3, 3.4 Hz for *trans*), 4.64 (m, 1H for *cis*). ^{13}C: 10.1 (*trans*), 11.5 (*cis*), 21.7, 22.0, 24.4, 24.7, 25.2, 26.4, 27.2, 28.9, 31.9, 40.6 (*cis*), 42.0 (*trans*), 86.3 (*cis*), 91.1 (*trans*). *m/z*: 157 (M^+).

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Ionic liquid soluble photosensitizers

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Abstract—The preparation and investigation of triplet photosensitizers designed to be preferentially soluble in room-temperature ionic liquids are reported. Photosensitizers prepared by covalent attachment of 1-methylimidazole to aryl ketones are soluble in ionic liquids and remain in the ionic liquid layer when the solution is extracted with an organic solvent. The photosensitized isomerization of *trans*-β-ionol to *cis*-β-ionol was efficiently carried out in ionic liquid solution with the product ionol being extracted and the sensitizer/ionic liquid mixture being re-used in additional photosensitization reactions. The scope and utility of the sensitizers in sensitizing other reactions are discussed.

1. Introduction

Ionic liquids have found increasing use in organic chemistry as chemists have improved their ability to tailor these solvents to a particular need.[1] Among the specialized ionic liquids developed are those that carry catalysts and reagents.[2] Ionic liquids have been designed for specific chemical function, such as fixing carbon dioxide.[3] Although ionic liquids are often discussed as a uniform class of material in the literature, the physical properties and chemical behavior of ionic liquids cannot be narrowly defined and may vary significantly from one ionic liquid to another.[4] Ionic liquids may be soluble in water, organics, or both.[1] Furthermore, ionic liquids may dissolve a wide range of solutes.[1] When partitioned against an organic solvent, ether for instance, many ionic species are retained in the ionic liquid layer. This property is useful in that it allows the rapid separation of neutral organics from charged molecules. This technique is often used to separate a metal catalyst or other reagent following a reaction in an ionic liquid.[2] Several examples using homogenous supports in ionic liquids have appeared in the last few years.[5] A substrate is loaded onto a support that is preferentially soluble in an ionic liquid, which then becomes a fluid solid support. The substrate then undergoes a series of transformations, until the final product is cleaved from the support and extracted with an organic solvent. Though fewer examples are known, homogenously supported reagents have also been reported.[2] The idea is analogous to work done in fluorous media where a perfluorinated catalyst or reagent is used to carry out a transformation on a 'normal' organic substrate.[6] After the reaction, the catalyst can easily be separated from the substrate/product by extraction.

While studying photochemical reactions in ionic liquids, we noted that ionic liquids are excellent media in which to perform anaerobic reactions.[7] The negligible vapor pressure of the ionic liquids allows solutions to be degassed readily without numerous cycles of freeze–pump–thaw. If none of the reagents or reactants are volatile, the reaction mixture can simply be placed on a vacuum line prior to irradiation. A number of reports discussing photochemistry in ionic liquids have appeared in the past several years.[8] These observations led to the idea of an ionic liquid containing a photosensitizing chromophore so that the ionic liquid itself might be a photosensitizer. Photochemical energy transfer in ionic liquids has been reported previously.[9] Unfortunately, all attempts at preparing such a liquid have failed, as the chromophore invariably rendered the salt a solid. However, the ability of ionic liquids to retain other ionic compounds suggested the idea of doping an ionic liquid with a sensitizer bearing a charge, thereby making it preferentially soluble in the ionic liquid. The result would be a homogenously supported photosensitizer. We sought to attach a dialkylimidazolium cation to a photosensitizer and then use a solution of this new sensitizing salt dissolved in an ionic liquid to carry out a photosensitized reaction. If successful, the photoproduct could be separated from the sensitizer by extraction, leaving the ionic liquid/sensitizer mixture to be reused.

A suitable photosensitizer would need three properties: (1) a partition coefficient of zero between an ionic liquid and an

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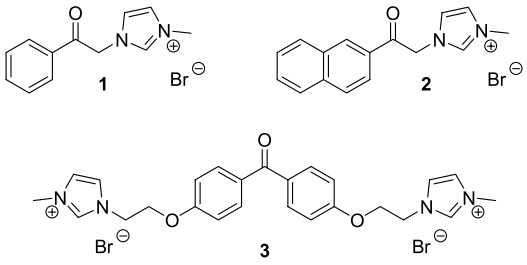


Chart 1.

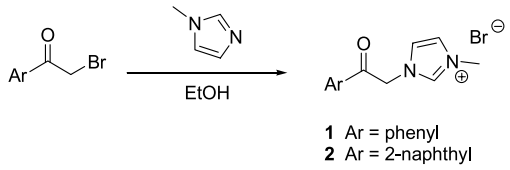
organic solvent, (2) photochemical inertness, other than triplet energy transfer, and (3) ease of preparation. We chose the common ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) and diethyl ether as representative example of an ionic liquid/organic solvent pair. The sensitizers prepared are shown in Chart 1.

2. Results and discussion

Our first target was acetophenone **1**, which was readily prepared by treating 1-methylimidazole with phenacyl bromide in ethanol (Scheme 1). Acetophenones have been widely used as triplet sensitizers and absorb well at $\lambda >$ 300 nm, which is critical in protecting the ionic liquid itself from excitation. Compound **1** was soluble in [bmim][BF_4] and insoluble in diethyl ether. When **1** was dissolved in [bmim][BF_4] (1 mg/mL) and extracted three times with an equivalent volume of diethyl ether, no **1** was present in the combined ether layers. Thus, **1** fulfilled two of the three criteria listed above.

Unfortunately, **1** did not meet criterion 2, photochemical inertness. In the photosensitized isomerization of *trans*-β-ionol,[10] **1** invariably led to complex mixtures and low mass recoveries. Isomerization of the ionol was observed, but it was clear that a number of other undesired processes were occurring. In retrospect, these side reactions might have been anticipated. Acetophenone triplets are highly reactive as hydrogen abstractors and electron acceptors. In addition, the expulsion of leaving groups from the α position following photoinduced electron transfer has also been observed in related systems.[11] Consistent with the latter reaction, acetophenone was observed in small amounts in the organic extracts from these attempted photoisomerizations. Decomposition of **1** could also be surmised because isomerization in recycled [bmim][BF_4]/**1** mixtures was markedly less efficient.

Both hydrogen abstraction and expulsion of leaving groups, such as the alkylimidazole in **1**, from α-positions are efficient processes in n,π^* triplets. Considering this reactivity, we next prepared two additional photosensitizers, **2** and **3**. Sensitizer **2** was prepared by alkylation



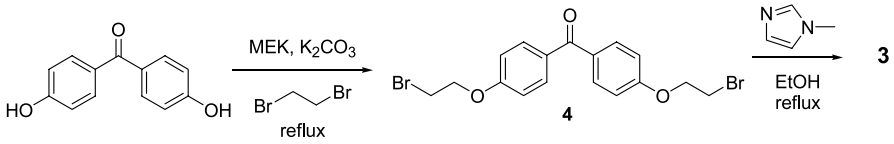
Scheme 1.

of 1-methylimidazole with 2-bromoacetonaphthone (Scheme 1). Sensitizer **3** was prepared as shown in Scheme 2. Alkylation of 4,4′-dihydroxybenzophenone with excess 1,2-dibromoethane gave **4** in 66% yield. Dibromide **4** was then used to alkylate 1-methylimidazole to give **3** in 68% yield. The chromophore in both **2** and **3** was modified such that the lowest triplet state should be the π,π^* configuration. Also, imidazole expulsion was not possible in **3**. As was the case with **1**, neither **2** nor **3** was soluble in diethyl ether.

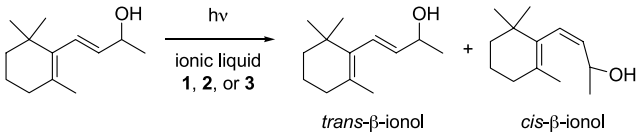
Both **2** and **3** sensitized the isomerization of *trans*-β-ionol (Scheme 3) when irradiated with a medium pressure Hg lamp with a UO_2 doped glass filter ($\lambda > 350$ nm). In degassed methanol, the photostationary state achieved in sensitization with **3** was 65% *cis*-β-ionol (see below). This photostationary state was reached in 48 h when 5 mol% of **3** (relative to ionol) was used. Under the same conditions, sensitizer **2** gave a photostationary state of 100% *cis*-β-ionol, which was reached in 24 h.

Irradiation of *trans*-β-ionol in the [bmim][BF_4]/**2** or [bmim][BF_4]/**3** (5 mol% of **2** or **3** in each case) mixture led to formation of *cis*-β-ionol much more effectively than in reactions with **1**. Interestingly, the reaction was much slower in the ionic liquid than in methanol when sensitized with **3**, though no similar reduction in the rate of reaction with **2** was observed in going from methanol to ionic liquid. In all cases, the β-ionol product could be easily extracted from the ionic liquid, with recovered yields between 85 and 100%. No sensitizer was observed in any ether extracts.

The sensitizer/ionic liquid mixture could be recycled several times with no noticeable effect on rate, photostationary state composition or yield. Three successive 24 h runs were conducted using **2** (5 mol%) in 10 mL [bmim][BF_4]. In each case, the composition of β-ionol after 24 h of irradiation ($\lambda > 350$ nm) was 100% *cis*. By simple extraction, pure β-ionol could be obtained from the ionic liquid. Yields for the three runs were 92, 89 and 83%. Between each run, the recovered ionic liquid was concentrated in vacuo. A ^{1}H NMR spectrum of the ionic liquid portion after each run indicated pure [bmim][BF_4] (the sensitizer was present in too low concentration to be seen by NMR). For the fourth attempt, the ionic liquid layer from the third reaction was allowed to sit unused for ten days at ambient temperature and in room light prior to the sensitization reaction. Again,



Scheme 2.



Scheme 3.

the isomerization was complete in less than 24 h and the yield of *cis*-β-ionol was 97%. The variation in actual recovered yields was slight and was consistently between 80 and 100%.

In all cases described thus far, diethyl ether was used as the organic solvent in extracting ionol from the ionic liquid. Attempts to extract the ionic liquid ([bmim][BF_4]) with benzene or ethyl acetate were problematic, as the ionic liquid itself was partially soluble in the organic solvent. This led to significant quantities of ionic liquid, and some sensitizer, crossing into the organic layer. Significant accumulation of the organic solvent in the ionic liquid layer also occurred, requiring removal of the solvent under reduced pressure. Though the suitability of an organic solvent will vary according to the ionic liquid used, in most cases, ether or hexane will be the most effective extraction solvent.

Recoveries of β-ionol from reactions sensitized by **3** in [bmim][BF_4] were also high (>80%). However, these reactions were very slow when irradiated at $\lambda>350$ nm. The photostationary state (88% *cis*) was only reached after 410 h. The reduced rate is best explained by the relative absorptivities of **2** and **3**. Benzophenone **3** absorbs strongly between 260 and 320 nm ($\varepsilon_{313}=5000$) but poorly at 350 nm ($\varepsilon=28$). At the principal emission line of the filtered lamp (366 nm), **3** has practically no absorbance. In contrast, **2** absorbs very well between 300 and 350 nm ($\varepsilon_{350}=1410$ and $\varepsilon_{366}=41$). The rate of sensitization using **3** did appear to be slower in [bmim][BF_4] than in organic solvent, as the photostationary state using 4,4′-dimethoxybenzophenone (an analog of **3**) in benzene was reached in only 72 h ($\lambda>$ 350 nm). In methanol, a photosensitization reaction using **3** did not proceed to the usual photostationary state. Instead, this reaction stopped after approximately a 3:1 ratio of *cis* to *trans*-β-ionol had been obtained. When this reaction was concentrated, NMR analysis indicated an absence of **3**, which had apparently undergone photoreduction by the solvent, as determined by the aromatic signals moving upfield by 0.1–0.4 ppm.

Figure 1 shows a comparison of rates of ionol isomerizaton in [bmim][BF_4] and methanol, using **2** as the sensitizer. No significant difference in the rate of sensitization in the two solvents was observed. The rate appeared to slow as the reaction progressed when the reaction was conducted in methanol. However, consumption of **2** was not detected when the residue was analyzed following removal of the methanol.

The usefulness of **2** and **3** in ionic liquids other than [bmim][BF_4] was also examined. Two additional ionic liquids were chosen: 1-butyl-4-methylpyridinium tetrafluoroborate ([BuPic][BF_4]) and *N*-butyl-*N*-methylpyrrolidinium triflimide ([BMPyr][Tf_2N]). The sensitization of ionol

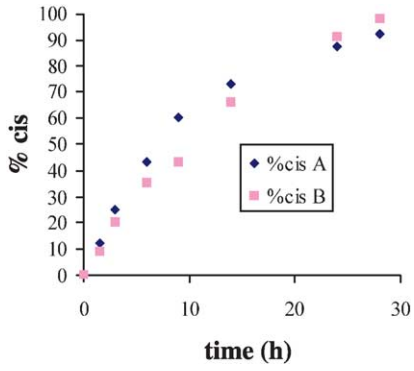
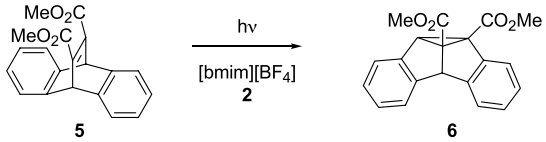


Figure 1. Amount of *cis*-ionol present in reactions sensitized by **2** in methanol (A) and in [bmim][BF_4] (B). The two samples were irradiated using a medium pressure Hg lamp and UO_2 filter ($\lambda>350$ nm).

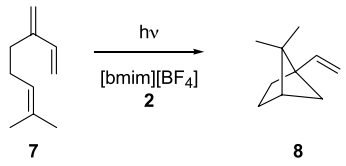
isomerization using **2** was tested in both solvents. This reaction gave a very complex mixture when carried out in [BuPic][BF_4]: GC analysis registered 13 volatile products in greater abundance than the total amount of ionol. However, after 24 h ($\lambda>350$ nm), the ionol was 87% *cis*, indicating that the sensitized isomerization did occur. NMR confirmed that a complex mixture had resulted. We have observed numerous undesired photoreactions in pyridinium based ionic liquids.[7a] Given our past results and the recent data, we suggest avoiding pyridinium based ionic liquids as solvents for photoreactions, unless a specific interaction with the solvent is desired.

Photoisomerization reactions (5 mol% **2**) run in [BMPyr][Tf_2N] proceeded smoothly. In our first attempt, isomerization was complete after 24 h and *cis*-β-ionol was recovered in 87% yield. The recovered [BMPyr][Tf_2N] was used in a second reaction, and again, isomerization went to completion in 24 h with 91% recovery of *cis*-β-ionol. This ionic liquid was not as attractive for use in the sensitized ionol isomerization because of its higher hydrophobicity relative to [bmim][BF_4]. Extraction with ether was impossible, as the ether dissolved significant quantities of the ionic liquid, contaminating the recovered ionol with both [BMPyr][Tf_2N] and sensitizer **2**. Instead, hexane was used to extract the ionol from the ionic liquid.

Other sensitized photoreactions were examined to determine the scope of the ionic liquid soluble photosensitizers. The sensitized di-π-methane rearrangement of **5**[12] was successfully carried out by irradiation ($\lambda>350$ nm) of a solution of **2** in [bmim][BF_4] for 18 h (Scheme 4). The solution was prepared and irradiated exactly as were the ionol photoisomerization reactions. The starting dibenzobarrelene was remarkably soluble in the ionic liquid, 75 mg of **5** dissolving readily in 10 mL of [bmim][BF_4]. The product, **6**,[13] was extracted from the ionic liquid with ether and recovered in 87% yield. No starting material remained and no other products were extracted with the product.



Scheme 4.



Scheme 5.

In contrast, the sensitized photolysis of myrcene (**7**) did not proceed when irradiated similarly in [bmim][BF_4] (Scheme 5).[14] Extraction of the ionic liquid with ether following photolysis gave a complex mixture of products that could not be separated, though signals of the desired product (**8**) were identified in a ^{1}H NMR spectrum of the crude product. An explanation for this result could be found in the behavior of the hydrocarbon upon addition to the ionic liquid; when myrcene is added to [bmim][BF_4] (or other ionic liquids), visible globules form. The insolubility of myrcene in the ionic liquid most likely produces a higher effective concentration than indicated by the relative amounts of substrate and solvent. Liu and Hammond reported that at higher concentrations photosensitization of myrcene led to dimerization, rather than photocyclization.[14] We believe that in this case, the phase separation that occurs between myrcene and [bmim][BF_4] results in dimerization and oligomerization in addition to the desired reaction. Thus, ionic liquids are suitable solvents for photosensitization reactions only when they dissolve the substrates at reasonable concentrations. Unfortunately, use of the more hydrophobic ionic liquid, [BMPyr][Tf_2N], did not provide improvement in the sensitized photoisomerization of myrcene.

3. Conclusion

Imidazole-tagged aryl ketones have been developed that can efficiently sensitize photochemical reactions in ionic liquids. The product can be isolated simply by extraction of the ionic liquid solution with an appropriate organic solvent, with the sensitizer remaining in the ionic liquid layer. The ionic liquid/sensitizer mixture can be recycled a number of times with little loss of energy transfer efficiency or recovered yield of the product. Due to apparent participation of the solvent, [BuPic][BF_4] was not an effective ionic liquid for these reactions. The most effective combination was acetonapthone **2** in [bmim][BF_4], using ether as the solvent for extraction, though this will likely vary according to reaction and substrate. This method is effective for rapid isolation of product and as a means of avoiding chromatography of sensitive photoproducts in cases where photosensitization via energy transfer is required.

4. Experimental

4.1. General methods

^{1}H NMR (300, 500 MHz) and ^{13}C NMR (75, 125 MHz) spectra were recorded on Bruker Avance 300 and 500 MHz spectrometers. Unless otherwise indicated, all reagents and solvents were obtained commercially and used without further purification: all compounds were purchased from Sigma-Aldrich or Fisher Scientific. CH_2Cl_2 was distilled over calcium hydride. Thin-layer chromatography was performed on silica gel (250 μm thickness doped with fluorescein) unless otherwise indicated. The chromatograms were visualized with UV light (254 nm) unless otherwise indicated. GC analyses were conducted on an Agilent 6890 GC and analytes detected by FID following elution from a 30 m β-cyclodextrin column (Supelco 24304). Elemental analyses were performed by Atlantic Microlabs, Inc. in Norcross, GA. High-resolution mass spectrometry was performed at Ohio State University by the laboratory of Christopher Hadad in Columbus, OH. Photochemical reactions were conducted using a 450 W medium pressure Hg vapor lamp (Hanovia) in conjunction with a UO_2 doped glass filter.

4.1.1. 3-Phenacyl-1-methylimidazolium bromide (1). Phenacyl bromide (15.0 g, 76 mmol) was dissolved in ethanol (300 mL) and the solution cooled to 0 °C by an external ice/water bath. 1-Methylimidazole (6.2 mL, 84 mmol) was added dropwise to the stirring reaction mixture via addition funnel. The resulting solution was allowed to warm to ambient temperature with the bath. After 36 h, solvent was removed in vacuo to give a yellowish sludge. The sludge was poured into rapidly stirring diethyl ether and a white precipitate formed. The precipitate was collected and triturated extensively with ether and dried in vacuo to give the desired salt as a yellow powder (19.2 g, 68 mmol, 90%). ^{1}H NMR (300 MHz, CD_3OD) δ 4.01 (s, 3H), 6.00 (s, 2H (exchanges with CD_3OD)), 7.63 (m, 5H), 8.08 (s, 1H), 8.11 (s, 1H), 8.99 (s, 1H (exchanges with CD_3OD)); ^{13}C NMR (75 MHz, CD_3OD) δ 192.1, 139.4, 135.7, 135.2, 130.2, 129.4, 125.4, 124.5, 56.3, 36.8. HRMS calcd for $C_{12}H_{13}N_2O^+$: 201.102239. Found: 201.10241.

4.1.2. 3-Acenapthyl-1-methylimidazolium bromide (2). 2′-Bromo-2-acetonaphthone (2.49 g, 10 mmol) was dissolved in ethanol (50 mL). To this stirring solution was added 1-methylimidazole (0.836 mL, 10.5 mmol). The reaction mixture warmed during addition of 1-methylimidazole; an ice/water bath was used to keep the reaction cool. After addition was complete, the bath was removed and the mixture allowed to warm to ambient temperature overnight. Solvent was removed in vacuo to give a yellow-orange powder. The residue was recrystallized from boiling $CHCl_3$ to give the desired salt as small yellow crystals (2.41 g, 7.3 mmol, 73%). ^{1}H NMR (300 MHz, CD_3OD) δ 4.02 (s, 3H), 6.15 (s, 2H (exchanges with CD_3OD)), 7.68 (m, 4H), 7.99 (m, 4H), 8.74 (s, 1H), 9.01 (s, 1H, exchanges with CD_3OD)); ^{13}C NMR (75 MHz, CD_3OD) δ 191.3, 139.5, 137.7, 134.0, 132.5, 130.9, 130.5, 130.0, 129.4, 129.0, 128.7, 125.4, 124.6, 124.3, 56.3, 36.8. HRMS calcd for $C_{16}H_{15}N_2O^+$: 251.117889. Found: 251.11685.

4.1.3. 4,4′-(2-Bromoethoxy)benzophenone (4). To a stirring solution of 4,4′-dihydroxybenzophenone (2.14 g, 10 mmol) in MEK (200 mL) was added K_2CO_3 (3 g, 22 mmol) followed by 1,2-dibromoethane (38 g, 200 mmol) in one portion. The solution was heated to reflux for 5 h. TLC indicated no starting material remained. The solution was filtered through a plug of basic alumina, which was

washed with MEK (100 mL). The filtrate was concentrated to give a white solid (2.82 g, 6.6 mmol, 66%), which was used without further purification. An analytically pure sample of **4** was obtained by recrystallization from hot ethanol. Mp 124–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.68 (t, 4H, $J=6.6$ Hz), 4.38 (t, 4H, $J=6.6$ Hz), 6.98 (d, 4H, $J=9.0$ Hz), 7.78 (d, 4H, $J=9.0$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.2, 161.3, 132.3, 131.3, 114.2, 67.9, 28.6. Anal. Calcd for $C_{21}H_{16}Br_2O_3$: C, 47.69; H, 3.77; Br, 37.33. Found: C, 48.06; H, 3.72; Br, 36.99.

4.1.4. 4,4′-(2-(1-Methylimidazolium)ethoxy)benzophenone dibromide (3). To a stirring solution of **4** (2.28 g, 5.4 mmol) in MEK (50 mL) was added 1-methylimidazole (7 mL, 84 mmol) in one portion. The solution was heated to reflux for 72 h. Solvent was removed in vacuo to give a gummy residue that was partitioned between EtOAc (50 mL) and water (100 mL). The layers were separated and the aqueous layer washed repeatedly (5×50 mL) with EtOAc. The aqueous layer was then dried in vacuo to give an amorphous paste that was crystallized from ethanol/EtOAc to give the desired salt (2.18 g, 3.68 mmol, 68%). ^{1}H NMR (300 MHz, CD_3OD) δ 3.99 (s, 6H), 4.52 (m, 4H), 4.74 (m, 4H), 7.11 (m, 4H), 7.71 (m, 8H), 9.18 (s, 2H, exchanges with CD_3OD)); ^{13}C NMR (75 MHz, CD_3OD) δ 196.2, 162.8, 138.3, 133.0, 132.6, 125.0, 124.2, 115.5, 67.6, 36.7, 33.9. HRMS calcd for $C_{25}H_{28}BrN_4O_3Na^+$: 511.133928. Found: 511.13426.

4.2. Method for photoisomerization of ionol

To 10 mL RTIL was added a measured amount of sensitizer followed by 100 μL *trans*-β-ionol (94 mg, 0.48 mmol). The mixture was then vigorously stirred while evacuated on a high-vacuum line (50 mTorr). The solution was stirred for 1 h or until the sensitizer was fully dissolved, whichever took longer. The solution was then irradiated with a medium pressure Hg lamp through Pyrex and a UO_2 doped glass filter ($\lambda>350$ nm). Photolyses were followed by removal of 500 μL aliquots from the reaction mixture, extraction of the aliquot with 500 μL diethyl ether and GC analysis of the extract. For preparatory scale reactions, the photolysis mixture was extracted three times with an equivalent volume of diethyl ether, the combined organic extracts washed with 10 mL water and conc. in vacuo.

4.3. Method for sensitized di-π-methane of dibenzobarrelene 5.[13]

To 10 mL [bmim][BF_4] was added 16 mg of **2** (0.05 mmol) and the solution stirred vigorously while being gently heated until the sensitizer had dissolved. To the resulting solution was added **5** (75 mg, 0.23 mmol).[12] The mixture was then evacuated on a high-vacuum line (50 mTorr) for 3 h. The solution was then irradiated with a medium pressure Hg lamp through Pyrex and a UO_2 doped glass filter ($\lambda>$ 350 nm) while being vigorously stirred for 18 h. The photolysis mixture was extracted four times with 20 mL of diethyl ether, the combined organic extracts washed with 10 mL water and conc. in vacuo to give **6** (65 mg, 0.20 mmol, 87%). The ^{1}H NMR spectrum for **6** isolated from ionic liquid supported reactions matched exactly that of **6** made by literature methods.[13]

4.4. Method for sensitized photolysis of myrcene

To 10 mL [bmim][BF_4] was added 15 mg of **2** (0.05 mmol) and the solution stirred vigorously while being gently heated until the sensitizer had dissolved. To the resulting solution was added 125 μL of freshly distilled myrcene (100 mg, 0.74 mmol). The mixture was then evacuated on a high-vacuum line (50 mTorr) for 30 min. The solution was then irradiated with a medium pressure Hg lamp through Pyrex and a UO_2 doped glass filter ($\lambda>350$ nm) while being vigorously stirred for 24 h. The photolysis mixture was extracted three times with 15 mL of diethyl ether, the combined organic extracts washed with 10 mL water and conc. in vacuo to give the crude product.

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Effective consideration of ring structures in CAST/CNMR for highly accurate ^{13}C NMR chemical shift prediction

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Abstract—A new function that effectively takes into account ring structural environments achieves extensive highly accurate prediction of ^{13}C NMR chemical shift in the CAST/CNMR system. The approach adapts a fast and flexible ring perception algorithm and a new CAST coding method for the ring information. ^{13}C NMR chemical shift prediction is performed for complicated polycyclic natural products and their synthetic intermediates as the demonstration, which shows the reliability of the function in extending the scope of the practically accurate ^{13}C NMR prediction for wide range of organic compounds.

1. Introduction

The NMR chemical shift is influenced by several factors such as diamagnetic, paramagnetic, and neighbor-atom effects. The neighbor-atom effects vary according to the bond distance, namely, the effects consist of the electronegativity of directly bonded substituent (α-effects) and also steric interaction with non-bonded atoms (γ- and δ-effects). ^{13}C nuclei in the same partial structure within the δ-position show close chemical shift values, and most database-oriented ^{13}C NMR chemical shift prediction systems, including CAST/CNMR[1], accordingly calculate chemical shift values based on the NMR data of carbons in the same structural environment.

In the use of the NMR prediction systems in practical chemical research fields such as in natural products chemistry, both prediction of a chemical shift within 1 ppm accuracy and the guarantee of the predicted values are essential, because general ^{13}C NMR experiments in the same solvent have precision within 1 ppm and therefore more than 1 ppm difference is a sufficient level of significance to point out misdetermination of the chemical structure or misassignments of the chemical shifts and requires that they be reinvestigated. This is an important problem still remaining in the computer-assisted NMR prediction especially for supporting structural elucidation studies on natural products and their synthetic intermediates. In the last four decades, many excellent systems for NMR prediction have been reported and/or marketed,[2–20] but none of them can give sufficient solutions for the practical analysts even though they are well-developed and elaborate systems.

CAST/CNMR is the first system that ensures highly accurate prediction of ^{13}C NMR chemical shift values, which are sufficient for highly experienced structure analysts in the practical field. The accurate prediction was achieved by careful deliberation of significant factors in the NMR prediction including stereochemistry from the viewpoint of practical analysis of complicated structures and stereochemistry. We demonstrated that the CAST/CNMR system was practically applied to stereochemically complicated compounds. The development of CAST/CNMR gave confirmation that stereochemistry was essential information for accurate prediction of the NMR chemical shift.

A ring system gives a long-range influence to the NMR chemical shift, and the results from our investigation for many compounds on the degrees of accuracy with respect to partial structural features considered in the CAST/CNMR prediction were consistent with the ring effects. Namely, in specific cases, the effects of some features including a ring system should be taken into account, whereas in general,

Keywords: ^{13}C NMR chemical shift prediction; A ring structure effect; Natural products; Database.
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Table 1. Reported ^{13}C NMR chemical shift values in compounds **1–5**

Compound	Carbon no.	^{13}C NMR chemical shift values/ppm	Carbon no.	^{13}C NMR chemical shift values/ppm	Carbon no.	^{13}C NMR chemical shift values/ppm
1a	1	25.8				
1b	1	27.0				
1c	1	28.7				
1d	1	26.8				
2a	1	72.9	2	34.6		
2b	1	69.1	2	35.1		
2c	1	72.0	2	37.3		
2d	1	70.6	2	34.0		
3a	1	79.7	2	41.2	1-Me	28.2
3b	1	69.8	2	39.7	1-Me	29.5
3c	1	72.2	2	42.4	1-Me	30.5
4a	1	219.6	2	37.9		
4b	1	209.7	2	41.5		
4c	1	215.0	2	43.9		
5a	5	50.7	6	213.2	19	13.4
5b	5	46.9	6	216.3	19	15.3
5c	5	51.2	6	220.2	19	14.6

both planar and configurational partial structures within the γ-position are necessary, and those within the δ-position are sufficient for accurate prediction.

Structures that differ in ring size generally show different chemical shift values. Reported chemical shift values of structures **1–5** are shown in Table 1[21,22] as instances, where the structures with the same number are different only in the ring size, for example, **1a**, **1b**, **1c**, and **1d** are cyclopentane, cyclohexane, cycloheptane, and cyclooctane, respectively. The ^{13}C NMR chemical shift differences among **1a–d** are from 0.2 (between **1b** and **1d**) to 2.9 (between **1a** and **1c**) ppm. A similar range of differences is shown in **2a–d**. Larger differences are found in **3**, **4**, **5**, for example, 9.9 ppm difference is found between C-1 of **4a** and **4b**, and 7.0 ppm difference is found between C-6 of **5a** and **5c**. It is clear that ring size influences the ^{13}C NMR chemical shift, although there is no simple relationship between the ring size and the differences in the chemical shift values.

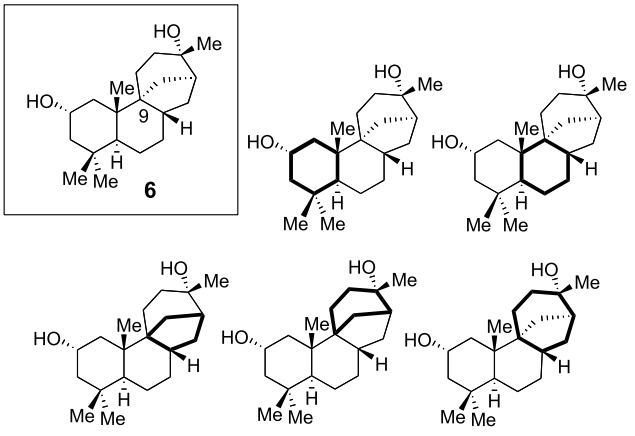
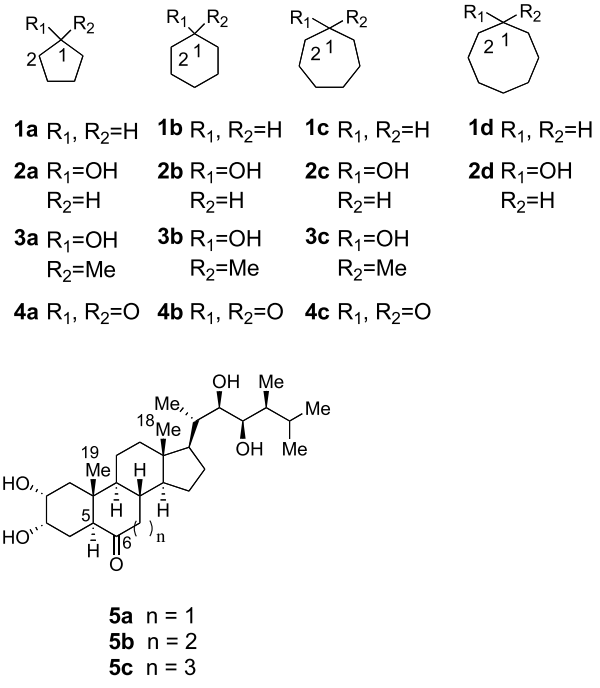


Figure 1. An example of the ring perception. These rings are recognized in **6**.



The CAST/CNMR system has therefore been adapted to consider the ring structures so as to extend the range of compound types for which highly accurate ^{13}C NMR chemical shifts can be predicted. This paper describes the method of handling the ring effects in CAST/CNMR together with the applications to complicated ring systems.

```
05060607*0606;050607;0506;0607*0606;06;0,06;0507;0,050607;0;0,0607;0;0
*06;06;0,06;0;0,0;0;0,&1;0;0,&2;0;0,,&2;0607;0,,,&3;0;0,,*06;0;0,&1;0;
0,,&4;0;0,,,,,,,,,,,,,&3;0;0,,,,*&4;0;0,0;0;0,0;0;0,,,,0,,,0;0;0,0*
```

Figure 2. An example of the ring CAST coding. The ring CAST notation started from C-9 of **6**.

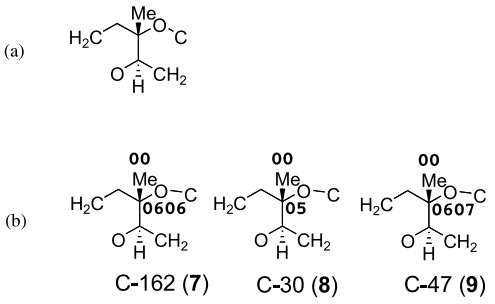


Figure 3. Partial structures and the ring CAST codes for C-162 (**7**), C-30 (**8**), and C-47 (**9**). (a) They have the same partial structures with respect to both planar and configurational partial structures within three bonds. (b) The methyl carbons are distinguished with the ring CAST codes, which are denoted besides the corresponding atoms.

2. Results and discussion

2.1. CAST coding for rings

A new CAST coding method for ring systems was developed, where rings except inclusion relationships were adopted, namely, when ring-A was included in ring-B, only ring-A was taken into account. For example in coding of stemodin (**6**), 9-, 10-, 11-, 13-, and 15-membered rings are excluded and five rings are selected as shown in Figure 1, where perceived rings are drawn with a bold line in the structure of **6**.

The ring information was translated into a CAST notation style.[23] A ring CAST code is a combination of the size of the

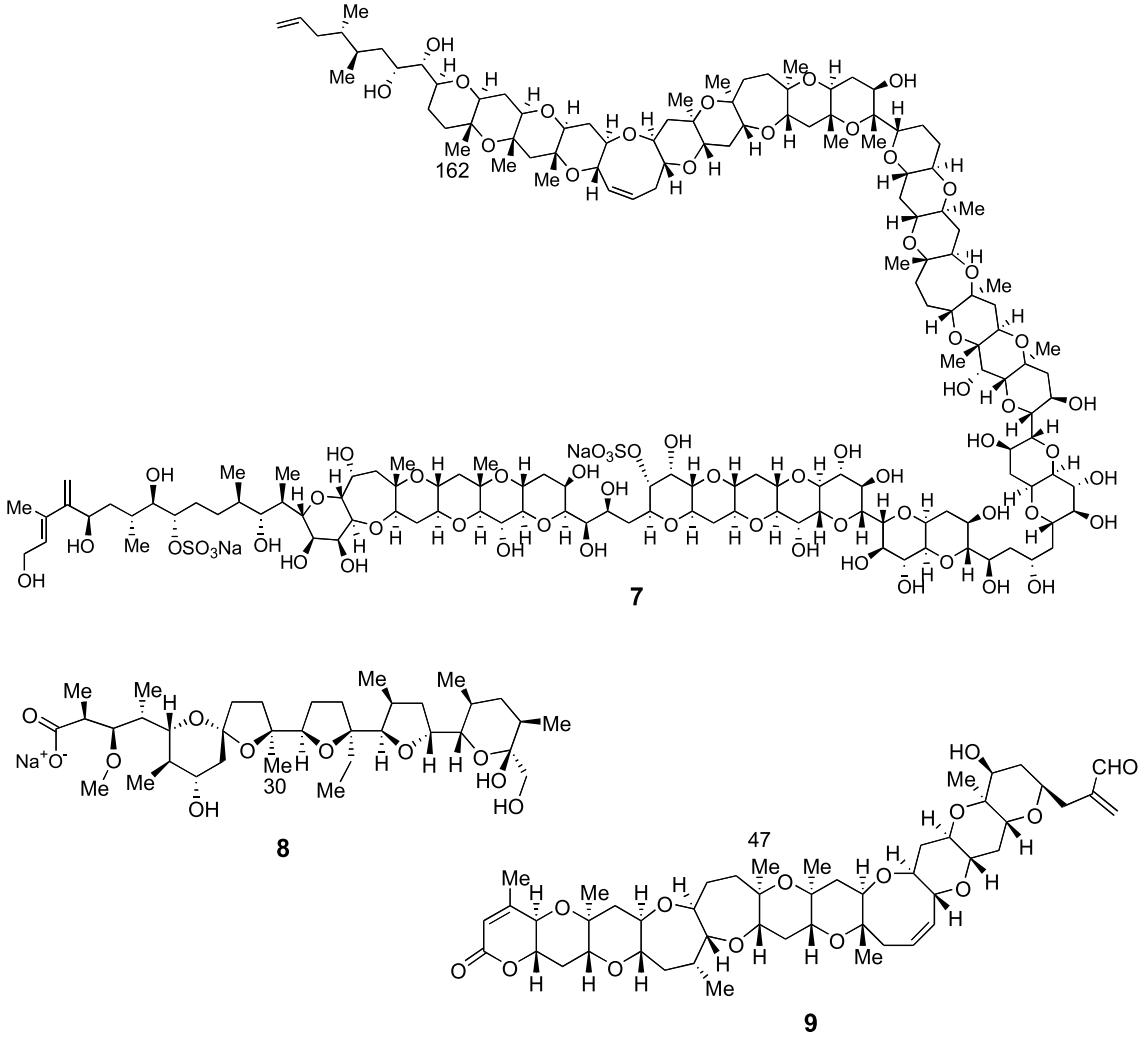


Table 2. Predicted and reported ^{13}C NMR chemical shift values in compounds **7–9**

Compound	Carbon no.	Reported values/ppm	Predicted values with conditions of planar and configurational level 4 (Δ from reported)/ppm	Predicted values with conditions of planar and configurational level 4, and ring level 2 (Δ from reported)/ppm
7	162	22.0	23.3 (1.3)	22.3 (0.3)
8	30	27.4	23.3 (−4.1)	27.4 (0.0)
9	47	22.1	23.3 (1.2)	22.0 (0.1)

ring including the atom to be coded, for example, a ring code '0607' is assigned to an atom when the atom is included in both six and seven membered rings. In the case of an atom fused with two rings that are the same size, a code with two notations of the ring size is assigned, such as '0606'. The ring CAST code was assigned to each of the corresponding atoms in a CAST notation generated according to the CAST coding rule.[23] Figure 2 shows the ring CAST notation starting from C-9 of **6** as an instance.

2.2. Ring consideration in the CAST/CNMR system

The ring CAST notations were implemented to the CAST/CNMR system so as to take into account ring structural environments in the ^{13}C NMR chemical shift prediction. The consideration of ring information is performed by simple string matching between the ring CAST linear notations by the same way in the other CAST notation matching in CAST/CNMR.[1]

Applications to maitotoxin (**7**),[24] monensin sodium salt (**8**),[25] and brevetoxin B (**9**)[26] are first attempts of ring consideration in CAST/CNMR. The carbon atoms of C-162 (**7**), C-30 (**8**), and C-47 (**9**) are in the same planar and configurational partial structure within three bonds (Fig. 3-a). The same ^{13}C NMR chemical shift value 23.3 ppm was therefore predicted for them in CAST/CNMR without ring information, whereas the reported chemical shift values were 22.0, 27.4, and 22.1 ppm for C-162 (**7**), C-30 (**8**), and C-47 (**9**), respectively, as listed in Table 2, where any absolute error was over 1 ppm and the maximum was over 4 ppm.

Taking into account the ring information within one bond, namely in this case, information on the rings including the quaternary carbon that attached to the methyl group, the predicted values were much improved, where even the largest difference from the reported ones was only 0.3 ppm (Table 2). The results were due to the data being selected by using the ring information as shown in Figure 3-b. They were namely distinguished with the ring codes of the quaternary carbon, of which the codes were '0606', '05', and '0607' for C-162 (**7**), C-30 (**8**), and C-47 (**9**), respectively.

The distribution of data that was selected in the prediction are shown in Figure 4, where (I), (II), and (III) are the results of C-162 (**7**), C-30 (**8**), and C-47 (**9**), respectively. Blue, red, and pinkish lines show reported, predicted, and used data, respectively. Results from the prediction without ring information are shown in Figure 4-(I)-a, (II)-a, and (III)-a, where clearly any of the selected data (pinkish lines) was widely distributed and gave predicted values that were far from the reported ones. The wide distribution then become narrow, which enabled highly accurate prediction when the ring information was taken into account, as shown in Figure 4-(I)-b, (II)-b, and (III)-b.

2.3. A practical application to a synthetic intermediate of brevetoxin B

A more practical application was performed to a synthetic intermediate (**10**) in a total synthesis of brevetoxin B.[27]

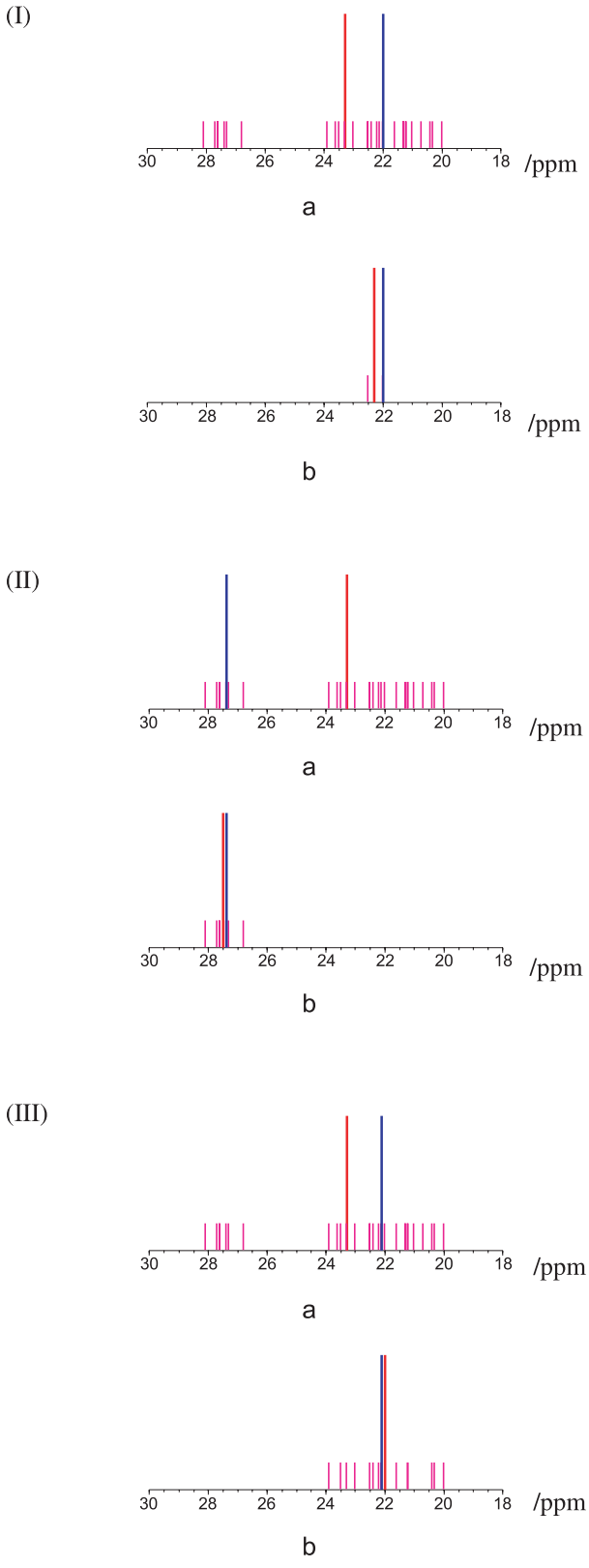


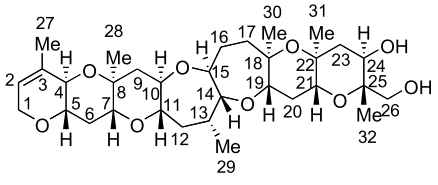
Figure 4. Distribution of data selected in the prediction. (I) Results of C-162 (**7**). (II) Results of C-30 (**8**). (III) Results of C-47 (**9**). (a) Without ring information. (b) With ring information. Blue, red, and pinkish lines show reported, predicted, and used data, respectively.

Table 3 shows the results from the ^{13}C NMR chemical shift prediction without and with ring information. Both planar and configurational partial structures within three bonds

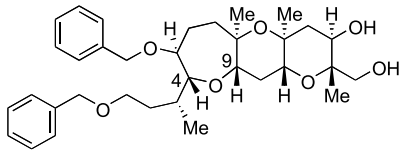
Table 3. Predicted and reported ^{13}C NMR chemical shift values in compound **10**

Carbon no.	Reported values/ppm	Predicted values without ring information/ppm	Δ (predicted−reported)/ppm	Predicted values with ring information/ppm	Δ (predicted−reported)/ppm
1	66.8	66.9	0.1	66.9	0.1
2	120.9	121.2	0.3	121.2	0.3
3	134.4	134.3	−0.1	134.3	−0.1
4	69.5	69.6	0.1	69.6	0.1
5	74.6	74.6	0.0	74.6	0.0
6	30.8	31.0	0.2	31.0	0.2
7	80.2	79.5	−0.7	79.5	−0.7
8	73.8	74.7	0.9	74.7	0.9
9	44.8	44.6	−0.2	45.0	0.2
10	83.8	83.5	−0.3	83.5	−0.3
11	85.2	84.7	−0.5	84.7	−0.5
12	35.2	35.4	0.2	35.4	0.2
13	33.1	33.0	−0.1	33.0	−0.1
14	87.5	88.4	0.9	88.4	0.9
15	83.7	83.3	−0.4	83.3	−0.4
16	29.2	29.8	0.6	29.8	0.6
17	37.7	36.8	−0.9	36.8	−0.9
18	78.0	77.2	−0.8	77.2	−0.8
19	87.8	86.0	−1.8	86.0	−1.8
20	28.6	29.9	1.3	29.9	1.3
21	73.6	73.5	−0.1	73.5	−0.1
22	73.4	73.2	−0.2	73.2	−0.2
23	43.2	44.4	1.2	44.4	1.2
24	66.5	67.3	0.8	67.3	0.8
25	78.7	78.6	−0.1	78.6	−0.1
26	67.7	68.4	0.7	68.4	0.7
27	17.0	17.1	0.1	17.1	0.1
28	16.1	17.7	1.6	16.5	0.4
29	18.3	17.0	−1.3	18.1	−0.2
30	21.2	23.4	2.2	22.0	0.8
31	19.9	21.3	1.4	20.8	0.9
32	13.9	13.5	−0.4	13.5	−0.4

were considered in the former execution, and in the latter one, ring structures including the atoms connecting to a target carbon of the prediction was added. In comparing of prediction results without and with ring information, the maximum of the absolute error was 2.2 and 1.8 ppm, the average was 0.64 and 0.51 ppm, and the root mean square (RMS) was 0.86 and 0.66, respectively, which show that the accuracy became higher by considering the ring information. The plot graph of the predicted data to the reported data is shown in Figure 5, which also demonstrates the high accuracy.



10



11

In more detail, the prediction to C-28, 29, 30, and 31 gave largely improved values, which originated in environments of the ring structures. The carbon atoms 28, 30, and 31 are all in angular methyl, ^{13}C NMR chemical shift of which influences can be taken by steric interaction. The 1,3-diaxial methyl carbons C-30 and 31 affect the ring conformation. The carbon atom 29 is a methyl carbon that may be sensitive to conformational changes of the ring structures. For C-19, the absolute error of 1.8 ppm was not improved even using ring information within one bond. The absolute error value then became 0.8 ppm when the ring information up to

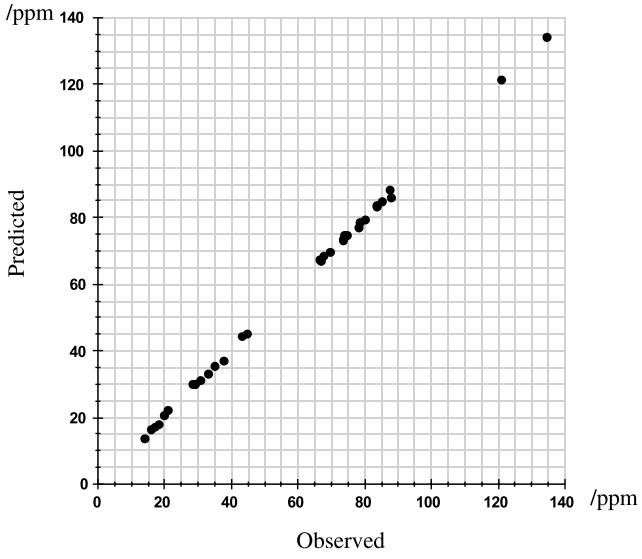


Figure 5. CAST/CNMR prediction for **10**. Plots of predicted ^{13}C NMR chemical shifts against the observed values, which show the high accuracy of the prediction.

within two bonds was taken into account. The improvement resulted the more restricted data selection with the ring codes at atoms that were two bonds away from the carbon to be predicted. For example, data of C-9 (**11**) was removed because of the difference between C-14 (**10**) and C-4 (**11**), the ring CAST codes of which are '0707' and '07', respectively. Actually, the observed ^{13}C NMR chemical shift of C-9 (**11**) was 80.8 ppm, whereas C-19 (**10**) was observed at 87.8 ppm. The results demonstrate that the data selection with the ring CAST is reasonable for accurate prediction in the CAST/CNMR system.

3. Conclusion

A function considering a ring structural effect has been implemented in the ^{13}C NMR chemical shift prediction system CAST/CNMR, which made it possible to provide more highly accurate prediction especially to complicated ring systems. Concrete applications to natural products and their synthetic intermediates having complicated ring structures proved the reliability of the new function, which extends the range of compounds for which practically accurate ^{13}C NMR chemical shift prediction can be carried out in the CAST/CNMR system. The CAST/CNMR system can be applied to correcting misassignments in chemical shifts and structures as well as to determining stereochemistry. We are currently preparing papers describing the more practical applications in organic chemistry. Further developments of CAST/CNMR are in progress to ensure the prediction of diverse compounds, including structures having other specific influences to NMR chemical shift, such as aromatic compounds, which will be reported elsewhere in future.

4. Experimental

4.1. Ring perception

Ring structures were recognized with a newly developed ring perception algorithm that effectively enumerates rings with recursive operations.[28] The algorithm runs in linear time to the number of rings to be recognized and implements options that flexibly cope with several kinds of ring perception problems containing smallest, all, and other restricted ring sets. The ring perception was automatically carried out. Rings except inclusion relationships were adopted in the CAST coding for rings.

4.2. Databases

The execution used a database constructed using about 1500 compounds, including terpenoids, steroids, macrolides, polyketides, polyethers, and their synthetic intermediates.

4.3. Execution of CAST/CNMR

All executions were performed by using the current version of CAST/CNMR, which run only by command-line typing without any graphic user interface (GUI) in the Cygwin[29] environment on the Windows© operating system.[30]

Acknowledgements

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30. The development of a GUI and the improvement of some algorithms for faster calculations are now in progress. If you are interested in the use of the current version for your academic research purposes, please contact the corresponding authors.

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Palladium catalyzed Suzuki–Miyaura coupling with aryl chlorides using a bulky phenanthryl *N*-heterocyclic carbene ligand

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Abstract—A novel bis-phenanthryl *N*-heterocyclic carbene (NHC) based palladium acetate catalyst was effective for the coupling of various aryl and vinyl chlorides with organoboron compounds. *N*,*N*-Bis-(2,9-dicyclohexyl-10-phenanthryl)-4,5-dihydroimidazolium chloride **8** ($H_2ICP{\cdot}HCl$) with $Pd(OAc)_2$ and KF·18-c-6 in THF at room temperature gave Suzuki–Miyaura coupling of aryl and vinyl chorides, including unactivated and di-*ortho* substituted substrates in high yields. Hindered tri- and tetra-*ortho* substituted products were also efficiently produced. Benzyl chloride was also found to be a useful coupling partner and trimethylboroxine was used to give methylated products. The effect of ligand, base, temperature, solvent, and reaction time are reported along with various substrates including halides and triflates.

1. Introduction

Coupling reactions with readily available aryl chlorides continue to be actively investigated in an effort to develop new catalysts and to provide economical routes to useful products.[1] Until recently, chloride coupling reactions were limited to activated, electron poor, and aromatic heterocyclic substrates. Typical conditions include the use of palladium–phosphine catalysts at elevated temperatures in polar solvents. The poor reactivity of aryl chlorides is attributed to the strength of the aryl–Cl bond compared to the more active bromides and iodides.[2] While advances have been made with aryl chlorides in a number of coupling procedures, the Suzuki–Miyaura reaction holds great promise due to the utility and versatility of the organoborane coupling partner.[3] Seminal studies by Buchwald[4] and Fu extended the process to unactivated, electron rich aryl chlorides using biaryl aminophosphane ligands with $Pd(OAc)_2$ and with the bulky tri-*t*-butylphosphine ligand at room temperature.[5] Building on previous work with *N*-heterocyclic carbene (NHC) ligands,[6] Herrmann and co-workers reported the use of a bis-adamantyl–NHC for Suzuki couplings with aryl chlorides used at ambient temperature.[7] Nolan and others have reported the use of imidazolium and diazabutadiene ligands with $Pd_2(dba)_3$ that perform well with aryl chloride substrates.[8] Fürstner and Leitner have also investigated NHC ligands for aryl chloride couplings with alkylboronates.[9] Copper carboxylate additives, reported by Liebeskind et al., can also be used at lower temperatures and have the advantage of being used without added base.[10] Most recently, Glorius and co-workers have developed a conformationally flexible bis-cyclohexyl substituted NHC ligand for aryl chloride coupling at room temperature.[11] In this case di- and tri-*ortho* substituted products were formed. We now report the use of a novel bis-phenanthryl NHC ligand for the efficient coupling of aryl chlorides at room temperature with KF·18-c-6 in THF where all substrate types including electron rich aryl chlorides and vinyl chlorides are shown to be effective. Hindered products including tri-*ortho* and tetrasubstituted products are readily formed from di-*ortho* substituted substrates.

Catalyzed reactions using NHC–palladium complexes continue to be the subject of intense interest due to their enhanced reactivity and stability compared to the more commonly employed phosphines.[12] Successful transformations now include Heck,[13] Suzuki–Miyaura,[14] Hiyama,[15] and Kumada[16] couplings together with asymmetric hydrogenation reactions.[17] We have previously reported base free conditions with NHC catalysts for efficient Heck and Suzuki couplings using reactive aryl diazonium ions,[18] as part of an effort to develop milder, low temperature conditions for these transformations.[19] Bulky bis-phenanthryl NHC

Keywords: Suzuki coupling; Imidazolium; Carbene; Palladium.
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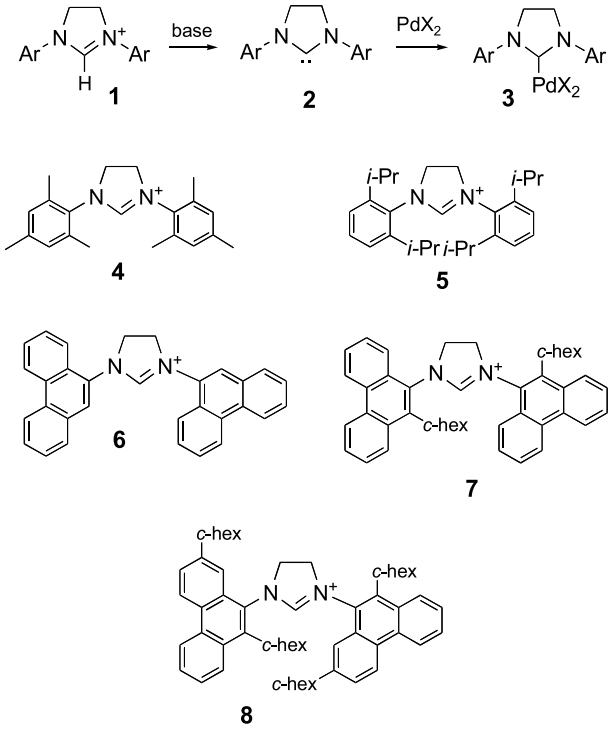
ligands, now reported in this study, were initially developed for use in copper-free Sonogashira coupling reactions.[20]

NHC imidazolium derived ligands provide enhanced stability and reactivity compared to phosphine ligands due to strong σ-bond donation to the metal coupled with attenuated back-bonding via *N*-lone pair donation.[12] This combined electronic effect renders the metal more electron rich, allowing for more favorable oxidative insertion. Typically, NHC complexes are formed by treatment of an imidazolium salt precursor **1** with base to give a free carbene **2** (Scheme 1). Free carbenes of this type, depending on the size of the nitrogen substituent, have been shown to be stable in solution and in crystalline form.[21] Treatment with a metal gives the carbene complex, many of which are air stable and can be chromatographed. Alternatively, the NHC–Pd complex can be formed in situ without added base from the imidazolium precursor. NHC precursors investigated in this study include the well-known non-aromatic *N*,*N*-bis-mesityl-4,5-dihydroimidazolium chloride **4** (H_2IMes) and *N*,*N*-bis-2,6-diisopropylphenyl-4,5-dihydro chloride **5** (H_2IPr) and the bis-phenanthryl imidazolium salts **6**, **7**, and **8** reported previously for the Sonogashira investigation.[20] These imidazolium ligands are made from the corresponding anilines following the established route of Arduengo.[21]

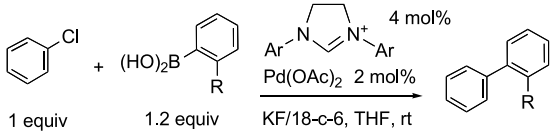


Scheme 1.

2. Results and discussion

Use of either H_2IMes **4** or H_2IPr **5** as ligand precursors produced low yields under all conditions explored for the coupling of phenyl chloride and aryl boronic acid (Table 1). Reaction with 2 mol% $Pd(OAc)_2$ and 4 mol% of the ligand in THF with various additives including KF·18-c-6, gave only a 10% yield of biaryl product after a 24 h. Use of *N*,*N*-9-phenanthryl-4,5-dihydroimidazolium chloride **6** gave an improved yield of 30%. The bis-9-cyclohexylphenanthryl ligand **7** showed further improvement with a 67% yield after 15 h. The optimal result was finally obtained using *N*,*N*-bis-(2,9-dicyclohexyl-10-phenanthryl)-4,5-dihydroimidazolium chloride **8** (H_2ICP·HCl) which gave an 89% isolated yield. This bulky ligand **8** was also found to the most effective complex for the copper-free Sonogashira coupling.[20] This finding is consistent with previous observations that have demonstrated that increased steric shielding of the NHC–palladium complex leads to increased reactivity.[12a,20] Use of the analogous 4,5-dehydro ligand H_2ICP·HCl, gave a somewhat lower yield of 86%. At a 2:1 ligand to palladium ratio, the loading of the catalyst was also explored. When 1 mol% $Pd(OAc)_2$ was used, together with 2 mol% **8**, the yield dropped to 83%. Lowering the catalyst further to 0.5 mol% gave a reduced yield of 61% after 15 h. By raising the catalyst amount to 5 mol%, the yield obtained was increased to 91%. When the ratio of palladium to ligand was changed to 1:1 (both at 2 mol%), the yield was again lowered to 77%. At 2:1 palladium to ligand **8** ratio, the yield was only slightly lowered to 81%. When more ligand was used, at a 1:3 palladium to ligand ratio, the yield was greatly lowered to 47%. Initial investigations also included *o*-methoxyphenylboronic acid and lower yields were generally obtained, but the similar trends were observed with the ligands and catalyst amounts.

Table 1. Effect of ligand and substrate

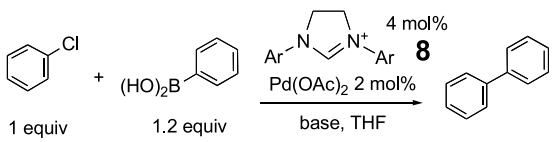


Ligand	R-	Time, h	Yield, %[a]
4	H	24	<1
5	H	24	10
6	H	24	30
7	H	15	67
8	H	15	89
8	OMe	15	68

[a] All yields are for isolated, chromatographed materials.

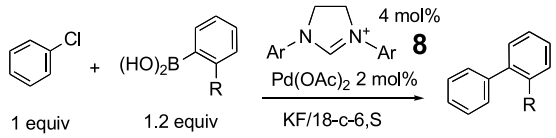
Numerous additives and bases were explored to optimize the process using H_2ICP·HCl **8** (Table 2) at room temperature and at 50 °C in THF. Aryl chloride and boronic acid substrates were used at 1:1.2 ratio. Stoichiometric cesium fluoride, potassium *t*-butoxide, potassium fluoride,

Table 2. Effect of base and temperature



Base	Time, h	Temperature, °C	Yield, %
CsF	15	Room temperature	11
KO-*t*-Bu	5	50	32
KF	5	50	32
KF/18-c-6	15	Room temperature	89
KF/18-c-6	5	50	96
Na_2CO_3/15-c-5	5	50	81

Table 3. Effect of solvent and time on coupling reactivity



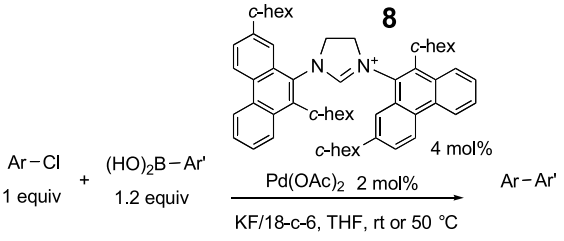
R-	Solvent	Time, h	Temperature, °C	Yield, %
H	THF	15	Room temperature	89
H	Diox.	15	Room temperature	56
H	MeOH	15	Room temperature	33
H	Tol.	15	Room temperature	21
H	THF	15	50	96
OMe	THF	24	50	98

and others when used alone gave product with low yields even at elevated temperature. Only when 18-c-6 crown ether was added in combination with base were high yields finally obtained. Potassium fluoride, a potent Lewis basic boronic acid activator in the presence of 18-c-6, gave an excellent yield of product at both 50 °C, 96% and at room temperature and in 89% yield after 15 h. The hindered NHC ligand conditions of Glorius include potassium *t*-butoxide or potassium hydride as base together with added cesium fluoride.[11] The bulky diadamantyl-NHC of Herrmann was used with excess cesium fluoride in dioxane.[7]

Solvents were screened along with reaction times using the new NHC **8**–palladium catalyst combination (Table 3). The time was held constant at 15 h for THF, dioxane, methanol, and toluene at room temperature. The highest isolated yield of product was obtained using THF at 89%. Dioxane, at 56%, was shown to be inferior. Methanol, and toluene showed less reactivity. The yield was dependent on temperature over the 15 h time period. At 30 °C, a 91% yield was obtained and at 50 °C the yield further improved to 96%. A linear response was obtained when time was varied and the temperature was maintained. Runs terminated at 6, 12, 18, and 24 h showed an exponential yield profile with corresponding 30, 71, 88, and 90% isolated product yields, respectively. Further extension of the reaction time did not lead to an improvement in yield. A similar response was noted with *o*-methoxyphenylboronic acid at 24 h over a temperature range of 30–50 °C. An excellent yield of 98% was obtained in this case at 50 °C.

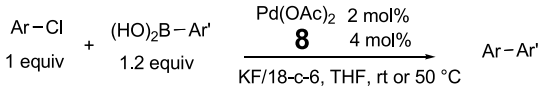
Various electron rich and deficient aryl chloride substrates were coupled to organoboron compounds using the palladium–NHC **8** catalyst conditions. Comparisons are made for each example for reactions performed at both room temperature and at 50 °C. Electron rich aryl chlorides have proven to be particularly unreactive and normally elevated temperatures are needed especially with *ortho*-substituted substrates.[1] Phenyl chloride coupled to *o*-tolyl boronic acid with excellent yields at both temperatures

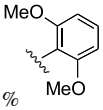
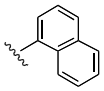
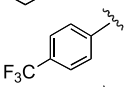
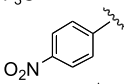
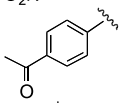
Table 4. Electron rich aryl chloride coupling



Ar=	Temperature, °C	Ar'= Ph		MeO, MeO	
		(Time, h), yield, %			
Ph—	Room temperature	(15) 89	(15) 89	(15) 89	(15) 89
	50	(6) 96	(6) 97	(6) 88	(6) 98
	Room temperature	(24) 89	(24) 87	(36) 57	(24) 84
	50	(6) 99	(8) 98	(8) 87	(8) 96
	Room temperature	(18) 51	(24) 43	(36) 37	(24) 51
	50	(12) 90	(16) 84	(12) 88	(12) 81
MeO	Room temperature	(24) 68	(12) 57	(24) 17	(24) 41
	50	(6) 98	(6) 91	(6) 86	(6) 94
MeO, MeO	Room temperature	(24) 51	(16) 37	(24) 11	(18) 37
	50	(12) 89	(12) 88	(24) 81	(12) 88
H_2N	Room temperature	(36) 51	(36) 50	(48) 31	(48) 33
	50	(24) 90	(24) 89	(24) 87	(24) 92

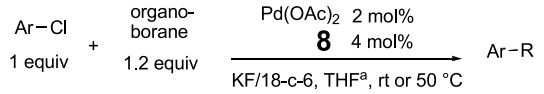
Table 5. Electron deficient aryl chloride coupling

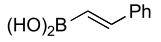
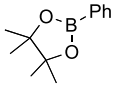
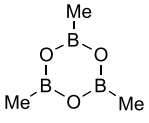
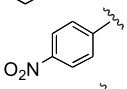
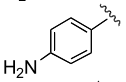
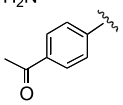


Ar=	Temperature, °C	Ar′=			
					
		(Time, h), yield, %			
	Room temperature	(24) 93	(24) 91	(48) 57	(36) 83
	50	(12) 99	(12) 99	(12) 95	(12) 98
	Room temperature	(16) 81	(24) 81	(24) 56	(12) 66
	50	(10) 96	(12) 96	(18) 91	(16) 95
	Room temperature	(6) 92	(12) 91	(18) 90	(8) 90
	50	(12) 99	(8) 97	(12) 97	(8) 97
	Room temperature	(9) 91	(8) 92	(16) 93	(8) 82
	50	(3) 97	(3) 96	(3) 97	(3) 99
	Room temperature	(24) 95	(34) 97	(36) 88	(24) 93

investigated (Table 4). 2,6-Dimethoxyphenyl and 1-naphthyl boronic acids also gave excellent yields. *O*-Tolyl chloride required extended reaction times. In particular, after 36 h with 2,6-dimethoxyphenylboronic acid, a low yield of 57% was obtained at room temperature. At 50 °C after 8 h, an 87% yield of tri-*ortho*-substituted product was obtained. Similar trends were observed with 1-chloro-2,6-dimethylbenzene. Low yields were seen with all substrates used at room temperature. However, at 50 °C with this hindered chloride, excellent yields were found including the tetra-*ortho*-substituted product from 2,6-dimethoxyphenylboronic acid. Previous to this result, only two other tetra-*ortho*-substituted Suzuki products have been reported, one generated from 9-chloroanthracene using a

Table 6. Vinyl boronic acid, pinnacolborane, and boroxazine



Ar=	Temperature, °C	Organo-borane		
				
		(Time, h), yield, %		
Ph—	Room temperature	(24) 68	(12) 31	(12) 57
	50	(6) 96	(6) 88	(6) 98
	Room temperature	(24) 81	(12) 51	(12) 78
	50	(5) 96	(10) 91	(10) 91
	Room temperature	(18) 94	(24) 93	(16) 91
	Room temperature	(8) 93	(16) 91	(8) 93
	50	(24) 87	(24) 81	(20) 87
	50	(5) 93	(5) 90	(5) 88

[a]Pinnacolatoborane and boraxazine couplings were performed in THF/H_2O (4:1) as solvent.

hindered phosphine–palladium catalyst at 110 °C,[22] and the other using a bulky, flexible carbene ligand with *t*-butoxide.[11b] The methoxy substituted chlorobenzenes also showed poor reactivity at room temperature with low yields. Again, the yields with these substrates were greatly improved at higher temperature. *p*-Chloroaniline, used previously as a substrate by Fu and Littke,[5b] also coupled with success under the bulky NHC **8** conditions. 50 °C proved to be the optimal temperature for high reactivity in this case.

Electron deficient arylchlorides reacted with excellent yields at room temperature and at 50 °C with the same range of arylboronic acids under NHC **8**–palladium catalysis (Table 5). The cyano and trifluoromethyl chlorobenzene substrates required longer reaction times. In contrast, *o*-chloronitrobenzene and 4-chloroacetophenone both gave excellent yields in much reduced time, 6–8 h. Near quantitative yields were obtained with these chlorides when the couplings were performed at 50 °C. 2-Chloropyridine gave excellent yields of Suzuki products with all the boronic acids tested. This result bodes well for the efficiency of the process with other aromatic heterocycles chlorides.

The coupling reaction conditions were extended to vinylboronic acid, phenyl pinnacolatoborane, and trimethylboroxine[23] for the generation of methylated products (Table 6). With chlorobenzene, the reaction at 50 °C gave higher yields, however, methoxy, cyano, and nitro chlorobenzene all gave excellent yields of stilbene products when reacted at room temperature with 2-styrylboronic acid. The pinnacol and boroxine couplings were performed in THF/H_20, 4:1, due to solubility problems when THF alone was used. In general, phenyl pinnacolatoborane did not perform as well as the corresponding phenylboronic acid giving lower yields with extended reaction times. Methylated, tolyl products were obtained in high yields with all the arylchlorides shown using trimethylboroxine. In general, the electron rich substrates again showed somewhat lower reactivity, and lower yields.

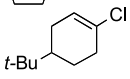
Vinyl chlorides were also shown to perform well as substrates for the coupling conditions with the bulky NHC ligand **8** (Table 7). Reflux temperature at 50 °C was needed due the lower reactivity of these halides. NHC ligands have not been used previously with vinyl chlorides. Tri-*t*-butylphosphine ligands have been successful for this class of substrates when used at elevated temperature. At room temperature with NHC **8**, coupling products were obtained in very low yields. At 50 °C however, good to excellent yields of products were obtained will all coupling partners shown. The only exception was with 2,6-dimethoxyphenylboronic acid, where the yields were only moderate. Benzyl chloride in contrast showed good reactivity even at room temperature after a 24 h period.

To round out the study, aryl and vinyl bromides, iodides, and triflates were also subjected to the new Suzuki conditions (Table 8). As expected all three showed higher reaction rates and isolated yields compared to the corresponding chlorides. When 3-methoxychlorobenzene was used at room temperature for 24 h with 2,6-dimethoxyphenylboronic acid, a low 17% yield was obtained. In contrast all the other halides, including the triflates were found to be superior.

3. Summary

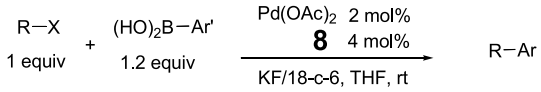
In summary, new conditions for efficient Suzuki–Miyaura coupling of organo boron compounds with aryl and vinyl chlorides have been developed. Key to the success of these reactions, at both room temperature and at 50 °C, is the use of the bulky NHC ligand *N*,*N*-bis-(2,9-dicyclohexyl-10-phenanthryl)-4,5-dihydroimidazolium chloride **8** (H_2-ICP·HCl) with $Pd(OAc)_2$ and KF·18-c-6 in THF. Electron rich substrates, including vinyl chlorides couple with aryl, vinyl, and methyl boron reagents in high yield. Tri and tetra-*ortho* substituted products are efficiently produced under mild conditions. Applications to other palladium catalyzed coupling reactions are currently under investigation using these reactive NHC ligands.

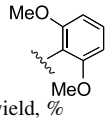
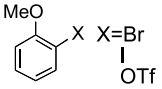
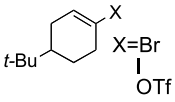
Table 7. Vinyl and benzyl chloride–arylborane coupling

Ar=	Temperature, °C	Aryl-borane		
		$(HO)_2B$–Ph	MeO, $(HO)_2B$, MeO	O, B, Ph, O
		(Time, h), yield, %		
	50	(16) 87	(24) 68	(24) 81
	50	(12) 92	(24) 58	(16) 91
	50	(16) 77	(24) 52	(24) 83
	Room temperature	(24) 71	(24) 51	
	50	(8) 89	(12) 80	

[a]Pinnacolatoborane and boraxazine couplings were performed in THF/H_2O (4:1) as solvent.

Table 8. Aryl and vinyl halide–arylborane coupling



RX	Ar′ = 	Ar′ =  (Time, h), yield, %
	(3) 97 (1.5) 98 (3) 94	(5) 89 (6) 92 (6) 87
	(3) 98	(8) 92
	(6) 90 (2) 98 (8) 89	(8) 62 (3) 89 (12) 61

4. Experimental

4.1. General method

To a flame dried flask under nitrogen were added the organo halide (0.100 mmol) and the organoboron (0.120 mmol), followed by Pd(OAc)2 (1.40 mg, 0.002 mmol, 2 mol%), dihydroimidazolium chloride salt (3.4 mg, 0.004 mmol, 4 mol%), 18-crown-6 (39.6 mg, 0.150 mmol) and KF (16.8 mg, 0.150 mmol) in anhydrous THF (5 mL). The resulting suspension was stirred at room temperature or at reflux temperature (as indicated) for the time shown in the tables. Progress of the reaction was monitored by TLC. Upon completion, to the reaction mixture was added water (15 mL) and the mixture was extracted with ethyl acetate (2×3 mL). The combined ethyl acetate fraction was washed 3 times with aqueous brine (5 mL) and dried over anhydrous magnesium sulphate. The solvent was removed by rotary evaporation and the crude material was purified by silica gel chromatography using ethyl acetate/hexanes (0–20%). The known compounds, with the isolated yields indicated in the table, were characterized by the individual data shown below.

4.1.1. Biphenyl. ^{1}H NMR ($CDCl_3$) 7.32–7.68 (m, 10H); MS (*m*/*z*) 154.

4.1.2. 2-Methylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.30 (s, 3H) 7.32–7.68 (m, 9H); MS (*m*/*z*) 168.

4.1.3. 2,6-Dimethylbiphenyl. ^{1}H NMR ($CDCl_3$) 1.92 (s, 6H), 7.00–7.03 (m, 1H), 7.07–7.37 (m, 6H); MS (*m*/*z*) 182.

4.1.4. 2-Methoxylbiphenyl. ^{1}H NMR ($CDCl_3$) 3.86 (s, 3H), 7.02 (d, 1H), 7.09 (t, 1H), 7.37–7.45 (m, 7H); ^{13}C NMR ($CDCl_3$) 56.5, 111.8, 119.6, 126.8, 127.9, 128.5, 129.9, 131.1, 139.0, 156.1; MS (*m*/*z*) 184.

4.1.5. 3,5-Dimethoxylbiphenyl. ^{1}H NMR ($CDCl_3$) 3.68 (s, 6H), 6.41 (m, 1H), 6.63–6.72 (m, 2H), 7.27–7.57 (m, 5H); ^{13}C NMR ($CDCl_3$) 56.1, 110.5, 118.2, 128.1, 128.5, 132.2, 139.1, 162.8; MS (*m*/*z*) 214.

4.1.6. 4-Trifluoromethylbiphenyl. ^{1}H NMR ($CDCl_3$) 7.40 (d, 1H, J=6.9 Hz), 7.47 (m, 2H), 7.60–7.68 (m, 6H); ^{13}C NMR ($CDCl_3$) 125.8, 127.5, 127.7, 128.7, 129.6, 139.6, 145.1; MS (*m*/*z*) 222.

4.1.7. 2-Cyanobiphenyl. ^{1}H NMR ($CDCl_3$) 7.41–7.50 (m, 3H), 7.59–7.71 (m, 6H); ^{13}C NMR ($CDCl_3$) 110.8, 118.6, 127.5, 127.9, 128.7, 128.9, 133.1, 137.7, 139.2, 146.0; MS (*m*/*z*) 179.

4.1.8. 4-Nitrobiphenyl. ^{1}H NMR ($CDCl_3$) 7.23–7.61 (m, 7H), 8.21–8.33 (m, 2H); ^{13}C NMR ($CDCl_3$) 124.3, 127.5, 128.5, 134.1, 136.3, 138.2, 145.0; MS (*m*/*z*) 199.

4.1.9. 2-Phenylpyridine. ^{1}H NMR ($CDCl_3$) 7.20–7.29 (m, 5H), 7.37–7.40 (m, 2H), 7.75 (m, 1H), 8.68 (m, 1H); MS (*m*/*z*) 155.

4.1.10. 4-Aminobiphenyl. ^{1}H NMR ($CDCl_3$) 5.51 (broad, 2H), 6.81–6.87 (m, 2H), 7.23–7.51 (m, 7H); ^{13}C NMR ($CDCl_3$) 117.2, 126.7, 126.9, 128.7, 128.9, 131.2, 137.7, 141.0, 146.1; MS (*m*/*z*) 169.

4.1.11. 4-Acetylbiphenyl. ^{1}H NMR ($CDCl_3$) 7.18–7.30 (m, 5H), 7.45 (d, 2H, J=8.6 Hz), 8.00 (d, 2H, J=8.6 Hz); ^{13}C NMR ($CDCl_3$) 27.1, 125.8, 129.1, 129.8, 135.9, 136.2, 141.0, 146.8, 196.7; MS (*m*/*z*) 196.

4.1.12. 2,2′-Dimethylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.31 (d, 6H) 7.17–7.57 (m, 8H); ^{13}C NMR ($CDCl_3$) 21.3, 125.6, 127.1, 129.2, 135.9, 141.1; MS (*m*/*z*) 196. MS (*m*/*z*) 182.

4.1.13. 2,2′,6-Trimethylbiphenyl. ^{1}H NMR ($CDCl_3$) 1.93 (s, 6H), 1.97 (s, 3H), 7.00–7.03 (m, 1H), 7.07–7.31 (m, 6H); ^{13}C NMR ($CDCl_3$) 19.6, 21.2, 126.3, 127.5, 128.1, 129.1, 129.8, 135.7, 135.9, 136.2, 141.3, 142.8; MS (*m*/*z*) 196.

4.1.14. 2-Methyl-2′-methoxylbiphenyl. ^{1}H NMR ($CDCl_3$)

2.30 (s, 3H), 368 (s, 6H), 6.41–6.57 (m, 3H), 7.13–7.74 (m, 4H); ^{13}C NMR ($CDCl_3$) 20.3, 56.2110.2, 112.8, 127.6, 130.2, 131.1, 141.9, 159.3; MS (*m*/*z*) 228.

4.1.15. 3,5-Dimethoxyl-2′-methylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.31 (s, 3H), 3.66 (s, 6H), 6.39 (m, 1H), 6.65–6.75 (m, 2H), 7.28–7.59 (m, 4H); ^{13}C NMR ($CDCl_3$) 20.5, 55.8, 111.2, 120.1, 127.3, 128.1, 128.7, 131.8, 138.7, 160.5; MS (*m*/*z*) 228.

4.1.16. 4-Trifluoromethyl-2′-methybiphenyl. ^{1}H NMR ($CDCl_3$) 2.30 (s, 3H), 7.37 (d, 1H, J=6.8 Hz), 7.43 (m, 2H), 7.58–7.63 (m, 5H); ^{13}C NMR ($CDCl_3$) 20.1, 125.3, 127.1, 127.3, 128.2, 129.0, 139.2, 144.8; MS (*m*/*z*) 236.

4.1.17. 2-Cyano-2′-methybiphenyl. ^{1}H NMR ($CDCl_3$) 2.28 (s, 3H), 7.39–7.48 (m, 3H), 7.58–7.70 (m, 5H); ^{13}C NMR ($CDCl_3$) 19.8, 111.0, 117.8, 127.1, 127.6, 128.0, 128.2, 133.3, 137.7, 139.0, 147.2; MS (*m*/*z*) 193.

4.1.18. 4-Nitro-2′-methybiphenyl. ^{1}H NMR ($CDCl_3$) 2.29 (s, 3H), 7.19–7.57 (m, 6H), 8.20–8.29 (m, 2H); ^{13}C NMR ($CDCl_3$) 19.8, 123.8, 126.7, 128.2, 133.7, 135.9, 138.2, 144.8; MS (*m*/*z*) 213.

4.1.19. 2-*o*-Tolylpyridine. ^{1}H NMR ($CDCl_3$) 2.36 (s, 3H), 7.22–7.31 (m, 4H), 7.35–7.40 (m, 2H), 7.75 (m, 1H), 8.70 (m, 1H); ^{13}C NMR ($CDCl_3$) 19.8, 126.8, 128.7, 129.2, 130.7, 135.9, 136.2, 140.3, 148.8, 160.1; MS (*m*/*z*) 169.

4.1.20. 4-Amino-2′-methylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.28 (s, 3H), 4.47 (broad, 2H), 6.72 (d, 2H, J=8.6 Hz), 7.12–7.30 (m, 6H); ^{13}C NMR ($CDCl_3$) 21.2, 114.2, 125.7, 126.2, 128.3, 128.7, 130.2, 137.1, 142.0, 145.3; MS (*m*/*z*) 183.

4.1.21. 4-Acetyl-2′-methylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.26 (s, 3H), 7.19–7.26 (m, 4H), 7.42 (d, 2H, J=8.6 Hz), 7.96 (d, 2H, J=8.6 Hz); ^{13}C NMR ($CDCl_3$) 20.3, 26.8, 125.2, 128.5, 129.3, 129.7, 135.3, 136.2, 146.2, 197.7; MS (*m*/*z*) 196.

4.1.22. 2,6-Dimethoxylbiphenyl. ^{1}H NMR ($CDCl_3$) 3.68 (s, 6H), 6.82 (m, 2H), 6.92–7.05 (m, 2H), 7.28 (m, 1H), 7.38 (m, 1H), 7.57 (m, 2H); ^{13}C NMR ($CDCl_3$) 21.1, 55.8, 91.3, 104.1, 121.3, 128.1, 133.2, 163.7; MS (*m*/*z*) 214.

4.1.23. 2,6-Dimethoxyl-2′-methylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.32 (s, 3H), 3.68 (s, 6H), 6.83 (m, 2H), 6.92–7.07 (m, 1H), 7.31 (m, 1H), 7.38 (m, 1H), 7.58 (m, 2H); ^{13}C NMR ($CDCl_3$) 56.0, 91.6, 104.3, 120.8, 128.0, 133.2, 163.8; MS (*m*/*z*) 228.

4.1.24. 2,6-Dimethoxyl-2′,6′-dimethylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.31 (d, 6H), 3.66 (d, 6H), 6.83 (m, 2H), 7.28 (m, 1H), 7.31 (m, 1H), 7.62 (m, 2H); ^{13}C NMR ($CDCl_3$) 20.6, 56.0, 91.6, 104.2, 120.5, 128.1, 133.2, 163.1; MS (*m*/*z*) 242.

4.1.25. 2,6,2′-Trimethoxylbiphenyl. ^{1}H NMR ($CDCl_3$) 3.81 (s, 3H), 3.88 (s, 6H), 6.82 (m, 2H), 7.01–7.12 (m, 3H), 7.20–7.25 (m, 2H); ^{13}C NMR ($CDCl_3$) 55.9, 100.1, 106.2, 113.2, 123.7, 123.9, 125.6, 127.1, 128.3, 155.7, 159.7; MS (*m*/*z*) 244.

4.1.26. 2,6,3′,5′-Tetramethoxylbiphenyl. ^{1}H NMR ($CDCl_3$) 3.68 (s, 6H), 3.80 (s, 6H), 6.27–6.42 (m, 2H), 6.73–6.84 (m, 3H), 7.17–7.21 (m, 2H); ^{13}C NMR ($CDCl_3$) 55.3, 55.8, 96.7, 105.2, 124.5, 127.6, 163.7, 165.2; MS (*m*/*z*) 274.

4.1.27. 2,6-Dimethoxyl-4′-trifluoromethylbiphenyl. ^{1}H NMR ($CDCl_3$) 3.68 (s, 6H), 6.76 (m, 2H), 7.03–7.11 (m, 2H), 7.26–7.62 (m, 3H); ^{13}C NMR ($CDCl_3$) 55.9, 96.2, 105.2, 122.9, 127.3, 130.8, 130.9, 135.2, 167.2; MS (*m*/*z*) 282.

4.1.28. 2,6-Dimethoxyl-2′-cyanobiphenyl. ^{1}H NMR ($CDCl_3$) 3.80 (s, 6H), 6.93 (m, 1H), 7.02–7.15 (m, 3H), 7.43 (m, 1H), 7.72 (m, 1H), 7.92 (m, 1H); ^{13}C NMR ($CDCl_3$) 55.9, 88.1, 104.2, 107.0, 125.9, 127.9, 135.2, 137.1, 166.9; MS (*m*/*z*) 239.

4.1.29. 2,6-Dimethoxyl-4′-nitrobiphenyl. ^{1}H NMR ($CDCl_3$) 3.80 (s, 6H), 6.82 (m, 2H), 7.13–7.17 (m, 1H), 7.52–7.63 (m, 2H), 8.03–8.12 (m, 2H); ^{13}C NMR ($CDCl_3$) 56.3, 125.8, 126.3, 128.2, 135.7, 135.9, 169.7; MS (*m*/*z*) 261.

4.1.30. 2-(2′, 6′-Dimethoxyl) pyridine. ^{1}H NMR ($CDCl_3$) 3.81 (d, 6H), 6.74 (m, 2H), 7.12–7.15 (m, 1H), 7.42 (m, 2H), 7.37–7.41 (m, 2H), 7.67 (m, 1H), 8.63 (m, 1H); ^{13}C NMR ($CDCl_3$) 55.9, 104.5, 117.0, 119.2, 122.9, 128.0, 138.6, 139.2, 143.5, 149.8, 162.7; MS (*m*/*z*) 215.

4.1.31. 2,6-Dimethoxyl-4′-aminobiphenyl. ^{1}H NMR ($CDCl_3$) 3.78 (s, 6H), 6.81–6.95 (m, 2H), 7.13–7.27 (m, 2H), 7.42–7.51 (m, 3H); ^{13}C NMR ($CDCl_3$) 55.8, 105.4, 120.7, 125.8, 127.3, 145.8, 161.2; MS (*m*/*z*) 229.

4.1.32. 2,6-Dimethoxyl-4′-acetylbiphenyl. ^{1}H NMR ($CDCl_3$) 2.46 (s, 3H), 3.66 (s, 6H), 6.67–6.81 (m, 2H), 7.08–7.15 (m, 3H), 7.73–7.95 (m, 2H); ^{13}C NMR ($CDCl_3$) 25.9, 56.0, 95.7, 105.1, 120.3, 132.2, 133.2, 162.6, 193.7; MS (*m*/*z*) 256.

4.1.33. 1-Phenylnaphthalene. ^{1}H NMR ($CDCl_3$) 7.43–8.07 (m, 12H); ^{13}C NMR ($CDCl_3$) 124.5, 126.0, 127.8, 128.2, 128.5, 131.2, 135.6, 143.7; MS (*m*/*z*) 204.

4.1.34. 1-*o*-Tolylnaphthalene. ^{1}H NMR ($CDCl_3$), 2.23 (s, 3H), 7.29–8.02 (m, 11H); ^{13}C NMR ($CDCl_3$) 21.0, 124.2, 126.3, 127.5, 128.1, 129.1, 129.8, 135.0, 142.1; MS (*m*/*z*) 218.

4.1.35. 1-(2′,6′-Dimethylphenyl) naphthalene. Yield: as in the table; ^{1}H NMR ($CDCl_3$) 1.97 (d, 6H), 7.12–8.07 (m, 11H); ^{13}C NMR ($CDCl_3$) 19.7, 20.3, 124.1, 126.7, 127.3, 128.1, 129.5, 129.8, 131.2, 137.1, 142.0; MS (*m*/*z*) 232.

4.1.36. 1-(2′-Methoxylphenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 3.66 (s, 3H), 6.46–6.71 (m, 3H), 7.40–8.17 (m, 8H); ^{13}C NMR ($CDCl_3$) 56.0, 110.9, 118.3, 126.3, 127.5, 128.1, 131.1, 131.7, 131.9, 133.2, 134.0, 160.3; MS (*m*/*z*) 234.

4.1.37. 1-(3′,5′-Dimethoxylphenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 3.65 (s, 6H), 6.31 (t, 1H), 6.73 (m, 2H), 7.46–8.29

(m, 7H); ^{13}C NMR ($CDCl_3$) 55.6, 111.0, 118.3, 126.2, 127.8, 131.4, 133.5, 134.0, 161.2; MS (*m*/*z*) 264.

4.1.38. 1-(4′-Trifluoromethylphenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 7.42–7.69 (m, 5H), 7.87–8.21 (m, 6H); ^{13}C NMR ($CDCl_3$) 120.2, 125.3, 125.9, 126.2, 127.5, 127.8, 133.1, 133.2, 135.6, 147.3; MS (*m*/*z*) 272.

4.1.39. 1-(2′-Cyanophenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 7.47–8.27 (m, 11H); ^{13}C NMR ($CDCl_3$) 118.5, 126.2, 126.9, 128.2, 130.5, 130.9, 131.0, 133.6, 134.1, 137.1; MS (*m*/*z*) 229.

4.1.40. 1-(4′-Nitrophenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 7.43–7.7.70 (m, 7H), 8.03–8.41 (m, 4H); ^{13}C NMR ($CDCl_3$) 124.1, 125.7, 126.3, 127.2, 127.7, 130.5, 136.8, 137.1, 140.6, 146.1; MS (*m*/*z*) 249.

4.1.41. 2-(1′-Naphthalyl) pyridine. ^{1}H NMR ($CDCl_3$) 7.27–8.05 (m, 10H), 8.63 (m, 1H); ^{13}C NMR ($CDCl_3$) 122.2, 124.5, 126.3, 127.1, 127.5, 128.1, 129.3, 139.5, 145.7, 151.2; MS (*m*/*z*) 205.

4.1.42. 1-(4′-Aminophenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 5.45 (broad, 2H), 6.65–6.71 (m, 2H), 7.43–7.68 (m, 7H), 8.12–8.21 (m, 2H); ^{13}C NMR ($CDCl_3$) 115.2, 124.1, 124.5, 125.7, 125.9, 126.2, 127.6, 127.8, 133.1, 134.8, 135.6, 140.4, 148.3; MS (*m*/*z*) 219.

4.1.43. 1-(4′-Acetylphenyl) naphthalene. ^{1}H NMR ($CDCl_3$) 2.67 (s, 3H), 7.43–7.87 (m, 9H), 8.09–8.21 (m, 2H); ^{13}C NMR ($CDCl_3$) 26.3, 124.1, 124.5, 126.2, 126.7, 128.4, 128.9, 129.8, 142.7, 192.7; MS (*m*/*z*) 246.

4.1.44. *trans*-Stillbene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 5.87 (d, 1H), 6.45 (d, 1H), 7.18–7.54 (m, 10H); MS (*m*/*z*) 180.

4.1.45. 2-Methoxyl-*trans*-stillbene. ^{1}H NMR ($CDCl_3$) 3.80 (s, 3H) 5.85 (d, 1H), 6.46 (d, 1H), 7.13–7.54 (m, 8H); ^{13}C NMR ($CDCl_3$) 26.3, 56.2, 118.5 124.0, 124.8, 125.1, 126.2, 126.7, 128.4, 128.9, 129.8, 160.0; MS (*m*/*z*) 210.

4.1.46. *trans*-2-Cyanostillbene. ^{1}H NMR ($CDCl_3$) 5.89 (d, 1H), 6.68 (d, 1H), 7.21–7.57 (m, 9H); ^{13}C NMR ($CDCl_3$) 110.2, 117.3, 126.5, 126.7, 127.7, 127.9, 128.4, 128.9, 129.8, 133.0, 136.4, 139.1; MS (*m*/*z*) 205.

4.1.47. *trans*-4-Aminostillbene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 5.47 (broad, 2H), 6.02 (d, 2H), 6.87 (m, 2H), 6.91 (d, 2H), 7.21–7.53 (m, 7H); ^{13}C NMR ($CDCl_3$) 110.2, 127.4, 127.7, 128.6, 130.9, 132.3, 148.7; MS (*m*/*z*) 246; MS (*m*/*z*) 195.

4.1.48. *trans*-4-Acetylstillbene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 2.32 (s, 3H), 5.45 (d, 1H), 6.41 (d, 1H), 7.11–7.34 (m, 7H), 7.87 (d, 2H); ^{13}C NMR ($CDCl_3$) 26.1, 128.4, 128.9, 129.8, 132.0, 132.6, 144.7, 195.7; MS (*m*/*z*) 246; MS (*m*/*z*) 222.

4.1.49. 1-Phenylcyclopentene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 2.01 (quintet, 2H), 2.47–2.58 (m, 2H), 2.66–2.73 (m, 2H), 6.11 (quintet, 1H), 7.10–7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) 21.2, 23.6, 33.5, 33.6, 125.1, 125.6, 128.9, 134.1, 136.7, 142.6; MS (*m*/*z*) 144.

4.1.50. 1-Phenyl-4-*t*-butylcyclohexene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 0.93 (s, 9H), 1.22–1.45 (m, 2H), 1.87–2.01 (m, 2H), 2.13–2.32 (m, 3H), 5.53–5.61 (m, 1H), 7.01–7.21 (m, 5H); ^{13}C NMR ($CDCl_3$) 20.1, 24.5, 27.3, 27.6, 32.1, 32.7, 45.2, 125.5, 125.8, 126.3, 128.3, 129.9, 135.7, 138.6, 142.8; MS (*m*/*z*) 214.

4.1.51. 2-Methyl-1-phenylpropene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 1.70 (d, 3H), 1.90 (d, 3H), 6.22 (m, 1H), 7.09–7.12 (m, 5H); ^{13}C NMR ($CDCl_3$) 19.7, 21.1, 26.5, 124.8, 125.3, 126.5, 129.1, 129.6, 135.1, 136.7, 138.2; MS (*m*/*z*) 132.

4.1.52. Diphenylmethane. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 3.32 (m, 2H), 7.03–7.15 (m, 10H); MS (*m*/*z*) 168.

4.1.53. 1-(2′,6′-Dimethoxylphenyl) cyclopentene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 2.03–2.35 (m, 2H), 2.38–2.51 (m, 2H), 2.62–2.68 (m, 2H), 3.86 (s, 6H), 6.17 (m, 1H), 6.72 (m, 2H), 7.32 (m, 1H); ^{13}C NMR ($CDCl_3$) 20.3, 23.3, 33.3, 33.5, 55.6, 125.2, 125.7, 129.1, 134.2, 136.7, 142.6, 162.1; MS (*m*/*z*) 204.

4.1.54. 1-(2′,6′-Dimethoxylphenyl)-4-*t*-butylcyclohexene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 0.91 (s, 9H), 1.21–1.43 (m, 2H), 1.85–1.99 (m, 2H), 2.13–2.31 (m, 3H), 5.58–5.62 (m, 1H), 6.72–6.77 (m, 2H), 7.31 (m, 1H); ^{13}C NMR ($CDCl_3$) 20.3, 24.2, 27.4, 27.7, 32.3, 32.7, 45.5, 55.7, 125.5, 125.8, 126.3, 128.3, 130.2, 135.7, 138.5, 142.8, 161.7; MS (*m*/*z*) 274.

4.1.55. 2-Methyl-1-(2′,6′-dimethoxylphenyl) propene. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 1.72 (d, 3H), 1.91 (d, 3H), 3.86 (s, 6H), 6.53 (m, 1H), 6.73 (m, 2H), 7.34 (m, 1H); ^{13}C NMR ($CDCl_3$) 19.2, 20.3, 26.1, 55.6, 114.2, 125.8, 125.9, 128.5, 129.1, 134.8, 138.2, 140.1, 160.0; MS (*m*/*z*) 192.

4.1.56. 2′,6′-Dimethoxylphenylmethane. Yield as indicated in the table; ^{1}H NMR ($CDCl_3$) 3.32 (m, 2H), 3.82 (s, 6H), 6.64–6.73 (m, 4H) 7.12–7.27 (m, 6H); ^{13}C NMR ($CDCl_3$) 27.5, 55.8, 108.1, 124.2, 125.2, 125.9, 128.5, 129.1, 134.8, 160.3; MS (*m*/*z*) 228.

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Supplementary data

Supplementary data associated with this article can be found at 10.1016/j.tet.2005.05.071

Characterization data, including ^{1}H and ^{13}C NMR spectra, are provided online with the paper at ScienceDirect.

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Synthesis of phthalascidin analogs

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Abstract—We report a new approach to obtain phthalascidin analogs. 6-Phthalimidomethylpyrazino[1,2-*b*]isoquinoline-1,4-dione (**5a**) was obtained in a one-pot *N*-alkylation/cyclization of the corresponding 1-acetyl-3-arylmethyl-2,5-piperazinedione with *N*-phthalylacetaldehyde dimethyl acetal. Chemoselective reduction of the C(1)-carbonyl group in the 3-arylmethyl-11,11a-dehydroderivative **9a** was followed by cyclization of an acyliminium intermediate, to give the 6,15-imino-7-oxo-14,14a-dehydroisoquino[3,2-*b*]3-benzazocin **11a**. Alternatively, the octacyclic compound **13a** was obtained through a novel double cyclization of a precursor in which the C(1)-carbonyl and one phthalimide carbonyl group were reduced.

1. Introduction

Many tetrahydroisoquinoline antitumor antibiotics, such as saframycins and ecteinascidins, in which an isoquinoline ring is fused to an iminobenzazocine moiety, are widely studied cytotoxic agents.[1] Their development as antitumor drugs, which is headed by ecteinascidin 743 (E-743) currently undergoing advanced clinical trials, has been limited by their natural scarcity and the complexity of their synthesis or semisynthesis.[2–7] It is known that most of the biological activity of ET-743 is maintained in simpler synthetic analogues such as phthalascidin,[8,9] but structure–activity correlations within these compounds are relatively unexplored[10–13] because most of the synthetic work carried out so far has focused on total syntheses (Fig. 1).

We are currently developing a synthetic approach to obtain 6-substituted 11,11a-dehydropyrazino[1,2-*b*]isoquinoline-1,4-diones assuming that these compounds, that contain rings A–C of the active products, will be transformed into an iminobenzazocine pentacyclic system and, by subsequent functional group manipulation, into antitumor agents. Readily accessible 1-acetyl-3-arylmethylpiperazine-2,5-diones (**1**)[14] cyclize to pyrazinoisoquinoline-1,4-diones after *N*-alkylation with acetals.[15] Because of the poor solubility of compounds **1** we used as starting materials their corresponding *O*-trimethylsilyllactims, which are also very convenient *N*-nucleophiles.[16–18] In our previous work, trimethylsilyl triflate was selected as a catalyst, and

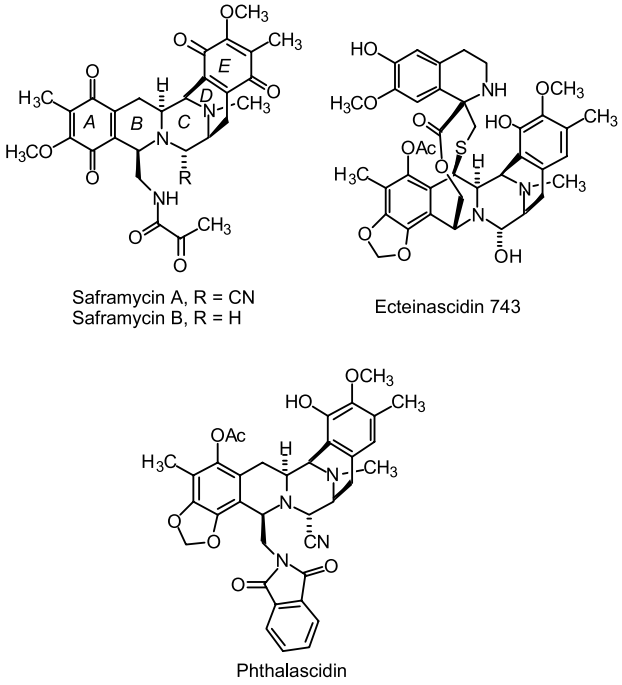

Figure 1. Structures of saframycins A and B, ecteinascidin 743, and phthalascidin.

reactions with acetaldehyde or benzaldehyde dimethyl acetals (**2**) gave diastereomeric mixtures of *N*-alkoxyalkyl derivatives **3**. Subsequent treatment with protic acids generated *N*-acyliminium intermediates,[19] that cyclized to 6-substituted pyrazinoisoquinoline-1,4-diones with a 6,11a-*trans*-relationship.[20] Some acid media were compatible with the *N*-acetyl group of compounds **5** while others gave

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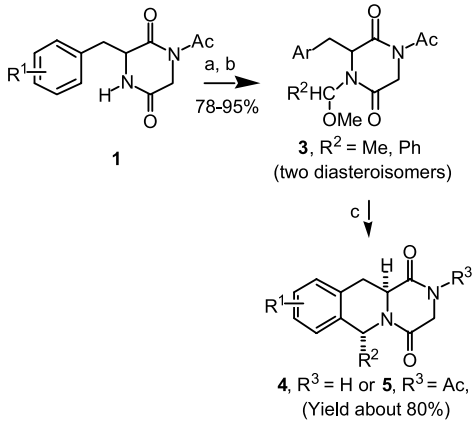


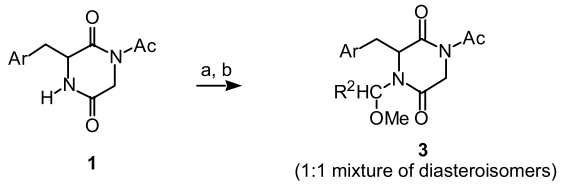
Scheme 1. Reagents and conditions: (a): TMSOTf, iPr$_2$NEt, CH_2Cl_2, −78 °C, 4 h; (b): $R^2CH(OCH_3)_2$ (**2**), TMSOTf (cat), −78 °C, 4 h; (c): p-TsOH, CH_2Cl_2, reflux, 14 h (or conc. H_2SO_4, rt, 30 min).

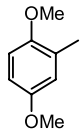
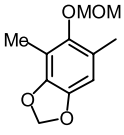
compounds **4** that were subsequently *N*-acetylated (Scheme 1).[21,22]

We report here the application of this methodology to the synthesis of phthalascidin precursors by *N*-alkylation of 3-arylmethylpiperazine-2,5-dioxosilyllactims with *N*-phthalimidoacetaldehyde acetal and the subsequent cyclization to 6-phthalimidomethylpyrazinoisoquinoline-1,4-diones. We also study the transformations of these tricyclic compounds to obtain 6,15-imino-7-oxo-14,14a-dehydroisoquino[3,2-*b*]-3-benzazocin derivatives following a reaction sequence that was first investigated with a 6-methylpyrazinoisoquinoline-1,4-dione.

2. Results and discussion

When 3-arylmethylpiperazine-2,5-dioxosilyllactims, where Ar=2,5-dimethoxyphenyl, 2,3,5-trimethoxy-4-methyl,[14] or 2-methoxymethoxy-3-methyl-4,5-methylenedioxyphenyl,[23] were treated with *N*-phthalimidoacetaldehyde dimethyl acetal in the previously reported conditions (method A, Scheme 2), we obtained very low yields of *N*-alkylation products (see compounds **3a** and **3b**). Reactions at room temperature with 1 equiv of TMSOTf gave similar yields (method B). To corroborate that these disappointing results were due to the acetal reactivity and not to the substitution pattern of the arene ring, we performed the alkylation of the *O*-trimethylsilyllactim derived from 1-acetyl-3(2-methoxy-3-methyl-4,5-methylenedioxibenzyl)-piperazine-2-5-dione with acetaldehyde dimethyl acetal and, as expected, we obtained **3c** in good yield.

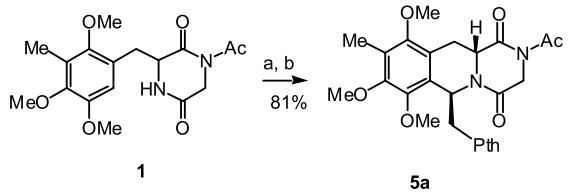


Comp.	Ar	R^2	Method	Yield (%)
3a		$PthNCH_2$	**A**	7
			B	13
3b		$PthNCH_2$	**A**	15
3c		CH_3	**A**	60

Method A, (a): TMSOTf, ^{i}Pr$_2$NEt, CH_2Cl_2, -78 °C, 4 h; (b): $R^2CH(OCH_3)_2$ (**2**), TMSOTf (cat), -78 °C, 4h.
Method B, (a): TMSCl, Et_3N, CH_2Cl_2, rt, 4 h; (b): $R^2CH(OCH_3)_2$ (**2**), TMSOTf (1 eq), rt, 14h.

Scheme 2.

In order to find a better method to obtain 6-phthalimidomethylpyrazinoisoquinolinediones, we planned a one-pot procedure, in which conveniently activated compounds **3** could be trapped in situ by the arene ring. We were delighted to find that treatment at room temperature of the *O*-trimethylsilyllactim derived from **1** (Ar=2,4,5-trimethoxy-3-methylphenyl) with excess of TMSOTf gave the desired tricyclic product in very good yield. The major isomer was compound **5a** that, as expected, showed a *trans*-relationship between H-6 and H-11a protons (Scheme 3).

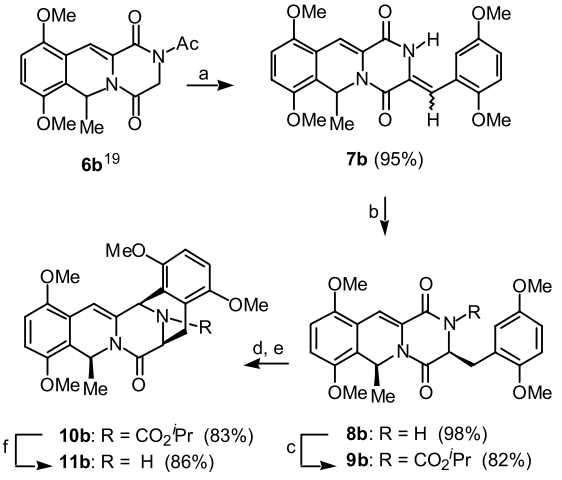


Scheme 3. Reagents and conditions. (a) TMSCl, Et_3N, CH_2Cl_2, rt, 2 h; (b) $PhtCH_2CH(OCH_3)_2$, TMSOTf (5 equiv), rt, 12 h.

Transformation of **5a** into the desired pentacycle was first assayed with the analog **5b** (Scheme 1, R^1=7,10(OMe)$_2$; R^2=Me). The planned protocol began by an aldol-type condensation with aromatic aldehydes followed by catalytic hydrogenation and subsequent reduction/cyclization. In the first step we obtained mixtures of stereoisomers due to the lability of the H-11a proton, a fact that is common in related dipeptide anhydrides. In order to circumvent this problem, we studied this protocol with the 11,11a-dehydroderivative **6b**, that we had previously used in the synthesis of tricyclic compounds with a 6,11a-*cis*-relationship.[21,22] Aldol-type condensation between **6b** and 2,4-dimethoxybenzaldehyde in the presence of potassium *tert*-butoxide gave **7b** as a mixture of *E*-*Z* diastereoisomers with simultaneous loss of the *N*-acetyl group. The exocyclic double bond was then chemo- and diastereoselectively hydrogenated to give **8b** as a single diastereoisomer. Because of the asymmetric induction of the C(6)-substituent, we expected in **8b** a *cis*-stereochemistry between H-3 and H-6 protons but, probably due to its planarity, this relationship could not be supported by conclusive NOE experiments. Following a reaction sequence described for other arylmethylpiperazine-2,5-diones,[24–26] we next enhanced the electrophilicity of the C(1)-carbonyl group by acylation of its vicinal NH group. Thus, we obtained **9b**, in which assignment of signals required low temperature NMR spectra to minimize the possible rotamers around the carbamate group.

Chemoselective reduction and subsequent treatment with formic acid of **9b** gave the pentacyclic compound **10b**, and the imino group was later restored by acid hydrolysis to give **11b**. This compound showed an all-*cis* relative stereochemistry that was corroborated by NOE experiments (Scheme 4). The closest precedent of this reduction/cyclization sequence in pyrazinoisoquinoline-1,4-diones is from Liu and coworkers,[27] that used L-DOPA and L-tyrosine as starting materials to obtain enantiopure analogs of compounds **5**. In this precedent, the feasibility to introduce an additional stereocenter on the 6-position of compounds **5** was claimed, but these authors have not yet described the application of its protocol to any 6-substituted compound. In our hands, the reduction/cyclization of 3-arylmethyl derivatives of compounds **5** ($R^2 \neq H$) was not a totally stereocontrolled process.

When this protocol was applied to the phthalimidomethyl unsaturated compound **6a**, we obtained its 3-arylmethylene derivative **7a**, also as a mixture of *E*/*Z* diastereoisomers. Probably because of the steric hindrance imposed by the *N*-phthalimidomethyl substituent, hydrogenation of its exocyclic double bond required stronger conditions than those used for **7b** (Scheme 5). Once established the *cis*-relationship between H-3 and H-6 protons in the product **8a** by NOE experiments (Fig. 2), it was derived to **9a** whose NMR spectra could only be studied when registered at low temperature. Treatment with lithium tri*ert*butoxyaluminium hydride and formic acid in the same reaction conditions used for **9b**, gave the expected pentacyclic product **10a** in a near equimolecular mixture with other product (**12a**), both of them as single diastereoisomers. Compound **10a** was chemoselectively obtained with less



Scheme 4. Reagents and conditions: (a) ArCHO, dry DCM, K*t*BuO/*t*BuOH, rt, 12 h; (b) Pd–C, MeOH, H_2, 1 bar, 16 h; (c) Et_3N, 4-DMAP, $ClCO_2iPr$, rt, 48 h. (d) Li(*t*BuO)$_3$AlH (3 equiv), THF, rt, 5 h; (e) HCO_2H, 80 °C, 30 min; (f) F_3CCO_2H/H_2SO_4, rt, 24 h.

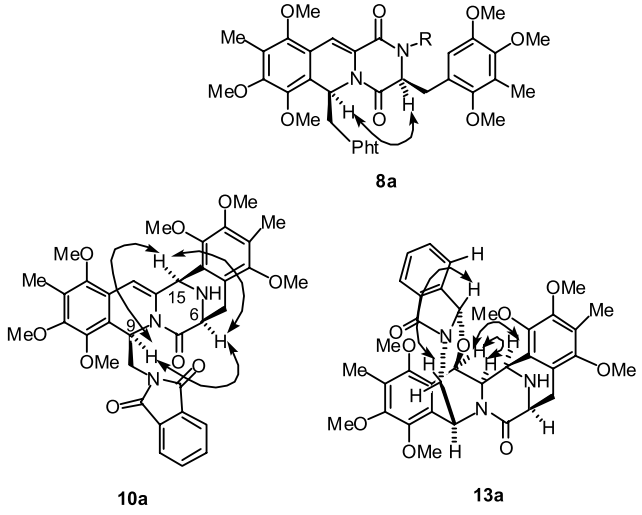
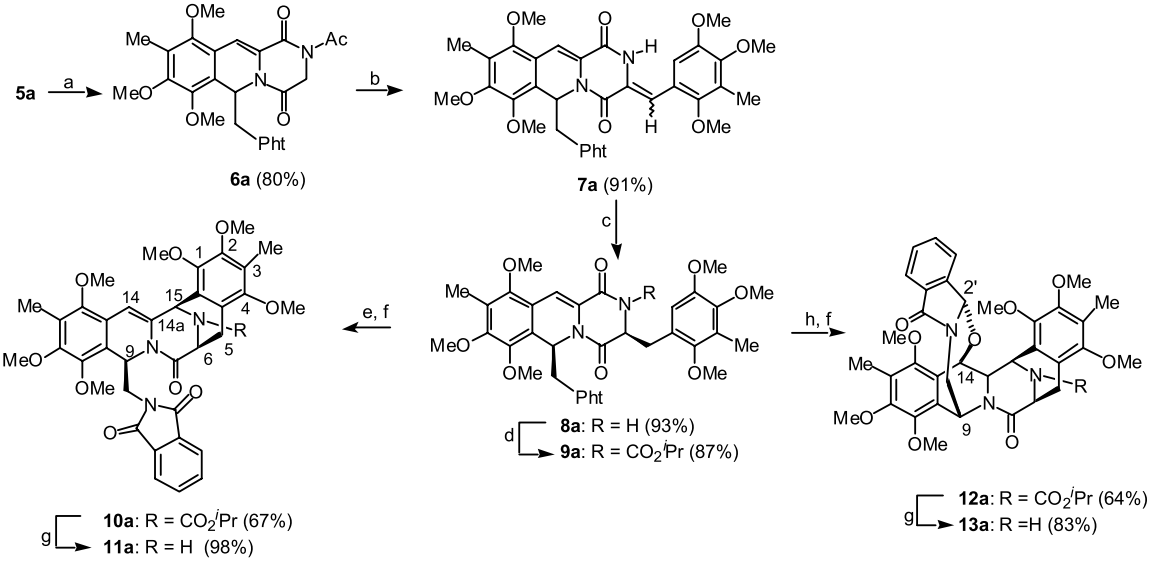
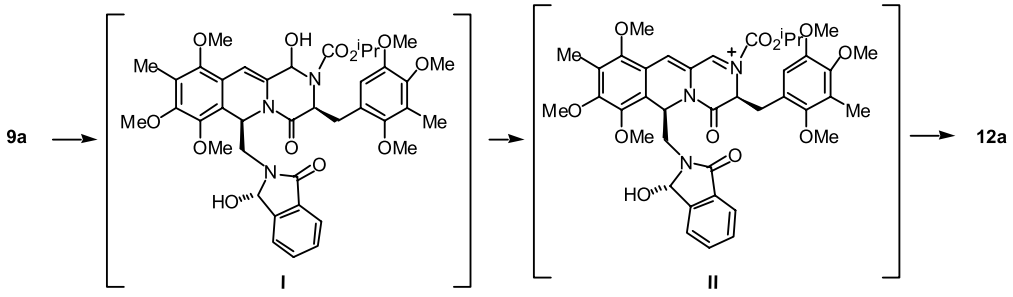


Figure 2. NOE and NOESY of compounds **8a**, **10a** and **13a**.



Scheme 5. Reagents and conditions: (a) AIBN, NBS, CCl_4, reflux, 14 h; (b) ArCHO, dry DCM, K*t*BuO/*t*BuOH, rt, 12 h; (c) Pd–C, MeOH, H_2, 3.5 bar, 30 h; (d) Et_3N, 4-DMAP, $ClCO_2iPr$, rt, 48 h. (e) Li(*t*BuO)$_3$AlH (3 equiv), THF, rt, 5 h; (f) conc. HCO_2H, 80 °C, 30 min; (g) F_3CCO_2H/H_2SO_4, rt, 24 h; (h) Li(*t*BuO)$_3$AlH (10 equiv), THF, rt, 20 h.



Scheme 6.

excess of reductive agent and shorter reaction times than those used to obtain the above mentioned mixture, while compound **12a** was the only reaction product using greater excess of reductive agent and longer reaction times. NOE experiments on **10a** showed the *cis*-relationship between its H-6, H-9 and H-15 protons. A subsequent hydrolysis of **10a** and **12a** gave the NH derivatives **11a** and **13a**. The ^{1}H NMR spectrum of **13a** showed the absence of the vinylic proton, as well as the presence of two CH–CH_2 portions and other four CH signals. Structural analysis through COSY, HMQC and HMBC NMR experiments was in accordance to the given structure, and NOESY spectra corroborated the *cis*-relationship between H-14, H-14a and H-15 protons, as well as the *exo*-position of the 'hemiaminal' proton H-2′ (Fig. 2).

The octacyclic compound **12a** must be formed from **9a** through hydrogenation of the activated C(1)=O group and one carbonyl of the phthalimide moiety. Apparently, the hydride attack to the later mentioned carbonyl group is diastereoselective to give intermediate **I**, because in the formation of **12a** we did not find any other reduced product. This dihydroxy intermediate may give in acid the acyliminium ion **II** that suffers the conjugate nucleophilic attack of the hemiaminal hydroxy group and is later captured by the arene ring of the arylmethyl side-chain. Alternatively, **II** may give first the pentacycle and, subsequently, the phthalimide hydroxy group would attack the benzyl cation formed in the protonation of the double bond (Scheme 6).

In conclusion, we have developed a short and versatile route to the unsaturated pentacyclic compound **11a**, from which we expect to obtain other phtalascidin analogues that contain the structural requirements of related antitumor agents. Additionally, a double reduction/cyclization sequence affords the new octacyclic compound **13a**. Our protocol differs from others that also used compounds **1** as starting materials in the synthesis of analog pentacyclic compounds in that we generate ring *B* in the first step, instead of on a rather elaborated system already containing rings *C–E*. The main advantage respect to similar approaches is that the use of 11,11a-dehydro compounds circumvents the stereochemical problems related to the lability of the 11a-stereocenter.

3. Experimental

All reagents were of commercial quality (Aldrich, Fluka, SDS, Probus) and were used as received. Solvents (SDS, Scharlau) were dried and purified using standard techniques. Reactions were monitored by thin layer chromatography, on aluminium plates coated with silica gel or aluminium oxide with fluorescent indicator (Merck 60 F_{254}). Separations by flash chromatography were performed on silica gel (Merck 60, 230–400 mesh) or aluminium oxide (Merck 90, 70–230 mesh). Melting points were uncorrected and were determined either using recrystallized samples or samples which crystallized during concentration of the chromatography eluents. Infrared spectra were recorded with solid compounds compressed into KBr pellets or as films over NaCl in the case of oils. NMR spectra were obtained in $CDCl_3$ at 250 MHz for ^{1}H and at 63 MHz for ^{13}C (Servicio de Resonancia Magnética Nuclear, Universidad Complutense). When necessary, assignments were aided by DEPT, COSY, NOESY and ^{13}C–^{1}H HMBC and HMQC correlation experiments. Elemental analyses were determined by the Servicio de Microanálisis Elemental, Universidad Complutense.

3.1. *N*-Alkylation of compounds 1 with acetals

Method A. A mixture of $^{i}Pr_2NEt$ (2.11 mmol) and TMSOTf (2.30 mmol) was added at −78 °C to a stirred solution of the corresponding compound **1** (1.92 mmol) in dry DCM (15 mL). The mixture was stirred under argon atmosphere for 4 h and then, a solution of dimethyl *N*-phthalimidoacetaldehyde acetal (2.3 mmol) in 10 mL of dry DCM was added and this solution was stirred for 4 h at −78 °C. Addition of a 10% $NaHCO_3$ aqueous solution (10 mL) was followed by extraction with DCM (30 mL× 3), and the extracts were washed with water (20 mL) and a saturated aqueous solution of NaCl (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The corresponding compounds **3** were purified by flash chromatography on silica gel with hexane/ethyl acetate as eluant. *Method B*. A mixture of triethylamine (0.40 mmol) and TMSCl (0.40 mmol) was added to a solution of the corresponding compound **1** (0.38 mmol) in dry DCM (4 mL). The mixture was stirred under argon atmosphere at room temperature for 4 h and then a solution of dimethyl *N*-phthalimidoacetaldehyde acetal (0.4 mmol) and TMSOTf in dry DCM (10 mL) was added. The solution was stirred under argon atmosphere for 14 h at room temperature and then a 10% aqueous solution of $NaHCO_3$ (3 mL) was added. After extraction with DCM (10 mL×3), the extracts were washed with water (20 mL) and a saturated aqueous solution of NaCl (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The corresponding compounds **3** were purified as above.

3.1.1. 1-Acetyl-3-(2,5-dimethoxybenzyl)-4-(1-methoxy-2-phthalimidoethyl)piperazine-2,5-dione (3a). The reaction was performed with 26 mg (0.05 mmol) of 1-acetyl-3-(2,5-dimethoxybenzyl)-piperazine-2,5-dione. Compound **3a** (1.6 mg, 7% yield) was obtained as a (1:1) mixture of diastereoisomers after flash chromatography with hexane/ethyl acetate (6:4) as eluant in as a yellow oil: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.90–7.70 (m, 4H), 6.80–6.60 (m, 3H), 5.85 (m, 1H), 4.86 (d, 0.5 H, J=17.9 Hz), 4.59 (d, 0.5H, J=17.6 Hz), 4.55 (m, 1H), 3.95 (m, 2H), 3.84 (s, 1.5H), 3.72 (s, 3H), 3.76 (d, 0.5H, J=17.9 Hz), 3.67 (s, 1.5H), 3.56 (d, 0.5H, J=17.6 Hz), 3.56 (s, 1.5H), 3.34 (m, 1H), 3.20 (m, 1H), 3.15 (s, 1.5H), 2.50 (s, 1.5H), 2.47 (s, 1.5H). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.6, 171.3, 167.7, 167.5, 167.4, 166.3, 166.0, 153.5, 153.4, 151.8, 151.8, 134.2, 134.1; 131.8, 124.0, 123.6, 123.4, 123.3, 117.3, 117.1, 113.4, 113.2, 111.1, 110.9, 82.5, 81.9, 58.2, 58.1, 56.4, 56.0, 55.6, 55.6, 55.5, 55.3, 46.0, 45.1, 39.1, 38.0, 34.4, 34.3, 26.8, 26.5. Anal. Calcd for, $C_{26}H_{27}N_3O_8$: C, 61.29; H, 5.34; N, 8.25. Found: C, 59.90; H, 5.16; N, 7.95.

3.1.2. 1-Acetyl-3-(2-methoxymethoxy-3-methyl-4,5-methylenedioxybenzyl)-4-(1-methoxy-2-phthalimidoethyl)piperazine-2,5-dione (3b). The reaction was performed with 172 mg (0.30 mmol) of 1-acetyl-3-(2-methoxymethoxy-3-methyl-4,5-methylenedioxy benzyl)-4-(1-methoxy-2-phthalimidoethyl)piperazine-2,5-dione. Compound **3b** (19 mg, 15% yield) was obtained as a (1:1) mixture of diastereomers after flash chromatography with hexane/ethyl acetate (1:1) as eluant as a yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.90–7.70 (m, 4H), 6.52 (s, 0.5H), 6.51 (s, 0.5H), 6.00 (s, 1H), 5.94 (s, 1H), 5.85 (m, 2H), 4.91 (m, 1H), 4.88 (d, 1H, J=13.6 Hz), 4.81 (d, 1H, J=13.8 Hz), 4.61 (d, 1H, J=18.0 Hz), 4.49 (m, 1H), 3.95 (dd, 2H, J=12.4, 4.6 Hz), 3.64 (d, 1H, J=18.0 Hz), 3.57 (s, 1.5H), 3.52 (s, 1.5H), 3.51 (s, 1.5H), 3.18 (s, 1.5H), 2.52 (s, 1.5H), 2.47 (s, 1.5H), 2.14 (s, 1.5H), 2.11 (s, 1.5H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.8, 171.5, 168.0, 167.7, 167.4, 167.1, 166.3, 166.2, 150.0, 149.9, 146.7, 146.5, 143.7, 143.6, 134.4, 134.3, 134.2, 134.0, 132.1, 132.0, 131.6, 123.7, 123.6, 123.5, 123.4, 120.4, 120.2, 113.9, 113.7, 107.2, 107.1, 101.4, 101.4, 100.0, 82.7, 82.4, 58.8, 58.2, 57.8, 57.7, 56.8, 56.7, 53.2, 46.4, 45.6, 40.2, 38.8, 38.1, 34.8, 34.5, 30.1, 29.7, 26.7, 26.6, 10.0. Anal. Calcd for, $C_{28}H_{29}N_3O_{10}$: C, 59.26; H, 5.15; N, 7.40. Found: C, 59.03; H, 4.98; N, 7.23.

3.1.3. 1-Acetyl-3-(2-methoxymethoxy-3-methyl-4,5-methylenedioxybenzyl)-4-(1-methoxyethyl) piperazine-2,5-dione (3c). The reaction was performed with 487 mg (1.15 mmol) of 1-acetyl-3-(2-methoxymethoxy-3-methyl-4,5-methylenedioxybenzyl)-4-(1-methoxy-2-phthalimido ethyl)piperazine-2,5-dione. Compound **3c** (391.2 mg, 60% yield). It was obtained as a (1:1) mixture of diastereomers after flash chromatography with hexane/ethyl acetate (1:1) as eluant as a white solid. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 6.47 ?s, 1H), 6.39 (s, 1H), 5.88 (s, 4H), 5.71 (q, 1H, J=6.1 Hz), 5.64 (d, 1H, J=6.2 Hz), 4.79 (m, 4H), 4.73 (d, 1H, J=18.1 Hz), 4.67 (d, 1H, J=18.0 Hz), 4.30 (dd, 1H, J=8.7, 5.5 Hz), 4.25 (dd, 1H, J=9.6, 5.6 Hz), 3.84 (d, 1H, J=18.1 Hz), 3.57 (d, 1H, J=18.0 Hz), 3.46 (s, 3H), 3.45 (s, 3H), 3.40 (s, 3H), 3.10 (s, 3H), 3.08 (m, 4H), 2.44 (s, 3H), 2.39 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.37 (d, 1H, J=6.8 Hz), 1.35 (d, 1H, J=6.3 Hz). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.3, 167.5, 167.1, 165.6, 165.3, 150.7, 150.6, 146.4, 146.2, 143.4, 143.3, 120.1, 119.8, 113.6, 113.4, 107.0, 106.8, 101.3, 101.2, 99.8, 81.3, 81.1, 58.2, 57.6, 57.5, 56.7, 56.4, 55.6, 46.2, 45.9, 34.4, 26.6, 26.5, 20.0, 18.5, 9.8, 9.7. Anal. Calcd for, $C_{20}H_{26}N_2O_8$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.88; H, 5.98; N, 6.49.

3.1.4. One-pot synthesis of (6*S,11a*R**)-2-acetyl-7,8,10-trimethoxy-9-methyl-6-phthalimidomethyl-2,3,11,11a-tetrahydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (5a).** A mixture of TMSCl (0.22 mL, 1.73 mmol) and triethylamine (0.24 mL, 1.73 mmol) was added to a stirred solution of the corresponding **1** (552 mg, 1.58 mmol) in dry DCM (10 mL). The mixture was stirred under argon atmosphere at room temperature for 2 h and a solution of diethyl *N*-phthalimidoacetaldehyde acetal (455.5 mg, 1.73 mmol) in dry DCM (6 mL) and TMSOTf (1.43 mL, 7.9 mmol) was added. This mixture was stirred for other 12 h at room temperature and then, a 10% aqueous solution of $NaHCO_3$ (10 mL) was added. After extraction with DCM (30 mL×3), the combined extracts were washed with H_2O (20 mL) and a saturated aqueous solution of NaCl (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo, to give a residue that was purified by flash chromatography on silica gel with hexane/ethyl acetate (7:3) as solvents to give **5a** (670 mg, 81%) as an off-white solid: mp 166–168 °C; IR (NaCl) ν_{max} 2942, 1772, 1715, 1682 and 1394 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.83 (m, 2H), 7.72 (m, 2H), 6.04 (dd, 1H, J=10.9, 3.5 Hz), 4.83 (dd, 1H, J=9.1, 5.6 Hz), 4.30 (d, 1H, J=18.0 Hz), 4.26 (dd, 1H, J=14.1, 3.6 Hz), 4.08 (d, 1H, J=18.0 Hz), 4.04 (s, 3H), 3.92 (1H, dd, J=14.1, 10.9 Hz), 3.89 (s, 3H), 3.75 (s, 3H), 3.42 (dd, 1H, J=16.6, 5.6 Hz), 3.22 (dd, 1H, J=16.6, 9.1 Hz), 2.25 (s, 3H), 2.22 (s, 3H). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 171.8, 168.5, 168.0, 163.5, 152.3, 150.7, 146.2, 134.2, 131.9, 125.8, 123.8, 123.4, 121.2, 60.7, 60.3, 60.1, 53.8, 48.3, 45.6, 38.8, 27.2, 25.6, 9.5. Anal. Calcd for $C_{27}H_{27}N_3O_8$: C, 62.18; H, 5.22; N, 8.06. Found: C, 61.96; H, 5.21; N, 7.95.

3.1.5. Bromination/dehydrohalogenation of 5a. Synthesis of 2-acetyl-7,8,10-trimethoxy-9-methyl-6-phthalimidomethyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (6a). A solution of **5a** (2.995 g, 5.75 mmol), AIBN (94.4 mg, 0.57 mmol) and NBS (1.023 g, 5,75 mmol) in CCl_4 (250 mL) was refluxed under an argon atmosphere for 14 h. The unreacted NBS was filtered from the cooled reaction, the solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography on silica gel with hexane/ethyl acetate (7:3) as solvents to give **6a** (2.385 g, 80%) as a yellow solid: mp 227–228 °C, IR (NaCl) ν_{max} 2942, 1772, 1704 and 1616 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.90–7.60 (m, 4H), 7.44 (s, 1H), 6.37 (dd, 1H, J=8.5, 3.4 Hz), 4.86 (d, 1H, J=17.4 Hz), 4.04 (s, 3H), 3.93 (dd, 1H, J=13.3, 8.5 Hz), 3.87 (s, 3H), 3.78 (d, 1H, J=17.4 Hz), 3.76 (dd, 1H, J=13.3, 3.4 Hz), 3.70 (s, 3H), 2.75 (s, 3H), 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 170.9, 167.2, 161.9, 160.1, 153.2, 151.3, 145.1, 133.3, 130.6, 124.8, 123.6, 122.2, 121.3, 117.3, 113.5, 61.2, 59.8, 59.2, 46.7, 43.6, 38.4, 23.4, 8.4. Anal. Calcd for $C_{27}H_{25}N_3O_8$: C, 62.42; H, 4.85; N, 8.09. Found: C, 62.26; H, 4.78; N, 7.98.

3.2. Condensation of compounds 6 with aromatic aldehydes (General procedure)

To a solution of **6** (0.18 mmol) and the corresponding aromatic aldehyde (0.23 mmol) in dry DCM (2 mL), under an argon atmosphere, was added K^{t}BuO (0.27 mmol) in ^{t}BuOH. The mixture was stirred at room temperature for 12 h and then, a saturated aqueous solution of NH_4Cl (5 mL) was added. The mixture was extracted with DCM (50 mL×3), the extracts were washed with H_2O (30 mL) and a saturated aqueous solution of NaCl (30 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give, after column chromatography, compounds **7**.

3.2.1. 7,8,10-Trimethoxy-9-methyl-3-(3-methyl-2,4,5-trimethoxybenzylidene)-6-phthalimidomethyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (7a). The reaction was performed with 3.818 g (7.3 mmol) of **6a**. Compound **7a** (4.44 g, 91% yield) was obtained as a 1/1 mixture of diastereoisomers after flash chromatography on silica gel with hexane/ethyl acetate (3:7) as solvents. The *Z*-isomer was obtained by further chromatographic purification using dichloromethane/ethyl acetate (9:1) as eluant as an off-yellow solid: mp 121–122 °C, IR (NaCl) ν_{max} 3240, 2939, 2835, 1682, 1627 and 1489 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.42 (s, 1H), 7.73 (m, 2H), 7.66 (m, 2H), 7.15 (s, 1H), 6.77 (s, 1H), 6.58 (m, 1H), 6.56 (s, 1H), 4.02 (s, 3H), 3.99 (m, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.66 (s, 3H), 3.62 (s, 3H), 2.20 (s, 3H), 2.17 (s, 3H). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 168.4, 158.0, 156.7, 153.9, 152.1, 150.0, 149.7, 149.2, 146.5, 134.3, 132.3, 127.0, 126.8, 126.7, 123.5, 122.1, 121.6, 119.0, 113.9, 112.3, 110.7, 62.5, 61.3, 61.2, 60.8, 60.5, 56.3, 49.3, 40.6, 9.9; 9.8. Anal. Calcd for $C_{36}H_{35}N_3O_{10}$: C, 64.57; H, 5.27; N, 6.27. Found: C, 64.84; H, 5.11; N, 6.47. The *E*-isomer was obtained as an off-yellow solid by using dichloromethane/ethyl acetate (8:2) as eluant: mp 264–266 °C, IR (NaCl) ν_{max} 3294, 2938, 1750, 1716 and 1694 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.76 (m, 2H), 7.66 (m, 2H), 7.18 (s, 1H), 6.53 (s, 1H), 6.37 (dd, 1H, J=7.4, 3.8 Hz), 6.28 (d, 1H, J=2.7 Hz), 5.85 (d, 1H, J=2.7 Hz), 4.02 (s, 3H), 3.89 (dd, 1H, J=17.4, 7.4 Hz), 3.81 (s, 3H), 3.80 (s, 3H), 3.73 (s, 3H), 3.69 (s, 3H), 3.67 (s, 3H), 3.65 (dd, 1H, J=17.4, 3.8 Hz), 2.19 (s, 3H), 2.14 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.0, 168.1, 163.0, 160.7, 151.7, 149.6, 149.4, 146.2, 134.0, 132.0, 126.4, 126.2, 123.3, 121.6, 118.5, 109.8, 107.5, 71.8, 62.1, 61.0, 60.7, 60.3, 60.2, 59.8, 56.2, 48.3, 20.6, 9.6, 9.5. Anal. Calcd for $C_{36}H_{35}N_3O_{10}$: C, 64.57; H, 5.27; N, 6.27. Found: C, 64.81; H, 5.57; N, 5.95

3.2.2. 7,10-Dimethoxy-3-(2,5-dimethoxybenzylidene)-6-methyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (7b). The reaction was performed with 176 mg (0.88 mmol) of **6b**. The mixture of *E* and *Z* diastereoisomers of compound **7b** (364.5 mg, 95% yield) was obtained after flash chromatography on silica gel with hexane/ethyl acetate (1:1) as eluant: ^{1}H NMR ($CDCl_3$, 250 MHz) δ (*Z* isomer): 8.79 (s, 1H), 7.43 (s, 1H), 6.98 (s, 1H), 6.91 (m, 2H), 6.83 (m, 2H), 6.75 (s, 1H), 6.30 (q, 1H, J=6.5 Hz), 3.89 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 1.34 (d, 3H, J=6.5 Hz). (*E* isomer): 9.03 (s, 1H), 7.40 (s, 1H), 7.18 (d, 1H, J=2.6 Hz), 6.99 (s, 1H), 6.84 (s, 1H), 6.80 (m, 1H), 6.70 (d, 1H, J=2.5 Hz), 6.54 (s, 1H), 6.21 (q, 1H, J=6.5 Hz), 3.81 (s, 3H), 3.78 (s, 3H), 3.77 (s, 6H), 1.22 (d, 3H, J=6.5 Hz). ^{13}C NMR ($CDCl_3$, 63 MHz) (*E*+*Z*). δ 157.1, 156.7, 154.4, 153.1, 151.9, 150.7, 150.2, 149.1, 126.3, 125.9, 125.4, 124.4, 124.2, 123.2, 117.8, 117.3, 116.6, 115.9, 114.9, 113.9, 113.5, 112.8, 112.7, 111.3, 111.2, 110.6, 110.2, 57.1, 56.3, 56.2, 55.1, 45.8, 45.4, 19.8, 19.4. Anal. Calcd for $C_{24}H_{24}N_2O_6$: C, 66.04; H, 5.54; N, 6.42. Found: C, 65.90; H, 5.77; N, 6.72.

3.2.3. 7,8,10-Trimethoxy-9-methyl-3-(3-methyl-2,4,5-trimethoxybenzyl)-6-phthalimidomethyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (8a). The diastereomeric mixture of compounds **7a** (773 mg, 0.56 mmol) was dissolved in methanol (50 mL) containing 50% palladium on-carbon (180 mg) and was vigorously stirred under 3.5 bar of hydrogen for 30 h. The catalyst was removed by filtration through celite, and the solvent was evaporated to give compound **8a**. This compound was purified by recrystallization in MeOH as an off-white solid (350 mg, 93%): mp 189–190 °C, IR (NaCl) ν_{max} 3304, 2944, 1775, 1716 and 1688 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (m, 2H); 7.67 (m, 2H), 7.15 (s, 1H), 6.53 (s, 1H), 6.42 (dd, 1H, J=7.2, 4.6 Hz), 6.18 (ws, 1H), 4.25 (dd, 1H, J=10.4, 3.8 Hz), 3.88 (s, 3H), 3.82 (m, 2H), 3.79 (s, 3H), 3.76 (s, 3H), 3.71 (s, 3H), 3.68 (s, 3H), 3.62 (s, 3H), 3.31 (dd, 1H, J=14.0, 3.8 Hz), 2.69 (dd, 1H, J=14.0, 10.4 Hz), 2.18 (s, 3H), 2.15 (s, 3H).^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.8, 165.1, 160.1, 153.1, 151.6, 150.9, 149.5, 147.3, 145.9, 133.8, 132.0, 126.2, 126.1, 123.7, 123.0, 121.6, 118.5, 111.3, 110.1, 61.9, 60.7, 60.5, 60.3, 60.1, 55.9, 55.0, 47.7, 39.6, 33.2, 9.6, 9.2. Anal. Calcd for $C_{36}H_{37}N_3O_{10}$: C, 64.37; H, 5.55; N, 6.26. Found: C, 64.04; H, 5.56; N, 6.24.

3.2.4. 7,10-Dimethoxy-3-(2,5-dimethoxybenzyl)-6-methyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*] isoquinoline-1,4-dione (8b). The diastereomeric mixture of compounds **7b** (1.3 g, 3.0 mmol) was dissolved in methanol (150 mL) containing 10% palladium on-carbon (130 mg) and was vigorously stirred under 1 bar of hydrogen for 16 h. The catalyst was removed by filtration through celite, and the solvent was evaporated to give compound **8b** that was purified by recrystallization in MeOH to give an off-yellow solid (1.287 g, 98%): mp 88–89 °C, IR (NaCl) ν_{max} 3250, 2926, 1684, 1628 and 1490 cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.34 (s, 1H), 6.82–6.77 (m, 4H), 6.70 (d, 1H, J=9.0 Hz), 6.17 (q, 1H, J=6.6 Hz), 4.40 (dd, 1H, J=8.5, 3.9 Hz), 3.82 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.76 (s, 3H), 3.62 (dd, 1H, J=13.8, 3.9 Hz), 2.98 (dd, 1H, J=13.8, 8.5 Hz), 1.21 (d, 3H, J=6.6 Hz). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 164.2, 160.8, 157.7, 151.7, 149.8, 148.7, 125.6, 125.0, 124.7, 118.5, 117.6, 113.1, 112.0, 111.6, 110.1, 109.7, 55.9, 55.8, 55.7, 55.6, 54.4, 45.1, 32.8, 19.2. Anal. Calcd for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.48; H, 6.21; N, 6.17.

3.3. *N*-Acylation of compounds 8

A solution of the corresponding compound **8** (0.32 mmol), triethylamine (0.98 mmol) and 4-dimethylaminopyridine (0.98 mmol) in dry DCM (10 mL) was cooled in ice water, and isopropyl chloroformiate (1.92 mmol) was added dropwise. The solution was stirred under argon atmosphere

for 48 h at room temperature and then, an aqueous solution of NH_4Cl (10 mL) was added. After extraction with ethyl acetate (20 mL×3), the extracts were washed with H_2O (30 mL) and a saturated aqueous solution of NaCl (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give compounds **9**.

3.3.1. 2-Isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-3-(3-methyl-2,4,5-trimethoxybenzyl)-6-phthalimidomethyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (9a). The reaction was performed with 600 mg (0.90 mmol) of **8a**. Compound **9a** was purified by flash chromatography on silica gel with hexane/ethyl acetate (1:1) as eluant to give a yellow oil (593 mg, 87% yield): IR (NaCl) ν_{max} 2932, 1773, 1722 and 1682 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (m, 2H), 7.66 (m, 2H), 7.08 (s, 1H), 6.73 (s, 1H), 6.38 (t, 1H, J=5.1 Hz), 5.27 (dd, 1H, J=7.6, 5.5 Hz), 4.88 (sept, 1H, J=6.3 Hz), 3.90 (m, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.76 (s, 3H), 3.73 (s, 3H), 3.54 (s, 3H), 3.54 (dd, 1H, J=13.7, 5.5 Hz), 3.50 (s, 3H), 3.34 (dd, 1H, J=13.7, 7.6 Hz), 2.21 (s, 3H), 2.11 (s, 3H), 1.23 (d, 3H, J=6.3 Hz), 1.15 (d, 3H, J=6.3 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 167.6, 163.5, 157.8, 153.3, 152.1, 151.6, 149.0, 147.4, 146.3, 133.9, 132.1, 126.7, 126.3, 125.6, 123.7, 123.1, 120.1, 119.0, 112.5, 110.8, 71.8, 62.1, 60.8, 60.6, 60.2, 59.7, 59.5, 56.1, 48.4, 39.9, 36.2, 21.7, 21.5, 9.8, 9.3. Anal. Calcd for $C_{40}H_{43}N_3O_{12}$: C, 63.40; H, 5.72; N, 5.55. Found: C, 63.51; H, 5.69; N, 5.38.

3.3.2. 2-Isopropyloxycarbonyl-7,10-dimethoxy-3-(2,5-dimethoxybenzyl)-6-methyl-2,3-dihydro-6*H*-pyrazino-[1,2-*b*]isoquinoline-1,4-dione (9b). The reaction was performed with 720 mg (1.70 mmol) of **8b**. Compound **9b** was purified by flash chromatography on silica gel with hexane/ethyl acetate (1:1) as eluant to give an off-yellow solid (708 mg, 82% yield): mp 145 °C, IR (NaCl) ν_{max} 2938, 2831, 1770, 1721, 1678 and 1488 cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ 7.20 (s, 1H), 6.74 (d, 1H, J=9.0 Hz), 6.71 (s, 3H), 6.65 (d, 1H, J=9.0 Hz), 6.00 (q, 1H, J=6.4 Hz), 5.18 (t, 1H, J=4.8 Hz), 5.07 (sept, 1H, J=6.3 Hz), 3.77 (s, 3H), 3.75 (s, 6H), 3.67 (s, 3H), 3.67 (dd, 1H, J=13.7, 4.9 Hz), 3.11 (dd, 1H, J=13.7, 4.9 Hz), 1.34 (d, 3H, J=6.3 Hz), 1.30 (d, 3H, J=6.3 Hz), 1.13 (d, 3H, J=6.4 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 162.8, 157.3, 152.8, 152.1, 151.5, 149.5, 148.4, 125.7, 123.5, 118.5, 113.0, 112.2, 110.7, 110.4, 109.7, 71.2, 68.3, 59.2, 55.8, 55.6, 55.4, 46.5, 34.8, 21.9, 21.6, 21.5, 19.6. Anal. Calcd for $C_{28}H_{32}N_2O_8$: C, 64.11; H, 6.15; N, 5.34. Found: C, 63.98; H, 6.20; N, 5.15.

3.4. Reduction/cyclization of compounds 9. Synthesis of 10a and 10b

A solution of the corresponding compound **9** (0.13 mmol) in 3 mL of dry THF was added over a solution of lithium tri*tert*-butoxyaluminium hydride (101 mg, 0.40 mmol) in 4 mL of dry THF cooled in ice water. After stirring at room temperature for 5 h, the reaction mixture was quenched by addition of ice and extracted with ethyl acetate. The extracts were washed with H_2O and a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the amino alcohol intermediate as a diastereomeric mixture. A solution of this mixture in 4 mL of formic acid was heated for 30 min at 80 °C and then, the reaction was quenched by addition over a 10% aqueous solution of $NaHCO_3$ and extraction with ethyl acetate. The extracts were washed with H_2O and a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo, to give the crude products **10**.

3.4.1. Isopropyl 1,2,4,10,11,13-hexamethoxy-3,12-dimethyl-7-oxo-9-phthalimidomethyl-(6*S,9*R**,15*S**)-5,6,9,15-tetrahydro-6,15-iminoisoquino[3,2-*b*]-3-benzazocine-16-carboxylate (10a).** The reaction was performed with 100 mg (0.13 mmol) of compound **9a**. The crude product **10a** was purified by flash chromatography on silica gel with hexane/ethyl acetate (1:1) as eluant to give an off-white solid (65 mg, 67%): mp 94–96 °C, IR (NaCl) ν_{max} 2939, 1776, 1722 and 1651 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz, 5 °C) (mixture of rotamers) δ 7.63 (m, 4H), 6.46 (s, 0.5H), 6.38 (s, 0.5H), 6.27 (t, 1H, J=6.7 Hz), 6.18 (s, 0.5H), 6.04 (s, 0.5H), 5.21 (d, 0.5H, J=6.1 Hz), 5.11 (d, 0.5H, J=6.2 Hz), 4.94 (m, 0.5H), 4.93 (m, 0.5H), 4.01 (s, 1.5H), 3.96 (s, 1.5H), 3.86 (s, 1.5H), 3.82 (s, 1.5H), 3.77 (s, 1.5H), 3.76 (s, 3H), 3.72 (s, 1.5H), 3.66 (s, 1.5H), 3.65 (s, 1.5H), 3.40 (m, 2H), 3.29 (s, 1.5H), 3.24 (s, 1.5H), 3.12 (d, 1H, J=16.6 Hz), 3.07 (dd, 0.5H, J=16.7, 6.2 Hz), 3.04 (dd, 0.5H, J=16.7, 6.2 Hz), 2.27 (s, 1.5H), 2.24 (s, 1.5H), 2.15 (s, 1.5H), 2.14 (s, 1.5H), 1.25 (m, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.2, 166.7, 166.5, 156.5, 153.2, 152.6, 152.4, 152.3, 150.7, 150.6, 150.1, 150.0, 149.9, 146.4, 146.3, 146.9, 145.8, 133.4, 132.8, 132.6, 131.9, 125.6, 125.5, 125.3, 124.8, 124.5, 122.8, 121.7, 121.1, 120.1, 119.9, 119.8, 119.6, 101.8, 101.2, 69.7, 69.5, 68.5, 61.5, 61.3, 60.5, 60.4, 60.12, 59.9, 59.8, 59.5, 59.5, 55.8, 53.4, 52.5, 49.8, 48.6, 45.6, 45.5, 38.0, 27.9, 22.2, 22.1, 22.1, 22.0, 9.4, 9.2. Anal. Calcd for $C_{40}H_{43}N_3O_{11}$: C, 64.77; H, 5.84; N, 5.66. Found: C, 64.65; H, 5.90; N, 5.74.

3.4.2. Isopropyl 1,4,10,13-tetramethoxy-9-methyl-7-oxo-(6*S,9*S**,15*S**)-5,6,9,15-tetrahydro-6,15-iminoisoquino-[3,2-*b*]-3-benzazocine-16-carboxylate (10b).** The reaction was performed with 234 mg (0.45 mmol) of compound **9b**. The crude product was purified by recrystallization in EtOH to give an off-white solid **10b** (190 mg, 83%): mp 162 °C, IR (NaCl) ν_{max} 2938, 2837, 1699, 1682, 1600 and 1488 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz, 5 °C) (mixture of rotamers) δ 6.80–6.60 (m, 4H), 6.51 (s, 0.6H), 6.39 (s, 0.4H), 6.14 (s, 0.6H), 5.97 (s, 0.4H), 5.93 (m, 1H), 5.11 (d, 0.6H, J=6.4 Hz), 5.20 (d, 0.4H, J=6.1 Hz), 4.92 (m, 1H), 3.86 (s, 1.2H), 3.85 (s, 1.2H), 3.84 (s, 1.8H), 3.83 (s, 1.8H), 3.78 (s, 3H), 3.77 (s, 3H), 3.26 (m, 1H), 3.04 (dd, 0.3H, J=20.2, 6.4 Hz), 3.00 (dd, 0.7H, J=17.5, 6.5 Hz), 1.28 (d, 1.2H, J=6.3 Hz), 1.26 (d, 1.2H, J=6.3 Hz), 1.23 (d, 1.8H, J=6.1 Hz), 1.22 (d, 1.8H, J=6.0 Hz), 0.78 (d, 2.2H, J=6.5 Hz), 0.76 (d, 0.8H, J=6.4 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.3, 166.2, 153.5, 152.7, 151.1, 150.9, 150.2, 149.9, 148.7, 148.6, 148.3, 148.2, 132.2, 131.9, 123.0, 122.8, 122.7, 122.1, 119.8, 109.4, 109.2, 108.7, 108.3, 108.2, 101.4, 100.8, 69.5, 69.3, 56.0, 55.9, 55.8, 55.5, 55.4, 53.1, 52.4, 49.6, 48.3, 43.9, 43.7, 28.1, 27.9, 22.1, 17.5, 17.4. Anal. Calcd for $C_{28}H_{32}N_2O_7$: C, 66.13; H, 6.34; N, 5.51. Found: C, 65.96; H, 6.39, N, 5.33.

3.4.3. Compound 12a. A solution of compound **9a** (186 mg, 0.25 mmol) in 4 mL of dry THF was added over a solution of lithium tri*tert*-butoxyaluminium hydride

(626 mg, 2.5 mmol) in 6 mL of dry THF cooled in ice water. After stirring at room temperature for 20 h, the reaction mixture was quenched by addition of ice and extracted with ethyl acetate. The extracts were washed with H_2O and a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the amino alcohol intermediate as a diastereomeric mixture. A solution of this mixture in 3 mL of formic acid was heated for 30 min at 80 °C and then, the reaction was quenched by addition over a 10% aqueous solution of $NaHCO_3$ and extraction with ethyl acetate. The extracts were washed with H_2O and a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo, to give the crude product **12a**, that was purified by flash chromatography on a silica gel column with hexane/ethyl acetate (3:7) as eluant to give an off-white solid (118 mg, 64%) yield: mp 248–249 °C, IR (NaCl) ν_{max} 2934, 1694, 1657 and 1469 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) (mixture of rotamers) δ 8.13 (dd, 1H, $J=8.0$, 7.8 Hz), 7.63 (dd, 1H, $J=8.0$, 7.8 Hz), 7.57 (dd, 1H, $J=7.0$, 6.6 Hz), 7.35 (dd, 1H, $J=7.5$, 7.4 Hz), 6.22 (s, 0.8H), 6.12 (s, 0.2H), 5.86 (s, 0.7H), 5.75 (s, 0.3H), 5.12 (d, 0.3H, $J=7.1$ Hz), 5.06 (d, 0.7H, $J=6.7$ Hz), 4.88 (m, 1H), 4.39 (m, 1H), 4.52 (s, 1H), 3.32 (m, 1H), 3.14 (m, 1H), 3.11 (s, 1H), 2.16 (s, 3H), 2.17 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.23 (m, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.1, 168.0, 167.9, 153.7, 153.4, 153.1, 152.6, 151.4, 151.3, 150.4, 150.3, 150.2, 149.9, 132.4, 131.9, 131.5, 128.4, 128.3, 127.0, 126.8, 125.1, 125.1, 125.0, 124.9, 124.7, 124.5, 124.1, 124.0, 123.1, 123.0, 69.7, 62.8, 62.5, 61.4, 61.4, 61.3, 61.0, 61.0, 60.9, 60.9, 60.8, 60.8, 60.3, 60.2, 60.1, 60.0, 57.8, 57.7, 52.2, 51.0, 48.0, 47.2, 46.9, 46.7, 46.5, 41.6, 41.4, 31.4, 30.0, 29.7; 29.4; 28.4; 27.7; 22.7, 22.2, 22.1, 22.0, 21.9, 14.2, 10.3, 10.2, 9.6, 9.5. Anal. Calcd for $C_{40}H_{45}N_3O_{11}$: C, 64.59; H, 6.10; N, 5.65. Found: C, 64.25; H, 6.20; N, 5.32.

3.5. Acid hydrolysis of carbamates 10a, 10b and 12a

To a stirred solution of the corresponding carbamate in 0.1 M trifluoroacetic acid was added 2 M H_2SO_4, and the resulting solution was stirred for 24 h at room temperature. The reaction mixture was poured into water and extracted three times with ethyl acetate. The extracts were washed with a 10% aqueous solution of $NaHCO_3$, H_2O and a saturated aqueous solution of NaCl, dried over Na_2SO_4, filtered and concentrated in vacuo, to give a solid residue that was purified by flash cromatography on silica gel. Ethyl acetate/DCM (1:9) for **11a** (**11b** (99%) and **13a** (83%), respectively.

3.5.1. 1-(6*S,9*R**,15*S**)-1,2,4,10,11,13-Hexamethoxy-3,12-dimethyl-9-phthalimidomethyl-5,6,9,15-tetrahydro-6,15-iminoisoquino[3,2-*b*]-3-benzazocin-7-one (11a).** The reaction was performed with 340 mg (0.46 mmol) of compound **10a**, 6.8 mL of conc. trifluoroacetic acid and 0.3 mL of conc. H_2SO_4. The crude product was purified by flash cromatography on silica gel with ethyl acetate/hexane (4:6) to give **11a** (295.4 mg, 98%) as an off-white solid: mp 144–145 °C, IR (NaCl) ν_{max} 2943, 1777, 1722 and 1682 cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ 7.61 (s, 4H), 6.48 (s, 1H), 6.28 (t, 1H, $J=6.8$ Hz), 5.52 (s, 1H), 4.48 (d, 1H, $J=2.3$ Hz), 3.90 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.66 (s, 3H), 3.62 (s, 3H), 3.40 (m, 2H), 3.37 (s, 3H), 3.27 (m, 2H), 2.25 (s, 3H), 2.12 (s, 3H). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 167.1, 163.5, 152.3, 151.7, 150.7, 150.6, 146.5, 145.8, 133.5, 131.9, 128.5, 126.8, 125.7, 122.9, 120.7, 120.2, 118.9, 118.5, 105.4, 61.5, 60.4, 60.1, 59.9, 59.8, 59.6, 51.9, 49.1, 46.4, 38.1, 27.1, 9.5, 9.2. Anal. Calcd for $C_{36}H_{37}N_3O_9$: C, 65.94; H, 5.69; N, 6.41. Found: C, 66.16; H, 5.72; N, 6.53.

3.5.2. 1,4,10,13-Tetramethoxy-9-methyl-(6*S,9*S**,15*S**)-5,6,9,15-tetrahydro-6,15-iminoisoquino[3,2-*b*][3]benzazocin-7-one (11b).** The reaction was performed with 200 mg (0.39 mmol) of compound **10b**, 4 mL of conc. trifluoroacetic acid and 0.2 mL of conc. H_2SO_4. The crude product was purified by flash cromatography on silica gel with ethyl acetate/DCM (1:9) as eluant to give **11b** (163 mg, 99%) as an off-yellow solid: mp 138–140 °C. IR (NaCl) ν_{max} 2953, 2838, 1681 and 1488 cm^{-1}. ^1H NMR ($CDCl_3$, 250 MHz) δ 6.74 (s, 2H), 6.67 (s, 2H), 6.56 (s, 1H), 5.91 (q, 1H, $J=6.4$ Hz), 5.50 (s, 1H), 4.51 (d, 1H, $J=4.8$ Hz), 3.83 (s, 3H), 3.80 (s, 3H), 3.77 (s, 3H), 3.75 (s, 3H), 3.42 (d, 1H, $J=18.3$ Hz), 3.28 (dd, 1H, $J=18.3$, 5.8 Hz), 0.75 (d, 3H, $J=6.4$ Hz). ^{13}C NMR ($CDCl_3$, 63 MHz) δ 162.6, 150.7, 150.0, 148.9, 148.7, 127.4, 123.0, 120.0, 118.8, 118.5, 110.6, 110.1, 109.8, 109.3, 105.2, 56.1, 56.0, 55.7, 55.5, 51.4, 48.4, 44.5, 26.8, 17.7. Anal. Calcd for $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63. Found: C, 67.91; H, 5.97; N, 6.43.

3.5.3. Compound 13a. The reaction was performed with 87 mg (0.12 mmol) of compound **12a**, 1.7 mL of conc. trifluoroacetic acid and 87 μL of conc. H_2SO_4. The crude product was purified by flash cromatography on silica gel with ethyl acetate/MeOH (9:1) as eluant to give **13a** (65.5 mg, 83%) as an off-white solid: mp 184–185 °C, IR (NaCl) ν_{max} 2937, 1689, 1643 and 1467 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.12 (d, 1H, $J=7.5$ Hz), 7.61 (t, 1H, $J=7.5$ Hz), 7.56 (d, 1H, $J=7.5$ Hz), 7.35 (t, 1H, $J=7.5$ Hz), 6.27 (d, 1H, $J=4.0$ Hz), 4.72 (s, 1H), 4.49 (s, 1H), 4.40 (dd, 1H, $J=13.8$, 4.0 Hz), 4.25 (s, 1H), 4.15 (s, 3H), 4.09 (d, 1H, $J=6.9$ Hz), 4.04 (d, 1H, $J=3.2$ Hz), 3.87 (s, 3H), 3.80 (s, 3H), 3.73 (s, 3H), 3.53 (s, 3H), 3.30 (d, 1H, $J=17.6$ Hz), 3.10 (m, 2H), 2.64 (s, 3H), 2.19 (s, 3H), 2.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.8, 168.0, 153.6, 151.4, 150.2, 150.1, 146.1, 145.7, 144.6, 132.2, 131.3, 128.2, 126.2, 125.6, 124.9, 124.8, 124.1, 123.4, 123.1, 64.6, 61.4, 60.8, 60.8, 60.6, 60.1, 60.0, 57.9, 52.7, 48.5, 46.9, 46.5, 41.7, 29.5, 10.1, 9.6. Anal. Calcd for $C_{36}H_{39}N_3O_9$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.83; H, 6.12; N, 6.21.

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Structure and absolute stereochemistry of novel C_{15}-halogenated acetogenins from the anaspidean mollusc *Aplysia dactylomela*

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Abstract—Three novel halogenated C_{15}-acetogenins, compounds **1**–**3**, have been isolated, together with known metabolites, from a South China Sea collection of the anaspidean mollusc *Aplysia dactylomela*. The structures have been suggested by both NMR analysis and comparison with literature data. The structure of **1** was confirmed by a single crystal X-ray study, which also allowed the establishment of its absolute stereochemistry.

1. Introduction

Opisthobranch molluscs, belonging to the order *Anaspidea*, are strictly herbivorous and widely distributed in both temperate and tropical waters. Generally these molluscs feed on red and brown algae from which they sequester selected bioactive secondary metabolites that are stored in the digestive gland and secreted in the mucus for defensive purpose.[1] Among anaspideans, different species belonging to the family Aplysiidae, which consists of nine genera including *Aplysia* and *Dolabella*, have been the object of several chemical studies that resulted in the finding of a great number of dietary compounds, usually typical algal halogenated metabolites.[2] We add here the isolation of three novel halogen-containing acetogenins, compounds **1**–**3**, along with known related cyclic ethers **4** and **5**, and brasilane-type sesquiterpenoid **6**, from a South China Sea collection of *Aplysia dactylomela* Rang, 1828. Worldwide distributed, this large mollusc and its secondary metabolite pattern have been deeply investigated. Previous chemical reports on *A. dactylomela* from distinct geographical areas, including the finding of sesquiterpenes with eudesmane,[3] chamigrane,[4] and cuparane[5] skeletons, dolabellane diterpenes[6] as well as C_{15} ethereal lipids,[7,8] suggest trophic relationship with red algae of the genus *Laurencia*. The function and the dynamics of the acquired algal metabolites have also been investigated in *A. dactylomela*.[9]

2. Results and discussion

The mollusc (four specimens) was collected along the coast of Hainan Island, in the South China Sea, during January 2002. Frozen individuals were carefully dissected into mantle and internal organs that were separately extracted by acetone using ultrasound. The ethereal soluble fractions from the extracts of the two distinct anatomical parts were analysed by silica-gel TLC chromatography, showing similar secondary metabolite patterns. In particular, two series of apolar compounds [spots at R_f 0.9–0.8 (light petroleum ether/diethyl ether, 9:1) and at R_f 0.85–0.70 (light petroleum ether/diethyl ether, 1:1)] were detected, along with the usual lipids and sterols, in the extract of both mantle and internal glands by spraying TLC plates with $CeSO_4$. A mixture of more polar terpenoid metabolites, the structure of which is under investigation, has been also found in both extracts.

The mantle ether extract (606 mg) was subjected to a silica gel column eluted with light petroleum ether/diethyl ether gradient to give pure compounds **4** (4.0 mg), **2** (0.8 mg), **3**

Keywords: Marine natural products; Molluscs; Acetogenins; Absolute stereochemistry.
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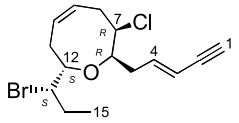
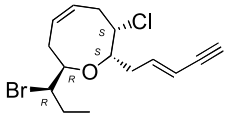
(0.2 mg), **5** (11.0 mg), **1** (6.4 mg), and **6** (4.0 mg), in order of increasing polarity. Compounds **1**–**3** were unprecedented enantiomers of known algal molecules, whereas the other metabolites were identified by their spectral data as (+)-3*E*-pinnatifidenyne (**4**), previously isolated from *Laurencia pinnatifida*,[7a] (+)-laurenyne (**5**), already reported from *Laurencia obtusa*,[8] and (+)-brasilenol (**6**), first isolated from both the mollusc *Aplysia brasiliana* and the red alga *Laurencia obtusa*.[10] The absolute stereochemistry of compounds **4**–**6**, previously established by either asymmetric synthesis[7d,10b] or X-ray analysis,[8] has been assigned by comparison of their $[\alpha]_D$ values with literature data.

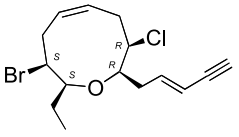
In order to get further amounts of minor metabolites **2** and **3**, the diethyl ether extract (16 g) of the internal glands was submitted to silica-gel column purification in the same conditions as used for the mantle extract, obtaining in a similar manner compounds **4** (13 mg), **2** (0.9 mg), **3** (1.8 mg), **5** (19 mg), **1** (16 mg), and **6** (13 mg).



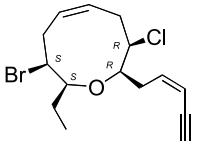
1, (-)-3*E*,6*R*,7*R*-pinnatifidenyne



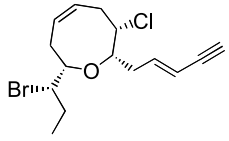
***ent*-1**, (+)-3*E*,12*R*,13*R*-pinnatifidenyne



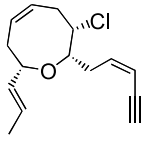
2, (+)-3*E*,6*R*,7*R*-obtusenyne



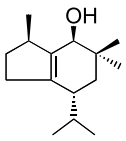
3, (+)-3*Z*,6*R*,7*R*-obtusenyne



4, (+)-3-*E*-pinnatifidenyne



5, (+)-laurenyne



6, (+)-brasilenol

Compound **1** showed the molecular formula $C_{15}H_{20}BrClO$, deduced by the sodiated molecular peak in the HRESMS spectrum at *m*/*z* 353.0290 $(M+Na)^+$. The ^1H NMR spectrum exhibited signals for a terminal methyl group [δ 1.09 (3H, t, $J=7.5$ Hz, H_3-15)], four olefinic protons [δ 5.62 (1H, dd, $J=16$, 5 Hz, H-3), δ 5.79 (1H, m, H-10), δ 5.87 (1H, m, H-9), and δ 6.18 (1H, m, H-4)], four deshielded methine groups [δ 3.96 (1H, m, H-13), δ 4.07 (2H, m, H-7 and H-12), and δ 4.34 (1H, m, H-6)], an acetylenic proton [δ 2.84 (1H, d, $J=2$ Hz, H-1)] and four methylenes [multiplets at δ 2.8–1.9 integrating for 8H], strongly suggesting a non-terpenoid halogen-containing structure. According to the molecular formula, the ^{13}C NMR spectrum contained 15 signals, attributed to two sp carbons, four sp^2 carbons, and the remaining to sp^3 carbons. The presence of a terminal conjugated enyne group was suggested by ^1H–^1H and long-range ^1H–^{13}C connectivities between the acetylenic proton at δ 2.84 (δ_C 77.2, C-1) and the olefinic signals at δ 5.62 (δ_C 112.0, C-3) and δ 6.18 (δ_C 141.4, C-4) and confirmed by the IR absorption at 3280 and 2140 cm^{-1}. The signal at δ 4.34 (δ_C 73.8, C-6) was coupled with both a methine at δ 4.07 (δ_C 63.8, C-7) and the methylene at δ 2.61–2.74 (δ_C 37.9, C-5) in turn coupled with the signal at δ 6.18. The proton at δ 4.07 was linked to another methylene at δ 2.58–2.73 (δ_C 33.7, C-8) coupled with the double bond protons at δ 5.87 (δ_C 129.9, C-9) and δ 5.79 (δ_C 127.9, C-10) in turn correlated with the methylene signal at δ 2.22 (δ_C 31.4, C-11). Another methine at δ 4.07 (δ_C 78.7, C-12) was cross-peak coupled with the latter methylene and with the methine proton at δ 3.96 (δ_C 62.1, C-13) which was linked to an ethyl group (δ_H 1.94–2.05, δ_C 29.3, H_2-14; δ_H 1.09, δ_C 12.5, H_3-15). Long range correlations between H-6 (δ 4.34) and C-12 (δ 78.7) suggested the presence of an ether linkage in agreement with the five degrees of unsaturation required by the molecular formula and by the absence of hydroxyl groups in the IR spectrum. The *trans* geometry of the C-3/C-4 double bond was suggested by the value of the coupling constant ($J=16$ Hz) between H-3 and H-4.

The NMR data of compound **1** (all assigned as reported in Section 3) were identical with those of (+)-(3*E*)-13-*epi*-pinnatifidenyne, recently isolated from *Laurencia obtusa*,[11] whereas the sign of $[\alpha]_D$ value was opposite suggesting that **1** should be its enantiomer.

With the aim of confirming the structure of **1**, a suitable single crystal, obtained by careful crystallization from *n*-hexane, was used for an X-ray diffraction study. The results are shown in Figure 1 and in Section 3. X-ray analysis confirmed the proposed structure, and indicated the orientation of substituents at the oxocene ring as follows: the five-carbon chain present at C-6 and containing the terminal enyne group was equatorially oriented, while the chlorine atom at C-7 and the bromo-containing propyl residue at C-12 were axially oriented but in opposite directions. Furthermore, the diffraction analysis allowed the establishment of the absolute stereochemistry at the four chiral centres 6*R*,7*R*,12*S*,13*S*. Therefore, compound **1** is a diastereoisomer of known (+)-(3*E*)-pinnatifidenyne (**4**)[7b] differing in the absolute configurations at C-6 and C-7 carbons which in the algal metabolite were both determined to be *S*.

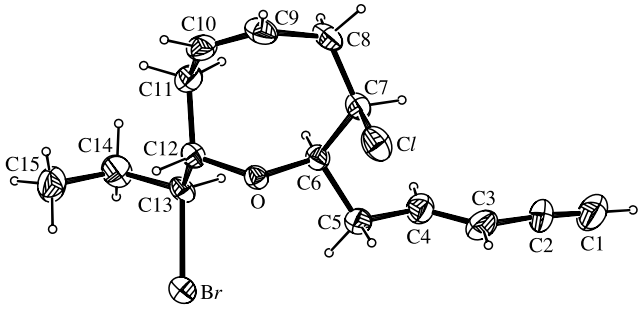


Figure 1.

On the basis of these data the structure of (3*E*)-13-*epi*-pinnatifidenyne[11] must be revised as the enantiomer of compound **1**, this being the (+)-3*E*,12*R*,13*R*-pinnatifidenyne (*ent*-**1**).

Compound **2** showed the molecular formula $C_{15}H_{20}BrClO$, the same as **1**, deduced by the sodiated molecular peak in the HRESMS spectrum at *m*/*z* 353.0281 $(M+Na)^+$. Analysis

of ^{1}H and ^{13}C NMR spectra indicated a nine-membered cyclic ether skeleton also revealing some structural analogies with **1**, in particular the same enyne-containing side-chain. Comparison of the spectral data of compound **2** with those reported by Norte et al. for (3*E*,12*R*,13*R*)-obtusenyne,[7b] the absolute stereochemistry of which has been determined by total synthesis,[12] indicated that **2** had the same structure, including the relative stereochemistry. However, the two molecules differed in the sign of the $[\alpha]_D$ value, indicating that **2** was the enantiomer of (3*E*)-Norte's obtusenyne, thus being the (+)-3*E*,6*R*,7*R*-obtusenyne.

Compound **3** displayed a close structural relationship with **2**, being the corresponding 3*Z*-isomer. Spectral data of **3** were identical with those reported by Norte et al. for (3*Z*,12*R*,13*R*)-obtusenyne,[7b,12] with the exception of the $[\alpha]_D$ value, that was opposite, thus indicating, analogously with **2**, that **3** was the enantiomer of (3*Z*)-Norte's obtusenyne.

In conclusion, chemical analysis of the lipid content of a population of *A. dactylomela* from South China Sea has confirmed, according to the chemical studies so far reported, the ability of the mollusc to accumulate from its diet typical algal secondary metabolites, halogenated acetogenins and brasilane sesquiterpenoids. The observed transfer of the algal metabolites from the digestive glands to the external part, the mantle, is an indirect evidence of the potential defensive role of these compounds. The novel acetogenins **1**–**3** are enantiomers of known molecules previously isolated from red algae of genus *Laurencia*. The absolute stereochemistry of **1** has been secured by X-ray diffraction study whereas the absolute configuration of compounds **2** and **3** have been suggested by comparison of their optical rotation values with those reported in the literature for the corresponding enantiomers, the structure of which have been demonstrated by total synthesis.[12]

In order to test biological properties of these molecules and to establish the probable role as deterrents against predators, feeding experiments with *Carassius auratus* were carried out on all of the isolated compounds, as described in the literature.[13] Compounds **1** and **5** were found to be active at a concentration of 50 μg/cm^2.

Even though no evidence of feeding preferences of Chinese *A. dactylomela* was detected in field, our results suggest, by chemical analogy, that this mollusc feeds on red algae belonging to the genus *Laurencia*, re-using algal metabolites in chemical defense. It could be a starting point for further field investigations aimed to assess the existence on the South China coast of a *Laurencia* species, which should contain enantiomers of C-15 halogenated acetogenins previously isolated from *Laurencia obtusa*.

3. Experimental

3.1. General experimental procedures

TLC plates (Merk Silica Gel 60 F254) were used for analytical TLC and Merck Kieselgel 60 was used for preparative column chromatography. HRESIMS were carried out on a Micromass Q-TOF micro. HPLC purifications were carried out on a Waters liquid chromotograph equipped with Waters 501 pump and a Waters R401 RI detector. Normal-phase purifications were conducted by using Kromasil Silica, 5 μ (250×4.60 mm, Phenomenex). NMR spectra, recorded at the NMR Service of Istituto di Chimica Biomolecolare of CNR (Pozzuoli, Italy), were acquired on a Bruker Avance-400 operating at 400 MHz, in $CDCl_3$ (δ values are reported referred to C*H*Cl$_3$ at 7.26 ppm) using an inverse probe fitted with a gradient along the *Z*-axis. ^{13}C NMR were recorded on a Bruker DPX-300 operating at 300 MHz (δ values are reported to C*D*Cl$_3$, 77.0 ppm) using a dual probe. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were measured on a Biorad FTS 155 FTIR spectrophotometer.

3.2. Biological material

A. dactylomela (four specimens) was collected by SCUBA diving at a depth of 20 m along the coast of Hainan Island, in South China Sea, during January 2002. Biological material was immediately frozen, then transferred to ICB Naples, and stored at −80 °C until extraction. A voucher specimen is stored for inspection at ICB (HN-59).

3.3. Extraction and isolation procedures

A. dactylomela individuals were carefully dissected into mantle and internal organs that were separately extracted by acetone using ultrasound. Filtration of the two homogenates gave an aqueous Me_2CO filtrate that was concentrated in vacuo to give a gummy residue. The residue was suspended in H_2O and extracted sequentially with diethyl ether and *n*-BuOH. The mantle ether extract (606 mg) was subjected to a silica gel column eluting with light petroleum ether/diethyl ether gradient to give the compounds **1** (6.4 mg), **4** (4.0 mg), **5** (11.0 mg), **6** (4.0 mg), and a less polar fraction that was purified on *n*-phase HPLC (eluent: *n*-hexane/AcOEt, 99.5/0.5, flow rate 1 ml/min) yielded **2** (0.8 mg) and **3** (0.2 mg). The digestive gland ether extract (16 g) was purified by a silica gel column eluting with light petroleum ether/diethyl ether gradient to give, in the same way, the compounds **1** (16 mg), **2** (0.9 mg), **3** (1.8 mg), **4** (13 mg), **5** (19 mg), **6** (13 mg).

The known compounds **4**–**6** have been identified by comparison of their spectral data (^{1}H NMR, ^{13}C NMR, MS) with those reported in the literature. Compound **4**: $[\alpha]_D$ +28.2 ($CHCl_3$, *c* 1.7); $[\alpha]_D$ lit.[7a] = +62.0 ($CHCl_3$, *c* 8.9). Compound **5**: $[\alpha]_D$ +14.2 ($CHCl_3$, *c* 3.0); $[\alpha]_D$ lit.[8] = +22.64 ($CHCl_3$, *c* 2.3). Compound **6**: $[\alpha]_D$ +25.0 ($CHCl_3$, *c* 1.7); $[\alpha]_D$ lit.[10a] = +33.4 ($CHCl_3$, *c* 1.6).

3.3.1. Compound 1. Crystal solid, R_f 0.8 (petroleum ether/diethyl ether 1:1); $[\alpha]_D$ −17.2 ($CHCl_3$, *c* 2.2); IR (KBr) ν_{max} 3280, 2140 cm^{-1}; HRESIMS *m*/*z* 353.0290 (M+Na), Calcd for $C_{15}H_{20}^{79}Br^{35}ClO$+Na 353.0285. ^{1}H NMR ($CDCl_3$): δ 6.18 (1H, m, H-4), δ 5.87 (1H, m, H-9), δ 5.79 (1H, m, H-10), δ 5.62 (1H, dd, J=5, 16 Hz, H-3), δ 4.34 (1H, m, H-6), δ 4.07 (1H, m, H-7), δ 4.07 (1H, m, H-12), δ 3.96 (1H, m, H-13), δ 2.84 (1H, d, J=2 Hz, H-1), δ 2.74 (1H, m, H-5a), δ 2.73 (1H, m, H-8a), δ 2.61 (1H, m, H-5b), δ

2.58 (1H, m, H-8b), δ 2.22 (2H, m, H_2-11), δ 2.05 (1H, m, H-14a), δ 1.94 (1H, m, H-14b), δ 1.09 (3H, t, 7.5, H_3-15). ^{13}C NMR ($CDCl_3$): δ 141.4 (C-4, CH), δ 129.9 (C-9, CH), δ 127.9 (C-10, CH), δ 112.0 (C-3, CH), δ 81.7 (C-2, C), δ 78.7 (C-12, CH), δ 77.2 (C-1, CH), δ 73.8 (C-6, CH), δ 63.8 (C-7, CH), δ 62.1 (C-13, CH), δ 37.9 (C-5, CH_2), δ 33.7 (C-8, CH_2), δ 31.4 (C-11, CH_2), δ 29.3 (C-14, CH_2), δ 12.5 (C-15, CH_3).

3.3.2. Compound 2. Pale yellow oil, R_f 0.8 (petroleum ether/diethyl ether 9:1); $[\alpha]_D$ +18 ($CHCl_3$, *c* 0.17); IR (KBr) ν_{max} 3320, 3000, 2920 cm^{-1}; HRESIMS *m/z* 353.0281 (M+Na) Calcd for $C_{15}H_{20}^{79}Br^{35}ClO$+Na 353.0285. ^{1}H NMR ($CDCl_3$): δ 6.12 (1H, ddd, *J*=7.6, 7.6, 15.5 Hz, H-4), δ 5.64 (1H, m, H-3), δ 5.61 (1H, m, H-10), δ 5.59 (1H, m, H-9), δ 4.21 (1H, m, H-12), δ 4.08 (1H, m, H-7), δ 3.51 (1H, m, H-6), δ 3.20 (1H, m, H-13), δ 2.84 (1H, d, *J*=2.2 Hz, H-1), δ 2.71 (1H, m, H-5a), δ 2.57 (2H, m, H-11), δ 2.51 (1H, m, H-5b), δ 2.45 (2H, m, H-8), δ 1.88 (2H, m, H-14), δ 0.85 (3H, t, *J*=7.5 Hz, H-15). ^{13}C NMR ($CDCl_3$): δ 140.6 (C-4, CH), δ 130.1 (C-10, CH), δ 128.5 (C-9, CH), δ 112.3 (C-3, CH), δ 83.3 (C-13, CH), δ 80.8 (C-6, CH), δ 77.0 (C-1, CH), δ 61.6 (C-7, CH), δ 54.2 (C-12, CH), δ 37.1 (C-5, CH_2), δ 34.2 (C-11, CH_2), δ 33.4 (C-8, CH_2), δ 27.7 (C-14, CH_2), δ 9.72 (C-15, CH_3).

3.3.3. Compound 3. Pale yellow oil, R_f 0.8 (petroleum ether/diethyl ether 9:1); $[\alpha]_D$ +10 ($CHCl_3$, *c* 0.2); IR (KBr) ν_{max} 3300, 3000, 2940 cm^{-1}; HRESIMS *m/z* 353.0288 (M+Na) Calcd for $C_{15}H_{20}^{79}Br^{35}ClO$+Na 353.0285. ^{1}H NMR ($CDCl_3$): δ 6.03 (1H, dt, *J*=7.5, 10.6 Hz, H-4), δ 5.60 (1H, m, H-10), δ 5.57 (1H, m, H-9), δ 5.56 (1H, d, *J*=10.6 Hz, H-3), δ 4.26 (1H, m, H-12), δ 4.06 (1H, m, H-7), δ 3.58 (1H, m, H-6), δ 3.22 (1H, m, H-13), δ 3.15 (1H, d, *J*=2.4 Hz, H-1), δ 2.84 (2H, m, H-5), δ 2.59 (2H, m, H-11), δ 2.44 (2H, m, H-8), δ 1.89 (2H, m, H-14), δ 0.85 (3H, t, *J*=7.4 Hz, H-15). ^{13}C NMR ($CDCl_3$): δ 140.1 (C-4, CH), δ 130.0 (C-10, CH), δ 128.6 (C-9, CH), δ 111.1 (C-3, CH), δ 83.4 (C-13, CH), δ 82.8 (C-1, CH), δ 80.9 (C-6, CH), δ 61.8 (C-7, CH), δ 54.1 (C-12, CH), δ 34.7 (C-5, CH_2), δ 34.1 (C-11, CH_2), δ 33.5 (C-8, CH_2), δ 27.6 (C-14, CH_2), δ 9.6 (C-15, CH_3).

3.4. X-ray structure determination of 1

Crystal data: colourless prismatic crystal from *n*-hexane; $C_{15}H_{20}BrClO$, MW 331.67; crystal dimensions 0.32×0.20×0.12 mm; crystal system orthorhombic; space group $P2_12_12_1$; unit cell dimensions *a*=5.0780(3) Å, *b*=16.1610(6) Å, *c*=19.191(3) Å; *V*=1574.9(3) Å^3; *Z*=4; calculated density 1.399 g/cm^3; F_{000}=680. A total of 10,798 reflections, 3614 of which were independent (R_{int}=0.0635), were measured at room temperature using graphite-monochromated Mo Kα radiation (λ=0.71073 Å) on a Bruker Kappa CCD diffractometer. Data were collected up to θ=27.67° (99.4% of completeness). The structure was solved by direct methods (SIR97) and refined with full-matrix least-squares calculations on F^2 using SHELXL. The *R* value was 0.0398 (R_w=0.1017) based on 2169 observed reflections ($I>2\sigma(I)$) and 163 variable parameters. The non-hydrogen atoms were refined anisotropically and hydrogen atoms were included at the ideal positions but not refined. The residual electron density is in the range −0.31–0.36 e/Å^3. The absolute configuration was determined using 1494 Friedel pairs with a final Flack parameter of 0.041(14). Bond distances and angles agree well with generally accepted values and there were no abnormally short intermolecular contacts. All the crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC number: 266836).

3.5. Biological assays

Feeding-deterrence tests against gold fish *Carassius auratus* were conducted according to literature procedures.[13] Compounds **1**–**6** were assayed at 50 μg/cm^2 and the activity was shown by compounds **1** and **5**.

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The authors are indebted to Professor V. Roussis for providing NMR spectra of (3*E*)-12*R*,13*R*-pinnatifidenyne. Thanks are due to the ICB NMR service and D. Melck of the staff service, C. Iodice for spectrophotometric measurements, R. Turco for the drawing support and G. Villani for the antifeedant bioassay. X-ray experiments were performed using the equipment of the 'Centro Regionale di Competenza Nuove Tecnologie per le Attività Produttive' of Campania Region. This research has been partially supported by an Italian-Chinese joint project (CNR/CAS 2001–2004) and by Regione Campania project: 'Valorizzazione di risorse naturali non tradizionali della Campania: studio chimico di invertebrati marini'.

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Efficient metathesis route to the B-ring of eleutherobin and other medium-sized cyclic ethers

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Dedicated to Professor K. K. Balasubramanian on the occasion of his 65th birthday

Abstract—A short and efficient RCM route is reported for the synthesis of the B-ring of eleutherobin and other medium-sized cyclic ethers from readily available 1,2,5,6-diisopropylidene-D-glucose. This strategy is successfully extended to the synthesis of a few bicyclic ethers, which may find applications in the synthesis of novel bicyclic nucleosides.

1. Introduction

The success of the anti-cancer drug taxol® (paclitaxel) has stimulated an intensive search for other drugs that operate by the same mode of action.[1] Successful examples, isolated from natural sources, include discodermolide,[2] the epothilones,[3] laulimalide[4] and eleutherobin.[5] Among them, the latest discovery, eleutherobin **1**[6] was isolated from a marine soft-coral found in the Indian ocean and found to be closely related to sarcodictyins **4**, **5**,[7] valdivone **6**[8] and eleuthesides (Fig. 1).[9] Later, several related congeners of eleutherobin were isolated[10] from the Caribbean soft coral *Erythropodium caribaeorum*. Eleutherobin has been shown to possess potent cytotoxicity with an IC_{50} of 10.7 nM, which is comparable to taxol. As eleutherobin was only available in scarce amounts from natural sources, chemical syntheses have become important to study its biological properties further. These prompted synthetic chemists to develop new strategies[11] to synthesize eleutherobin and their sustained efforts have culminated in two total syntheses.[12–14]

In continuation of our interest in the development of simple and efficient routes to the syntheses of tubulin binding anti-cancer agents, we became interested in the synthesis of eleutherobin and its congeners.[15]

2. Results and discussion

A strategy for the synthesis of eleutherobin was designed based on the retrosynthetic analysis involving metathesis reaction as the key step and is shown in Scheme 1.

With the discovery of air stable Grubbs' first[16] and second generation catalysts,[17] the last decade has witnessed a meteoric rise in the utility of the ring closing metathesis reaction (RCM)[18] in the formation of several types of alkenes, carbo- and heterocycles. The reaction tolerates a wide range of functional groups and with these reliable and practical catalysts, the RCM reaction has been widely used in the synthesis of a number of cyclic natural and unnatural products. However, construction of medium-size rings by ring closing metathesis has often been hampered by entropic and thermodynamic instability factors.[19] Nevertheless, there are quite a few successful reports of constructing medium size rings by ring closing metathesis reaction.[20] Encouraged by these reports, we envisaged that a RCM reaction would be an ideal key reaction to construct these natural product skeletons. Eleutherobin possesses three double bonds, which could be formed as a result of a RCM reaction. However, only disconnection of the B-ring double bond will simplify the target molecule leading to precursor **C**, which then can be further disconnected to the bicyclic enone **8** and the epoxide **9** (Scheme 1).

From this retrosynthetic modus operandi, it is clear that the success of our synthetic route to eleutherobin depends on the construction of the B-ring with the strategically placed double bond. Furthermore, there are several natural products, which have nine-membered cyclic ethers as their

Keywords: Eleutherobin; Cyclic ethers; Ring closing metathesis; Grubbs' catalyst; Bicyclic nucleosides.
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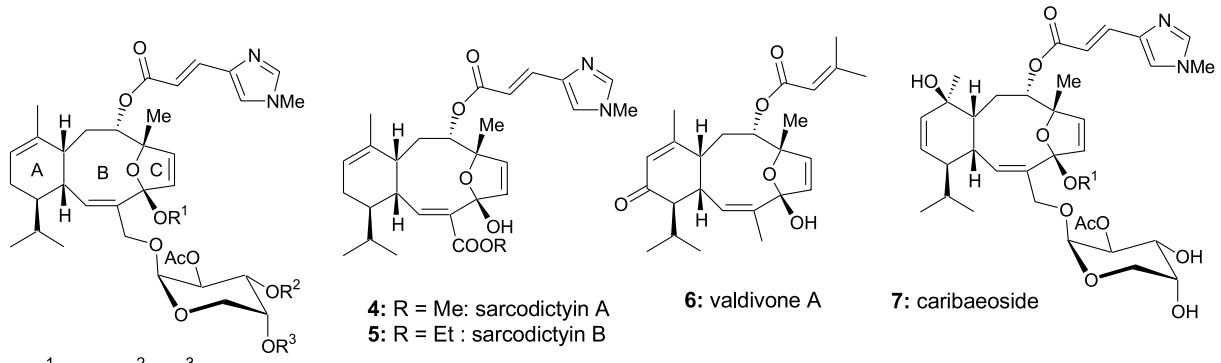
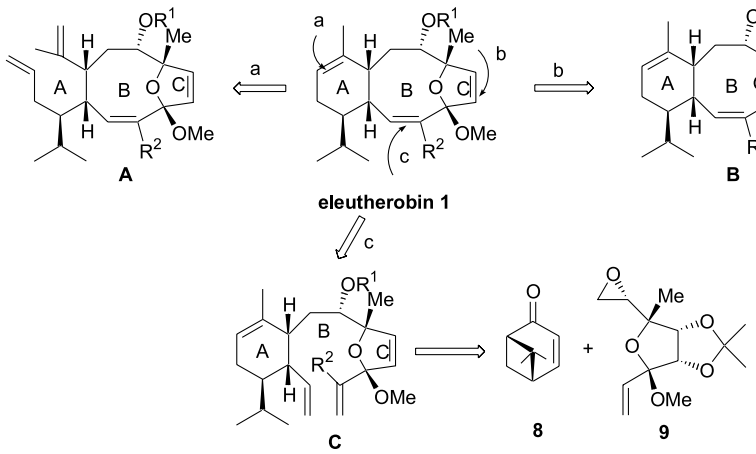


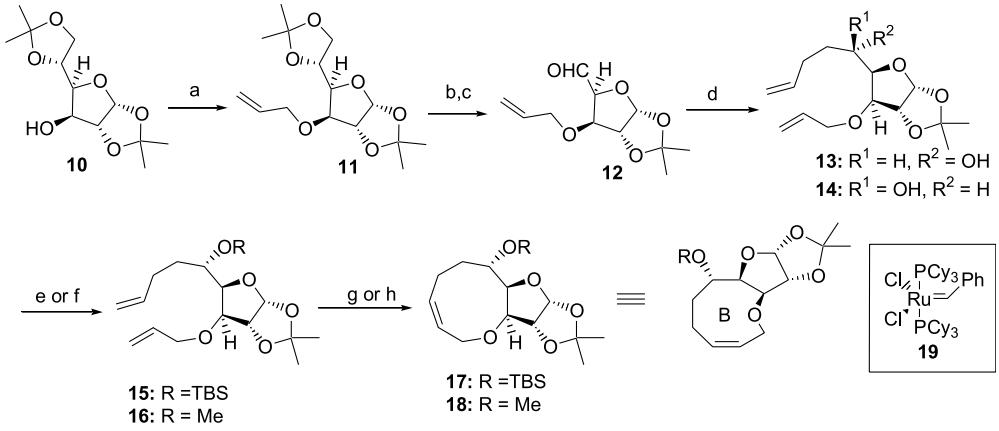
Figure 1.



Scheme 1.

key substructure. A convenient access to the fully functionalized nine-membered B-ring of eleutherobin as well as other related natural products in enantiomerically enriched form still remains a challenge to synthetic chemists. Thus, in view of the importance of these medium-size cyclic ethers, we planned to synthesize a highly oxygenated nine-membered cyclic ether[21] starting from the readily available 1,2,5,6-diisopropylidene-D-glucose and the details are reported here.

Our synthesis, as depicted in Scheme 2, starts with the classical etherification of the secondary alcohol **10** to afford **11**. Under mild acidic conditions, the more exposed 5,6-*O*-isopropylidene group was selectively deprotected to give a diol which was subsequently cleaved by treating with silica



Scheme 2. Reagents & Conditions: (a) NaH, allylbromide, THF, TBAI (cat.), RT, 2 h, 93%. (b) 60% *aq*. AcOH, H_2O, 12 h, 90%. (c) Silica gel supp. $NaIO_4$, CH_2Cl_2, 30 min, 86%. (d) 4-Bromo-1-butene, Mg, THF, 0°C to RT, 12 h, 83%. (e) TBSCl, imid., DMF, 0°C to RT, 10 h, 84%, 77%. (f) NaH, MeI, 0°C to RT, 2 h, 77%. (g) **19** (10 mol%), CH_2Cl_2, 40°C, 2 h, (30% for R=TBS, 33% for R=Me). (h) **19** (10 mol%), $Ti(O/Pr)_4$ (7.5 mol%), CH_2Cl_2, 40°C, (83% for R=TBS, 63% for R=Me).

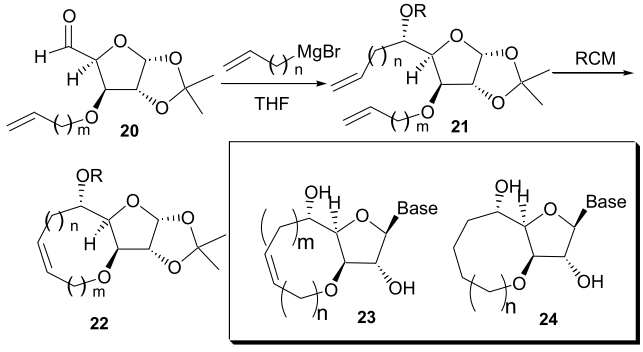
gel supported $NaIO_4$[22] to afford **12**. Addition of the Grignard reagent, derived from 4-bromo-1-butene and magnesium, to aldehyde **12** afforded two diastereomeric products **13** and **14** as a readily separable mixture in 3:2 ratio.[23] The stereochemistry of the major Grignard addition product was tentatively assigned as α-isomer as per the literature report.[23]

Moreover, the major isomer seems to be more polar than the other and this observation is consistent with all the Grignard reactions we have attempted on this system. All attempts to carry out this reaction at low temperature did not improve the ratio significantly. The major alcohol **13** was separated and subsequently protected as its TBS ether **15**, which sets the stage for the key RCM reaction. Unfortunately, when the RCM was carried out with Grubbs' first generation catalyst **19** under high dilution conditions (0.003 M solution with 10 mol% catalyst), only 30% of the desired cyclized product **17** was obtained, the remainder being unreacted starting material and presumably some cross-metathesis product. Assuming that the bulky TBDMS group would have provided a sterically crowed environment for the key RCM reaction, the major alcohol was protected as its methyl ether **16** and the key RCM reaction was subsequently attempted with Grubbs' first generation catalyst **19**. The observation of a similar result with the methyl ether suggested that the low yield could be presumably attributed to coordination of the metal center with the oxygen of the furanose ring as suggested by Fürstner.[24]

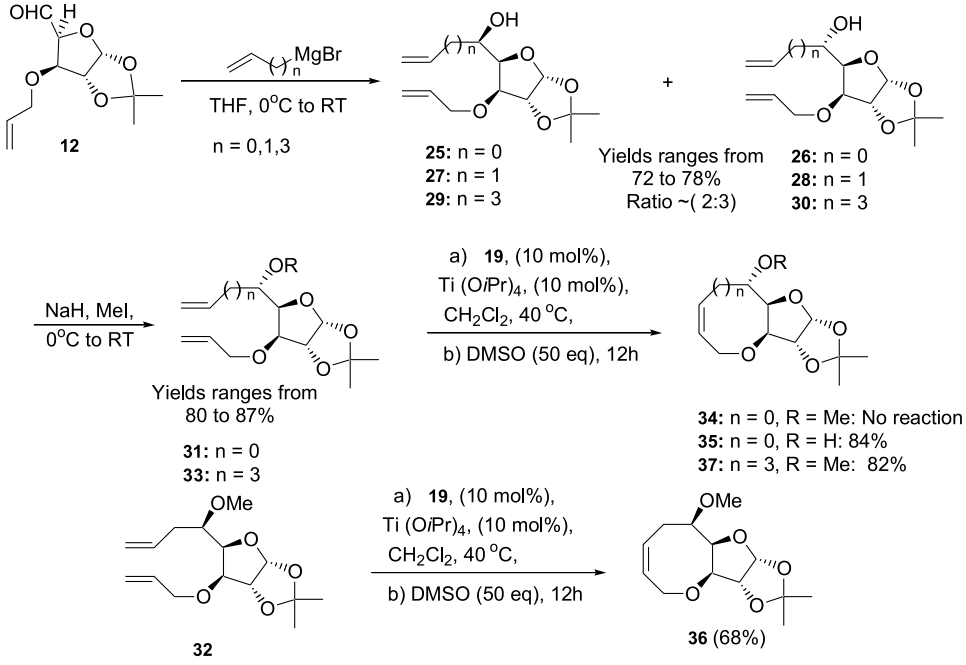
In order to destabilize this chelate structure, the RCM reaction of the dienes **15** and **16** was carried out with a catalytic amount of **19** in the presence of a sub-stoichiometric amount of $Ti(OiPr)_4$[24] and this modified protocol successfully led to the formation of the desired RCM products **17** and **18**, respectively, in high yields.

After successfully synthesising a model of the nine-membered B-ring of eleutherobin, we then examined the scope of utilizing this methodology to a diversity-oriented synthesis of various bicyclic ethers, which may find applications in the synthesis of novel bicyclic nucleosides (Scheme 3). Addition of Grignard reagents having terminal alkenes to the aldehyde **20**, followed by protection of the resultant alcohols should afford RCM precursors **21**. After the key RCM reaction, these bicyclic ethers could be used for the synthesis of several bicyclic nucleosides **23** and **24**.

Herein, we report the details of the synthesis of seven, eight and ten-membered cyclic ethers starting from the aldehyde **12**. The synthesis of five-seven fused bicyclic ether was started with the addition of vinyl magnesium bromide to aldehyde **12** at 0 °C in THF, and as expected, it afforded two diastereomeric products **25** and **26** as a readily separable mixture in 1:2 ratio. The major alcohol **26** was subsequently converted to its methyl ether **31** and unfortunately, when the RCM of diene **31** was carried out with Grubbs' catalyst **19** under high dilution conditions (0.003 M solution with 10% catalyst), in the presence of catalytic amount of $Ti(OiPr)_4$, only starting material was recovered. It has been reported that the protecting group in the allylic alcohol plays an



Scheme 3.



Scheme 4.

important role in the success of the RCM reaction[25] and so we attempted the RCM of the diene **26** (allylic alcohol) with Grubbs' catalyst **19** under our standard conditions. We were pleasantly surprised to see the formation of cyclised product **35** in 84% yield (Scheme 4).

The synthesis of five, eight-membered fused bicyclic ether was started with the addition of allyl magnesium bromide to aldehyde **12** at 0 °C in THF, and unfortunately it afforded **28** in 20% yield and the remainder being a complex mixture. Alternatively, a zinc mediated allylation was attempted with **12** and this protocol afforded two diastereomeric products **27** and **28** in 5:1 ratio. The alcohol **27** was subsequently converted to its methyl ether **32**, which was further subjected to RCM with Grubbs' catalyst **19** under our standard condition to give the cyclized product **36** in 68% yield. We also could successfully extend this method to synthesize the five, ten-membered fused bicyclic ether **37**. Addition of 5-bromo-1-pentene magnesium bromide in THF to aldehyde **12** afforded two diastereomeric products **29** and **30** in 1:1 ratio and the latter alcohol **30** was protected as its methyl ether **33**. Subsequently, RCM of the diene **33** was carried out with Grubbs' catalyst **19** in the presence of a catalytic amount of $Ti(OiPr)_4$ under high dilution conditions (0.003 M solution with 10% catalyst) to afford the desired product **37** in 82% yield. It is also very important to note that in this case, the RCM reaction exclusively provided the *cis* isomer.

3. Conclusion

In summary, we have successfully synthesized a model of the B-ring of eleutherobin utilizing the RCM reaction as the key step. The key factor involved in the success of this reaction was addition of $Ti(OiPr)_4$ which destabilised the possible formation of a chelated structure of ruthenium carbene with furanose oxygen. This methodology was then successfully extended to other medium-sized cyclic ethers through a common intermediate. This synthetic strategy has high potential in the synthesis of several related natural products and novel bicyclic nucleosides. Work is in progress in our laboratory to achieve this goal.

4. Experimental

4.1. General

Unless and otherwise noted, all starting materials and reagents were obtained from commercial suppliers and used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane and hexanes were freshly distilled from calcium hydride. DMF was distilled over calcium hydride and stored over Molecular Sieves 4 Å. Solvents for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried in oven at 100 °C for 12 h before use. Air and moisture sensitive reactions were performed under an argon/UHP nitrogen atmosphere. Column chromatography was performed using silica gel (100–200 mesh, Acme) with indicated solvents. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica plates (60F-254) using UV light as visualizing agent and 7% ethanolic phosphomolybdic acid and heat as developing agents. Optical rotation was recorded on Jasco DIP-370 digital polarimeter. IR spectra were recorded from Thermo Nicolet Avater 320 FT-IR and Nicolete Impact 400 machine. Mass spectra were obtained with Waters Micromass-Q-Tof micro™ (YA105) spectrometer. Elemental analysis was recorded on Thermo Finnigan Flash EA 1112. ^1H and ^{13}C NMR spectra were recorded either on Varian AS 400, Varian AS 500 or Varian ASM 300 instruments in $CDCl_3$ solutions. ^1H NMR data were reported in the order of chemical shift (δ in ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, and coupling constant J in Hertz (Hz).

4.2. General procedure for Grignard reactions

A solution of the aldehyde (1 mmol) in dry THF (5 mL) was cooled to 0 °C under an argon atmosphere. The Grignard reagent (3 mmol) was added dropwise and stirred for 2 h at 0 °C then for 12 h at room temperature. The reaction mixture was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo, and purified by silica gel chromatography (hexane/ethyl acetate) to give two diastereomeric alcohols.

4.3. General procedure for methylation of alcohol

To a suspension of sodium hydride (3 mmol, 60% dispersion in mineral oil) in dry THF (15 mL) was added a solution of alcohol (1 mmol) in THF (2 mL) dropwise at 0 °C and the mixture was stirred for 30 min. To this mixture iodomethane (2 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 1 h and then for 1 h at room temperature. The reaction mixture was quenched with saturated NH_4Cl and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo, and purified by silica gel chromatography (hexane/ethyl acetate) afforded the corresponding product.

4.4. General procedure for ring closing metathesis reaction

Method A. A 1 mmol portion of diene was dissolved in 325 mL of dry dichloromethane under argon and the solution was degassed. To this mixture, a solution of Grubbs' catalyst (10 mol%) in CH_2Cl_2 (10 mL) was added dropwise and the reaction mixture was refluxed for 2–12 h. The reaction mixture was cooled to room temperature, concentrated in vacuo, and purified by a silica gel column chromatography (hexane/ethyl acetate) to give the product.

Method B. A solution of diene (1 mmol) and $Ti(OiPr)_4$ (10 mol%) in dry CH_2Cl_2 (325 mL) was stirred under reflux for 1 h. To this mixture, a solution of Grubbs' catalyst (10 mol%) in CH_2Cl_2 (10 mL) was added dropwise and the reaction mixture was refluxed for 2–12 h. The reaction mixture was cooled to room temperature and DMSO (50 equiv with respect to catalyst) was added and stirred for 6 h. Evaporation of the solvent and purification

by column chromatography yielded the corresponding product.

4.4.1. (5*S*)-1,2-*O*-(-1-Methylethylidede)-3-*O*-allyl-5-*C*-buteyl-α-D-xylofuranose (13) and (5*R*)-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-buteyl-α-D-xylofuranose (14). Following the general procedure for Grignard reaction, a solution of aldehyde **12** (880 mg, 3.08 mmol) in THF was treated with 4-bromo-1-butenemagnesium bromide (9.32 mmol) to afford two diastereomeric products **13** (534 mg, 53%) and **14** (330 mg, 33%) as a readily separable mixture.

Compound **13**. Viscous colourless oil; R_f=0.6 (33%, EtOAc/hexane); $[\alpha]_D^{25}$ −53.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3443, 3075, 1641, 1077 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.97 (1H, d, *J*=3.9 Hz, anomeric C*H*), 5.93–5.78 (2H, m, 2×CH_2=C*H*), 5.34–5.22 (2H, m, CH_2=), 5.09–4.95 (2H, m, CH_2=), 4.58 (1H, d, *J*=3.9 Hz, C*H*), 4.22–4.15 (1H, m, C*H*), 4.05 (1H, dd, *J*=5.7, 3.3 Hz, C*H*), 4.01–3.94 (2H, m, CH_2), 3.92 (1H, d, *J*=3.3 Hz), 2.37–2.12 (2H, m, CH_2), 1.75–1.52 (2H, m, CH_2), 1.49 (3H, s, *Me*), 1.33 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 138.2, 133.2, 118.2, 114.7, 111.6, 104.7, 82.7, 82.6, 82.2, 70.7, 69.1, 32.1, 29.5, 26.7, 26.2. Anal. Calcd for $C_{15}H_{24}O_5$: C, 63.36; H, 8.51. Found: C, 63.47; H, 8.42.

Compound **14**. Colourless oil; R_f=0.7 (33%, EtOAc/hexane); $[\alpha]_D^{25}$ −36.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3467, 3076, 1641, 1077 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.96 (1H, d, *J*=4.2 Hz, anomeric C*H*), 5.94–5.79 (2H, m, 2×CH_2=C*H*), 5.36–5.24 (2H, m, CH_2=), 5.10–4.96 (2H, m, CH_2=), 4.58 (1H, d, *J*=4.2 Hz, C*H*), 4.24–4.17 (1H, m, C*H*), 4.05–3.93 (4H, m, CH_2 and 2×C*H*), 2.38–2.12 (2H, m, CH_2), 1.90–1.53 (2H, m, CH_2), 1.49 (3H, s, *Me*), 1.33 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 138.5, 133.6, 118.6, 114.9, 111.7, 105.2, 82.5, 82.3, 82.0, 70.9, 69.3, 33.9, 29.9, 26.8, 26.4; HRMS (EI) calcd for $C_{15}H_{24}O_5Na$ *m*/*z* 307.1521, found *m*/*z* 307.1510.

4.4.2. (5*S*)-5-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-(1-methylethylidede)-3-*O*-allyl-5-*C*-buteyl-α-D-xylofuranose (15). A solution of the alcohol **13** (400 mg, 1.40 mmol) in dry DMF (0.8 mL) was treated with imidazole (286 mg, 4.21 mmol), TBSCl (253 mg, 1.68 mmol) and a catalytic amount of DMAP at 0 °C and this solution was stirred for 10 h at room temperature. The reaction mixture was extracted with ethyl acetate and washed with water and brine, dried over anhydrous Na_2SO_4, concentrated in vacuo and purified by silica gel chromatography (hexanes/ethyl acetate 20:1) to give **15** (480 mg, 87%) as a colourless oil. R_f=0.8 (20%, EtOAc/hexane); $[\alpha]_D^{25}$ −46.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3085, 1643, 1255, 1097 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.92 (1H, d, *J*=3.7 Hz, anomeric C*H*), 5.89–5.75 (2H, m, 2×CH_2=C*H*), 5.31–5.18 (2H, m, CH_2=), 5.06–4.93 (2H, m, CH_2=), 4.53 (1H, d, *J*=3.7 Hz, C*H*), 4.17–4.09 (1H, m, C*H*), 3.99–3.86 (3H, m, CH_2 and C*H*), 3.76 (1H, d, *J*=2.9 Hz, C*H*), 2.28–2.09 (2H, m, CH_2), 1.58–1.51 (2H, m, CH_2), 1.47 (3H, s, *Me*), 1.31 (3H, s, *Me*), 0.89 (9H, s, SiCMe_3), 0.11 (3H, s, Si*Me*), 0.08 (3H, s, Si*Me*); δ_C (75 MHz, $CDCl_3$) 138.8, 133.8, 117.9, 114.5, 111.4, 104.8, 83.9, 82.2, 82.1, 70.9, 70.8, 32.7, 29.5, 26.8, 26.5, 26.1, 18.5, −3.9, −4.7. Anal. Calcd for $C_{19}H_{34}O_5Si$: C, 63.28; H, 9.61. Found: C, 62.59; H, 9.28; HRMS (EI) calcd for $C_{19}H_{34}O_5SiNa$ *m*/*z* 421.2386, found *m*/*z* 421.2391.

4.4.3. (5*S*)-5-*O*-Methyl-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-buteyl-α-D-xylofuranose (16). Following the general procedure for methylation reaction, a solution of **13** (880 mg 3.08 mmol) in THF was treated with sodium hydride (370 mg, 9.26 mmol, 60% in dispersion in oil) and methyl iodide (0.38 mL, 6.12 mmol) in presence of catalytic amount of TBAI. The crude product was purified by silica gel chromatography (hexanes/ethyl acetate 20:1) to give **16** (740 mg, 80%) as a colourless oil. R_f=0.8 (33%, EtOAc/hexane); $[\alpha]_D^{25}$ −49.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3085, 1643, 1091 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.94 (1H, d, *J*=3.9 Hz, anomeric C*H*), 5.89–5.72 (2H, m, 2×CH_2=C*H*), 5.31–5.19 (2H, m, CH_2=), 5.05–4.94 (2H, m, CH_2=), 4.53 (1H, d, *J*=3.9 Hz, C*H*), 4.17–4.07 (2H, m, CH_2), 3.88 (1H, dd, *J*=12.6, 6.1 Hz, C*H*), 3.76 (1H, d, *J*=3.1 Hz, C*H*), 3.52–3.45 (1H, m, C*H*), 3.48 (3H, s, O*Me*), 2.25–2.14 (2H, m, CH_2), 1.52–1.47 (2H, m, CH_2), 1.49 (3H, s, *Me*), 1.30 (3H, s, *Me*); δ_C (75 MHz, $CDCl_3$) 138.3, 133.5, 118.1, 114.7, 111.4, 104.9, 83.9, 82.1, 81.8, 79.3, 70.7, 59.3, 30.2, 29.2, 26.6, 26.2. Anal. Calcd for $C_{16}H_{26}O_5$: C, 64.41; H, 8.78. Found: C, 63.99; H, 8.73.

4.4.4. *tert*-Butyl-(12,12-dimethyl-8,11,13,15-tetraoxa-tricyclo[7.6.0.010,14]pentadec-5-en-2-yloxy)-dimethylsilane (17). *Procedure A*. Following the general procedure for ring closing metathesis reaction (method A), from 200 mg (0.50 mmol) of **15** and 50 mg (0.060 mmol) of Grubb's catalyst, 55 mg (30%) of **17** was obtained as a colourless oil.

Procedure B. Following the general procedure for ring closing metathesis reaction (method B), from 200 mg (0.50 mmol) of **15**, 0.015 mL (0.050 mmol) of Ti(O*i*Pr)$_4$ and 41 mg (0.050 mmol) of Grubb's catalyst, 160 mg (86%) of **17** was obtained as a colourless oil. R_f=0.8 (20%, EtOAc/hexane); $[\alpha]_D^{25}$ −39.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2947, 1650, 1255, 1091 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.90 (1H, d, *J*=3.7 Hz, anomeric C*H*), 5.84 (1H, q, *J*=9.2 Hz, C*H*=), 5.52 (1H, dt, *J*=9.2, 5.1 Hz, C*H*=), 4.47 (1H, d, *J*=3.7 Hz, C*H*), 4.35 (1H, dd, *J*=14.2, 3.7 Hz, C*H*), 4.08 (1H, d, *J*=2.9 Hz, C*H*), 3.96–3.82 (3H, m, CH_2 and C*H*), 2.39–2.14 (2H, m, CH_2), 1.91–1.51 (2H, m, CH_2), 1.47 (3H, s, *Me*), 1.30 (3H, s, *Me*), 0.88 (9H, s, SiCMe_3), 0.09 (3H, s, Si*Me*) 0.08 (3H, s, Si*Me*); δ_C (125 MHz, $CDCl_3$); δ 135.9, 125.7, 111.3, 104.6, 86.4, 84.1, 81.1, 73.8, 66.2, 33.2, 29.8, 26.9, 26.5, 26.0, 18.4, −4.3, −4.8. Anal. Calcd for $C_{19}H_{34}O_5Si$: C, 61.58; H, 9.25. Found: C, 61.29; H, 9.16.

4.4.5. 2-Methoxy-12,12-dimethyl-8,11,13,15-tetraoxa-tricyclo[7.6.0.010,14]pentadec-5-ene (18). *Procedure A*. Following the general procedure (method A) for ring closing metathesis, from 170 mg (0.56 mmol) of **16** and 56 mg (0.080 mmol) of Grubb's catalyst, 52 mg (33%) of **18** was obtained as a colourless oil.

Procedure B. Following the general procedure B, from 150 mg (0.50 mmol) of **16**, 0.015 mL (0.050 mmol) of Ti(O*i*Pr)$_4$ and 41 mg (0.050 mmol) of Grubb's catalyst, 90 mg (66%) of **18** was obtained as a colourless oil. R_f=0.4 (20%, EtOAc/hexane); $[\alpha]_D^{25}$ −63.9 (*c* 1.0, $CHCl_3$); IR

(neat) ν_{max} 2940, 1657, 1466 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.89 (1H, d, J=3.8 Hz, anomeric C*H*), 5.83 (1H, q, J=9.2 Hz, C*H*=), 5.48 (1H, dt, J=9.2, 4.2 Hz, C*H*=), 4.43 (1H, d, J=3.8 Hz, C*H*), 4.31 (1H, dd, J=14.1, 3.8 Hz, C*H*), 4.06 (1H, d, J=3.1 Hz, C*H*), 4.01–3.51 (2H, m, CH_2), 3.37 (3H, s, O*Me*), 3.37–3.32 (1H, m, C*H*), 2.34–2.13 (2H, m, CH_2), 1.94–1.83 (1H, m, C*H*) 1.41 (3H, s, *Me*), 1.40–1.31 (1H, m, C*H*), 1.25 (3H, s, *Me*); δ_C (75 MHz, $CDCl_3$) 135.6, 125.6, 111.5, 104.8, 85.3, 83.4, 82.5, 80.5, 65.9, 57.5, 27.9, 26.8, 26.4, 26.0. Anal. Calcd for $C_{14}H_{22}O_5$: C, 62.20; H, 8.20. Found: C, 62.30; H, 8.21.

4.4.6. (5*R*)-1,2-*O*-(-1-Methylethylidede)-3-*O*-allyl-5-*C*-vinyl-α-D-xylofuranose (25) and (5*S*)-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-vinyl-α-D-xylofuranose (26). Following the general procedure for Grignard reaction, a solution of aldehyde **12** (900 mg, 3.98 mmol) in THF was treated with vinylmagnesium bromide (12 mmol) to afford two diastereomeric products **25** (260 mg, 25%) and **26** (520 mg, 51%) as a readily separable mixture.

Compound **25**. Colourless oil; R_f=0.5 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −43.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3489, 3083, 1644, 1079, 1024 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 6.05–5.97 (1H, m, C*H*=), 5.99 (1H, d, J=3.6 Hz, anomeric C*H*), 5.93–5.86 (1H, m, C*H*=), 5.46–5.23 (4H, m, 2×CH_2=), 4.57 (1H, d, J=3.6 Hz, C*H*), 4.52 (1H, dd, J=5.2, 1.6 Hz, C*H*), 4.20–4.16 (1H, m, C*H*), 4.09 (1H, dd, J=6.4, 3.6 Hz, C*H*), 4.04 (1H, d, J=3.6 Hz, C*H*), 4.02–3.99 (1H, m, C*H*), 2.50 (1H, br s, O*H*), 1.49 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$) 137.7, 133.4, 118.4, 115.9, 111.7, 105.1, 82.7, 81.9, 81.4, 71.1, 70.6, 26.7, 26.2; LRMS (EI) $[M+Na]^+$279.0960; HRMS (EI) calcd for $C_{13}H_{20}O_5Na$ *m*/*z* 279.1208, found *m*/*z* 279.1207.

Compound **26**. Colourless oil; R_f=0.4 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −55.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3496, 3084, 1645, 1077, 1022 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.97 (1H, d, J=3.7 Hz, anomeric C*H*), 5.94–5.81 (2H, m, 2×CH_2=C*H*), 5.48–5.19 (4H, m, 2×CH_2=), 4.59 (1H, d, J=3.7 Hz, C*H*), 4.50 (1H, dd, J=7.3, 5.9 Hz, C*H*), 4.15 (1H, dd, J=12.8, 5.5 Hz, C*H*), 4.04 (1H, dd, J=6.9, 3.3 Hz, C*H*), 3.94 (1H, dd, J=12.5, 5.9 Hz, 1H), 3.86 (1H, d, J=3.3 Hz, C*H*), 2.97 (1H, br s, O*H*), 1.48 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (75 MHz, $CDCl_3$) 135.9, 133.6, 117.9, 117.1, 111.8, 104.9, 83.2, 82.3, 82.2, 71.0, 70.9, 26.8, 26.3. Anal. Calcd for $C_{13}H_{20}O_5$: C, 60.92; H, 7.87. Found: C, 60.97; H, 7.97.

4.4.7. (5*S*)-5-*O*-Methyl-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-vinyl-α-D-xylofuranose (31). Following the general procedure for methylation reaction, a solution of **26** (740 mg, 2.88 mmol) in THF was treated with sodium hydride (460 mg, 8.66 mmol, 60% in dispersion in oil) and methyl iodide (0.36 mL, 2.54 mmol) in the presence of a catalytic amount of TBAI. The crude product was purified by silica gel chromatography (hexanes/ethyl acetate 20:1) to give **31** (680 mg, 87%) as a colourless oil. R_f=0.6 (20%, EtOAc/hexane); $[\alpha]_D^{25}$ −42.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3081, 1645, 1078 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.97 (1H, d, J=3.7 Hz, anomeric C*H*), 5.93–5.80 (1H, m, CH_2=C*H*), 5.75–5.63 (1H, m, CH_2=C*H*), 5.43–5.18 (4H, m, 2×CH_2=), 4.54 (1H, d, J=3.7 Hz, C*H*), 4.13–4.07 (2H, m, C*H*), 3.98–3.84 (2H, m, CH_2), 3.72 (1H, d, J=2.9 Hz, C*H*), 3.36 (3H, s, O*Me*), 1.48 (3H, s, *Me*), 1.31 (3H, s, *Me*); δ_C (75 MHz, $CDCl_3$) 133.9, 133.8, 119.6, 117.5, 111.7, 105.3, 82.5, 81.9, 81.5, 81.0, 70.9, 56.7, 26.8, 26.3. Anal. Calcd for $C_{14}H_{22}O_5$: C, 62.20; H, 8.20. Found: C, 61.92; H, 8.17; HRMS (EI) calcd for $C_{14}H_{22}O_5Na$ *m*/*z* 293.1365, found *m*/*z* 293.1354.

4.4.8. 2,2-Dimethyl-3a,3b,5,8,8a,9a-hexahydro-1,3,4,9-tetraoxa-cyclopenta[*a*]azulen-8-ol (35). Following the general procedure B, from 120 mg (0.46 mmol) of **26**, 0.013 mL (0.046 mmol) of $Ti(OiPr)_4$ and 38 mg (0.046 mmol) of Grubb's catalyst, 90 mg (84%) of **35** was obtained as a colourless oil. R_f=0.3 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −18.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3483, 2929, 1652, 1087 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.96 (1H, d, J=3.6 Hz, anomeric C*H*), 5.59–5.56 (1H, m, CH_2=C*H*), 5.36–5.33 (1H, m, CH_2=C*H*), 4.89–4.85 (1H, m, C*H*), 4.58 (1H, d, J=3.6 Hz, C*H*), 4.51 (1H, dd, J=17.2, 2.4 Hz, C*H*), 4.44 (1H, dd, J=8.4, 4.8 Hz, C*H*), 4.15 (1H, dd, J=7.2, 2.4 Hz, C*H*), 4.05 (1H, d, J=3.6 Hz, C*H*), 2.44 (1H, br s, O*H*), 1.48 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$); δ 130.3, 126.0, 112.2, 105.2, 88.2, 85.9, 83.3, 72.3, 70.9, 27.3, 26.7; LRMS (EI) $[M+Na]^+$251.0827; HRMS (EI) calcd for $C_{11}H_{16}O_5Na$ *m*/*z* 251.0895, found *m*/*z* 251.0898.

4.4.9. (5*R*)-1,2-*O*-(-1-Methylethylidede)-3-*O*-allyl-5-*C*-allyl-α-D-xylofuranose (27) and (5*S*)-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-allyl-α-D-xylofuranose (28). A stirring solution of the aldehyde **12** (1.30 g, 5.75 mmol) and allyl bromide (1.47 mL, 17.25 mmol) was added a saturated aqueous solution of NH_4Cl/THF (5:1, 25 mol). The reaction mixture was cooled to 0 °C and zinc dust (2.25 g, 34.51 mmol) was slowly added to the reaction mixture and stirring was continued at 0 °C for 1 h. The reaction mixture was filtered and extracted with ethyl acetate, and the extracts were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo, and purified in a silica gel column chromatography afforded two diastereomeric products **27** (931 mg, 60%) and **28** (186 mg, 12%) as a readily separable mixture.

Compound **27**. Colourless oil; R_f=0.6 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −53.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3504, 3077, 1642, 1077, 1025 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.96–5.84 (2H, m, 2×CH_2=C*H*), 5.93 (1H, d, J=3.6 Hz, anomeric C*H*), 5.35–5.12 (4H, m, 2×CH_2=), 4.58 (1H, d, J=3.6 Hz, C*H*), 4.19 (1H, dd, J=12, 4.8 Hz, C*H*), 4.07–3.96 (4H, m, CH_2 and 2×C*H*), 2.54–2.29 (3H, m, CH_2 and OH), 1.48 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (100 MHz, $CDCl_3$); δ 134.3, 133.7, 118.0, 117.9, 111.5, 104.9, 82.1, 81.9, 81.7, 70.9, 68.2, 39.0, 26.7, 26.2. Anal. Calcd for $C_{14}H_{22}O_5$: C, 62.20; H, 8.20. Found: C, 62.67; H, 8.88; HRMS (EI) calcd for $C_{14}H_{22}O_5Na$ *m*/*z* 293.1365, found *m*/*z* 293.1368.

Compound **28**. Colourless oil; R_f=0.5 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −63.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3510, 3076, 1642, 1077, 1018 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.98 (1H, d, J=3.6 Hz, anomeric C*H*), 5.95–5.81 (2H, m, 2×CH_2=C*H*), 5.34–5.09 (4H, m, 2×CH_2=), 4.58 (1H, d, J=3.6 Hz, C*H*), 4.19 (1H, dd, J=12.6, 5.1 Hz, C*H*), 4.09–3.95

(3H, m, CH_2 and C*H*), 3.92 (1H, d, J=3.3 Hz, C*H*), 2.29 (2H, t, J=6.3 Hz, CH_2), 1.49 (3H, s, *Me*), 1.33 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$) 134.5, 133.3, 118.3, 117.5, 111.8, 104.8, 82.9, 82.4, 81.8, 70.8, 69.6, 37.8, 26.8, 26.4; HRMS (EI) Calcd for $C_{14}H_{22}O_5Na$ *m/z* 293.1365, found *m/z* 293.1368.

4.4.10. (5*R*)-5-*O*-Methyl-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-allyl-α-D-xylofuranose (32). Following the general procedure for methylation reaction, a solution of **27** (700 mg, 2.58 mmol) in THF was treated with sodium hydride (310 mg, 7.76 mmol, 60% in dispersion in oil) and methyl iodide (0.32 mL, 5.17 mmol) in presence of catalytic amount of TBAI. The crude product was purified by silica gel chromatography (hexanes/ethyl acetate 20:1) to give **32** (680 mg, 92%) as a colourless oil. R_f=0.8 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −75.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3077, 1642, 1079, 1023 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.96–5.86 (3H, m, anomeric C*H* and 2×CH_2=C*H*), 5.33–5.10 (4H, m, 2×CH_2=), 4.55 (1H, d, J=3.6 Hz, C*H*), 4.15 (1H, dd, J=12.4, 6.4 Hz, C*H*), 4.05–4.00 (2H m, CH_2), 3.93 (1H, d, J=2.8 Hz, C*H*), 3.65–3.54 (1H, m, C*H*), 3.39 (3H, s, O*Me*), 2.64–2.60 (1H, m, C*H*), 2.35–2.29 (1H, m, C*H*), 1.46 (3H, s, *Me*), 1.31 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$); δ 134.1, 133.9, 117.6, 117.4, 111.7, 104.9, 82.2, 81.4, 80.5, 76.5, 71.1, 57.4, 34.9, 26.8, 26.4. Anal. Calcd for $C_{15}H_{24}O_5$: C, 63.36; H, 8.51. Found: C, 64.04; H, 8.64; HRMS (EI) calcd for $C_{15}H_{24}O_5Na$ *m/z* 307.1521, found *m/z* 307.1518.

4.4.11. 9-Methoxy-2,2-dimethyl-3a,5,8,9,9a,10a-hexahydro-3b*H*-1,3,4,10-tetraoxa-cycloocta[*a*]pentalene (36). Following the general procedure for ring closing metathesis (method B), from 200 mg (0.70 mmol) of **32**, 0.020 mL (0.07 mmol) of Ti(O*i*Pr)$_4$ and 57 mg (0.07 mmol) of Grubb's catalyst, 122 mg (68%) of **36** was obtained as a colourless oil. R_f=0.40 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −81.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 2929, 1656, 1138, 1097 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 5.95 (1H, d, J=3.9 Hz, anomeric C*H*), 5.87–5.76 (1H, m, C*H*=), 5.43–5.37 (1H, m, C*H*=), 4.62–4.59 (1H, m, C*H*), 4.55 (1H, dd, J=3.9, 1.5 Hz, C*H*), 4.34 (1H, t, J=2.7 Hz, C*H*), 4.03–3.94 (1H, m, C*H*), 3.86–3.85 (1H, m, C*H*), 3.49–3.41 (1H, m, C*H*), 3.41 (3H, s, O*Me*), 3.40–3.16 (1H, m, C*H*), 2.09–2.01 (1H, m, C*H*), 1.51 (3H, s, *Me*), 1.33 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$) 128.3, 127.7, 111.9, 104.5, 85.9, 85.1, 81.2, 72.2, 56.5, 29.7, 27.0, 26.9, 26.5; HRMS (EI) calcd for $C_{13}H_{20}O_5Na$ *m/z* 279.1208, found *m/z* 279.1209.

4.4.12. (5*R*)-1,2-*O*-(-1-Methylethylidede)-3-*O*-allyl-5-*C*-pentenyl-α-D-xylofuranose (29) and (5*S*)-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-pentenyl-α-D-xylofuranose (30). Following the general procedure for Grignard reaction, a solution of aldehyde **12** (280 mg, 1.23 mmol) in THF was treated with 5-bromo-1-pentenemagnesium bromide (9.32 mmol) afforded two diastereomeric products **29** (134 mg, 38%) and **30** (138 mg, 39%) as a readily separable mixture.

Compound **29**. Semisolid; R_f=0.6 (33%, EtOAc/hexane); $[\alpha]_D^{25}$ −33.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3497, 3077, 1641, 1077 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.96 (1H, d, J=3.9 Hz, anomeric C*H*), 5.94–5.75 (2H, m, 2×CH_2=C*H*), 5.36–5.23 (2H, m, CH_2=), 5.06–4.93 (2H, m, CH_2=), 4.58 (1H, d, J=3.9 Hz, C*H*), 4.24–4.17 (1H, m, C*H*), 4.04–3.91 (3H, m, CH_2 and C*H*), 3.99 (1H, d, J=3.3 Hz, C*H*), 2.11–2.09 (2H, m, CH_2), 1.72–1.65 (2H, m, CH_2), 1.55–1.51 (2H, m, CH_2), 1.49 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$) 138.7, 133.4, 118.5, 114.6, 111.5 105.0, 82.5, 82.2, 81.9, 71.8, 69.7, 34.1, 33.7, 26.8, 26.2, 24.9; HRMS (EI) calcd for $C_{16}H_{26}O_5Na$ *m/z* 321.1678, found *m/z* 321.1678.

Compound **30**. Colourless oil; R_f=0.5 (33%, EtOAc/hexane); $[\alpha]_D^{25}$ −74.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3510, 3077, 1641, 1078 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.97 (1H, d, J=3.9 Hz, anomeric C*H*), 5.93–5.75 (2H, m, 2×CH_2=C*H*), 5.34–5.22 (2H, m, CH_2=), 5.05–4.93 (2H, m, CH_2=), 4.58 (1H, d, J=3.9 Hz, C*H*), 4.21–4.14 (1H, m, C*H*), 4.05–3.92 (3H, m, CH_2 and C*H*), 3.91 (1H, d, J=3.3 Hz C*H*), 2.44 (1H, br s, OH), 2.13–2.06 (2H, m, CH_2), 1.70–1.51 (4H, m, 2×CH_2), 1.49 (3H, s, *Me*), 1.33 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$) 138.7, 133.3, 118.3, 114.6, 111.8, 104.8, 82.8, 82.7, 82.4, 70.9, 69.7, 33.5, 32.4, 26.8, 26.3, 24.7; HRMS (EI) calcd for $C_{16}H_{26}O_5Na$ *m/z* 321.1678, found *m/z* 321.1678.

4.4.13. (5*S*)-5-*O*-Methyl-1,2-*O*-(-1-methylethylidede)-3-*O*-allyl-5-*C*-pentenyl-α-D-xylofuranose (33). Following the general procedure for methylation reaction, a solution of **30** (300 mg, 1.0 mmol) in THF was treated with sodium hydride (120 mg, 3.0 mmol, 60% in dispersion in oil) and methyl iodide (0.125 mL, 2.0 mmol) in the presence of catalytic amount of TBAI. The crude product purified by silica gel chromatography (hexanes/ethyl acetate 20:1) to give **33** (270 mg, 85%) as colourless oil. R_f=0.80 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −22.9 (*c* 1.0, $CHCl_3$); IR (neat) 3077, 1641, 1078, 1022 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.96 (1H, d, J=3.6 Hz, anomeric C*H*), 5.92–5.76 (2H, m, 2×CH_2=C*H*), 5.31–5.21 (2H, m, CH_2=), 5.04–4.95 (2H, m, CH_2=), 4.54 (1H, d, J=3.6 Hz, C*H*), 4.17–4.08 (2H, m, CH_2), 3.89 (1H, dd, J=13.2, 6 Hz, C*H*), 3.76 (1H, d, J=3.6 Hz, C*H*), 3.54–3.44 (1H, m, C*H*), 3.49 (3H, s, O*Me*), 2.10–2.05 (2H, m, CH_2), 1.64–1.35 (4H, m, 2×CH_2), 1.49 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$) 138.7, 133.6, 118.1, 114.6, 111.5, 105.0, 83.8, 82.2, 81.5, 79.3, 70.7, 59.2, 33.7, 30.3, 26.7, 26.3, 24.3; HRMS (EI) calcd for $C_{17}H_{28}O_5Na$ *m/z* 335.1834, found *m/z* 335.1822.

4.4.14. 11-Methoxy-2,2-dimethyl-3a,5,8,9,10,11,11a,12a-octahydro-3b*H*-1,3,4,12-tetraoxa-cyclodeca[*a*]pentalene (37). Following the general procedure for ring closing metathesis reaction (method B), from 120 mg (0.38 mmol) of **33**, 0.011 mL (0.038 mmol) of Ti(O*i*Pr)$_4$ and 31 mg (0.038 mmol) of Grubb's catalyst, 90 mg (82%) of **37** was obtained as a colourless oil. R_f=0.70 (25%, EtOAc/hexane); $[\alpha]_D^{25}$ −121.9 (*c* 1.0, $CHCl_3$); IR (neat) 2983, 1649, 1078 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 5.99 (1H, d, J=3.9 Hz, anomeric C*H*), 5.69–5.60 (2H, m, 2×C*H*=), 4.46 (1H, d, J=3.9 Hz, C*H*), 4.39–4.32 (1H, m, C*H*), 4.13 (1H, dd, J=9.3, 3.0 Hz, C*H*), 3.91 (1H, d, J=3.3 Hz, C*H*), 3.83 (1H, dd, J=6.0, 3.0 Hz, C*H*), 3.78–3.51 (1H, m, C*H*), 3.49 (3H, s, O*Me*), 2.81–2.70 (1H, m, C*H*), 2.02–1.98 (1H, m, C*H*), 1.81–1.51 (4H, m, 2×CH_2), 1.48 (3H, s, *Me*), 1.32 (3H, s, *Me*); δ_C (125 MHz, $CDCl_3$) 137.9, 125.9,

111.6, 105.7, 83.9, 81.8, 80.4, 78.9, 77.6, 63.8, 58.6, 28.9, 27.1, 26.8, 26.4; LRMS (EI) $[M+Na]^+$307.1321; HRMS (EI) calcd for $C_{15}H_{24}O_5Na$ *m/z* 307.1521, found *m/z* 307.1512.

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New antiproliferative 14,15-secopregnane glycosides from *Solenostemma argel*

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Abstract—Eight new 14,15-secopregnane glycosides, namely argelosides C–J, possessing two ketal functions involved in three five-membered rings, have been isolated from the hairy seeds of *Solenostemma argel*. Their structures have been established by MS and NMR experiments, combined with quantum mechanical calculations of the ^{13}C chemical shifts for the interpretation of the experimental data. On the basis of the obtained results, the structures of argelosides A and B have been revised. Additionally, the effect of these compounds on the VEGF-induced in Kaposi's sarcoma cell proliferation was evaluated. Results indicated that all the compounds reduced the cell proliferation in a dose-dependent manner.

1. Introduction

Plants belonging to the family Asclepiadaceae are frequently used in traditional medicine and have been reported to be rich in steroidal glycosides.[1,2] *Solenostemma argel* Hayne (Asclepiadaceae) is an Egyptian wild perennial erect shrub growing in the eastern desert and along the Nile in South Egypt,[3] whose leaves are commonly used in traditional medicine as a purgative, antipyretic, expectorant, antispasmodic and in cases of bile congestion.[4] Previous studies have reported the occurrence of monoterpenes,[5] pregnane glycosides,[6,7] and acylated phenolic glycosides in the leaves.[8] In previous communications, we reported the isolation of 15-keto pregnane glycosides[9] and 14,15-secopregnane glycosides[10] from the methanol extract of the pericarps. Since pregnane and their glycosides are attracting attention in recent years because of their antitumor and cytotoxic activities,[11–14] we continued with our studies on the pregnane glycoside constituents of the plant.

Here we report the occurrence of eight new 14,15-secopregnane glycosides, namely argelosides C–J (**1**–**8**), from the hairy seeds of *S. argel*. Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D NMR (DQF-COSY, HSQC, HMBC, and HOHAHA) experiments as well as ESI-MS analysis. To further validate the structural assignments, the proposed structures were confirmed by DFT calculations of the ^{13}C chemical shifts.

Recently, we reported the isolation of two 14,15-secopregnane glycosides, namely argelosides A and B, from the pericarps of *S. argel*. These glycosides were characterized by the presence of two hemiketalic functions on C-14 and C-20 involved in two five-membered rings. The structural elucidation was based on ESI-MS and NMR experiments (^{1}H, ^{13}C, 2D-HOHAHA, DQF-COSY, HSQC, HMBC). In particular, their relative configuration was defined by combining the 2D-ROESY and the ^{13}C NMR data with quantum chemical calculations of the geometries and ^{13}C chemical shifts, respectively. The presence of two hemiketalic functions on C-14 and C-20 was plausible by the observed HMBC correlations and from the molecular formulae obtained from HR-ESI-MS. On the basis of the evidence from structural elucidation of argelosides C–J, and for their structural homology with argelosides A and B, we

Keywords: *Solenostemma argel*; 14,15-Secopregnane; GIAO NMR; DFT calculations; Cell proliferation; Kaposi's sarcoma.
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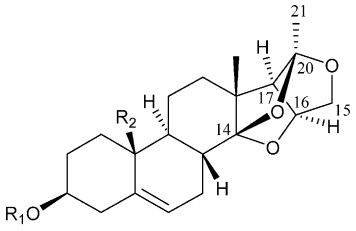
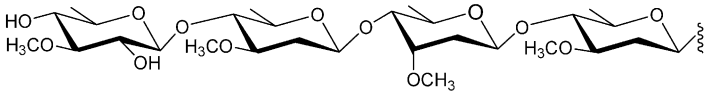
also focused our attention on the structural reinvestigation of the two latter products. In particular, our ongoing investigations on 14,15-secopregnane glycosides isolated from *S. argel* demonstrated the structures of argelosides A (**9**) and B (**10**) to be incorrect. The structural revision of argeloside A and B, based on the reexamination of the 1D, 2D NMR and MS data, was accompanied by quantum mechanical calculations of the ^{13}C chemical shifts. These results clearly indicated that the correct structures of argelosides A and B are characterized by the occurrence of two ketal functions in C-14 and C-20 involved in three five-membered rings as representated in **11** and **12**, respectively.



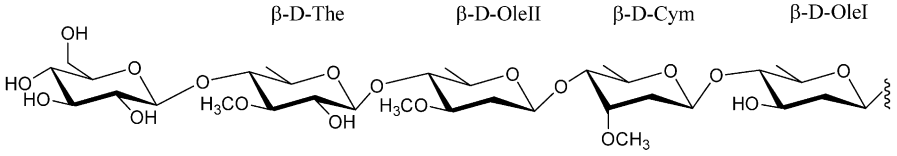
1	R_1 = A	R_2 = Me	**5**	R_1 = C	R_2 = Me
2	R_1 = A	R_2 = CH_2OH	**6**	R_1 = C	R_2 = CH_2OAc
3	R_1 = A	R_2 = CH_2OAc	**7**	R_1 = D	R_2 = Me
4	R_1 = B	R_2 = Me	**8**	R_1 = D	R_2 = CH_2OAc

A



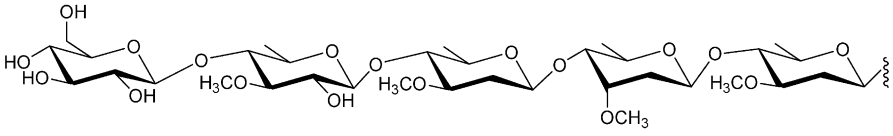
β-D-The β-D-OleII β-D-Cym β-D-OleI

B



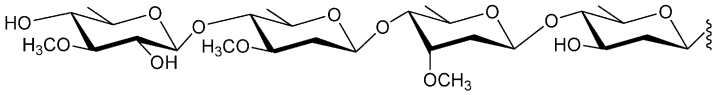
β-D-Glc β-D-The β-D-Ole β-D-Cym β-D-Can

C

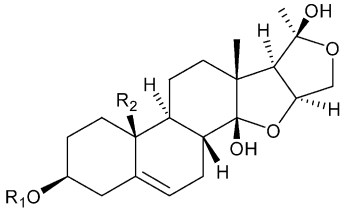


β-D-Glc β-D-The β-D-OleII β-D-Cym β-D-OleI

D

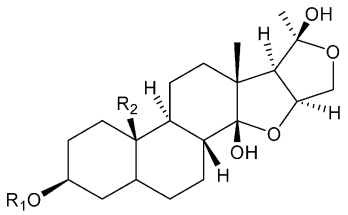


β-D-The β-D-Ole β-D-Cym β-D-Can



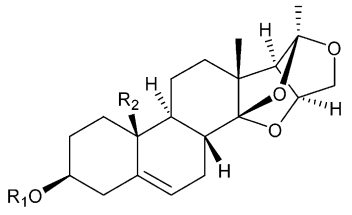
9 R_1 = B R_2 = CH_2OAc

argeloside A (unrevised structure)

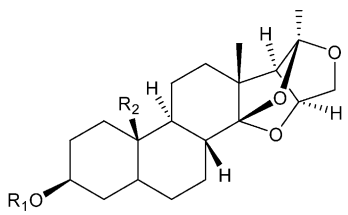


10 R_1 = E R_2 = CH_2OAc

argeloside B (unrevised structure)

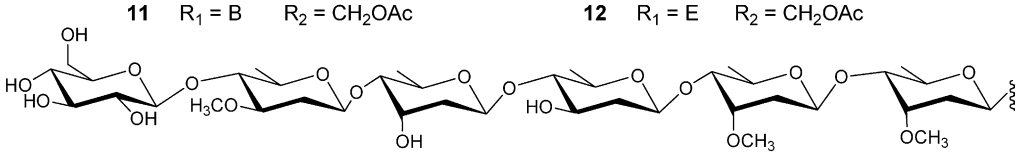


11 R_1 = B R_2 = CH_2OAc



12 R_1 = E R_2 = CH_2OAc

E



β-D-Glc β-D-Ole β-D-Dig β-D-Can β-D-CymII β-D-CymI

Finally, since pregnanes and their glycosides have shown to possess antitumor and cytotoxic activities[11–14] we examined whether compounds **1**–**8**, along with the previously isolated argelosides A and B,[10] can be useful in the control of tumor cell proliferation. Kaposi's sarcoma (KS) cells were used as a model to test the anti-proliferative properties of these compounds.

2. Results and discussion

2.1. Structural elucidation

The hairy seeds of *S. argel* were extracted with EtOH 70% and fractionated on Sephadex LH-20. The fractions containing pregnane glycosides were chromatographed by Si-gel MPLC and reversed-phase HPLC to yield eight new compounds **1**–**8** (see Section 3 for details).

Compounds **1**–**3** (Chain A). A detailed comparison of the sugar region NMR data (^{1}H, ^{13}C, HSQC, HMBC, DQF-COSY, 2D-HOHAHA) and ESI-MS data of compounds **1**, **2**, and **3** showed that the saccharide chain was identical in the three compounds. In particular, for the sugar portion, compound **1** showed in the ^{1}H NMR spectrum signals corresponding to four doublet methyls at δ 1.41 (3H, d, J=6.1 Hz), 1.30 (3H, d, J=6.1 Hz), 1.29 (3H, d, J=6.1 Hz) and 1.26 (3H, d, J=6.1 Hz), four methoxy groups at δ 3.65 (3H, s), 3.46 (3H, s), 3.45 (3H, s) and 3.44 (3H, s) as well as signals for four anomeric protons at δ 4.93 (1H, dd, J=9.5, 2.0 Hz), 4.68 (1H, dd, J=9.5, 2.0 Hz), 4.63 (1H, dd, J=9.6, 2.0 Hz) and 4.46 (1H, d, J=7.9 Hz) (Table 1). All these data indicated that the sugar chain of compound **1** consisted of four sugars, three of them being 2,6-dideoxy sugars and one of them being a 6-deoxy sugar. The chemical shifts of all the individual protons of the four sugar units were ascertained from a combination of 2D-HOHAHA and DQF-COSY spectral analysis, and the ^{13}C NMR chemical shifts of their attached carbons could be assigned unambiguously from the HSQC spectrum (see Table 1). These data showed the presence of one β-D-cymaropyranosyl unit (δ 4.93), two β-D-oleandropyranosyl units (δ 4.68 and 4.63), and one β-D-thevetopyranosyl unit (δ 4.46). Glycosidation shifts were observed for C-4$_{oleI}$ (δ 83.9), C-4$_{oleII}$ (83.8) and C-4$_{cym}$ (δ 83.6) suggesting that β-D-thevetopyranosyl was a terminal unit. An unambiguous determination of the sequence and

Table 1. ^{13}C and ^{1}H NMR data (J in Hz) of the sugar portions of compounds **1**–**8** (CD_3OD, 600 MHz)

	1–**3**, Chain A		**4**, Chain B		**5**–**6**, Chain C		**7**–**8**, Chain D	
		β-D-OleI		β-D-Can		β-D-OleI		β-D-Can
1	98.7	4.68 dd (9.5, 2.0)	98.9	4.72 dd (9.2, 1.6)	98.7	4.68 dd (9.6, 1.8)	98.9	4.72 dd (9.2, 1.5)
2	37.8	2.26 ddd (13.0, 4.0, 2.0)	39.7	2.14 ddd (13.0, 9.2, 9.0)	37.9	2.23 ddd (13.0, 4.0, 1.8)	40.2	2.14 ddd (13.0, 9.2, 9.0)
		1.41 ddd (13.0, 9.5, 9.0)		1.46 ddd (13.0, 4.0, 1.6)		1.39 ddd (13.0, 9.6, 9.0)		1.47 ddd (13.0, 4.0, 1.5)
3	80.0	3.39 ddd (9.5, 9.0, 4.0)	70.6	3.60 ddd (9.5, 9.0, 4.0)	80.1	3.38 ddd (9.5, 9.0, 4.0)	70.9	3.60 ddd (9.5, 9.0, 4.0)
4	83.9	3.15 dd (9.5, 9.5)	88.8	2.97 dd (9.5, 9.5)	83.9	3.14 dd (9.5, 9.5)	89.1	2.97 dd (9.5, 9.5)
5	72.1	3.35 dq (9.5, 6.1)	71.6	3.36 dq (9.5, 6.0)	72.0	3.36 dq (9.5, 6.1)	71.8	3.37 dq (9.5, 6.1)
6	18.3	1.29 d (6.1)	18.3	1.27 d (6.0)	18.1	1.28 d (6.1)	18.3	1.27 d (6.1)
OMe	57.4	3.44 s			57.3	3.45 s		
		β-D-Cym		β-D-Cym		β-D-Cym		β-D-Cym
1	99.4	4.93 dd (9.5, 2.0)	100.7	4.81 dd (9.5, 2.0)	99.2	4.92 dd (9.2, 2.0)	100.7	4.80 dd (9.6, 2.0)
2	36.2	2.17 m	36.1	2.21 m	36.2	2.16 m	36.4	2.22 m
		1.57 m		1.65 m		1.57 m		1.65 m
3	78.4	3.88 br m	78.2	3.89 br m	78.2	3.87 br m	78.3	3.91 br m
4	83.6	3.30 dd (9.5, 3.0)	83.3	3.36 dd (9.5, 3.0)	83.6	3.30 dd (9.5, 3.0)	83.8	3.36 dd (9.5, 3.0)
5	69.9	3.85 dq (9.5, 6.1)	70.3	3.95 dq (9.5, 6.0)	70.0	3.85 dq (9.5, 6.1)	70.5	3.96 dq (9.5, 6.1)
6	18.3	1.26 d (6.1)	18.4	1.27 d (6.0)	18.1	1.26 d (6.1)	18.2	1.27 d (6.1)
OMe	58.2	3.46 s	58.6	3.48 s	58.2	3.46 s	58.7	3.47 s
		β-D-OleII		β-D-Ole		β-D-OleII		β-D-Ole
1	102.5	4.63 dd (9.6, 2.0)	102.4	4.64 dd (9.0, 2.0)	102.4	4.62 dd (9.0, 2.0)	102.8	4.63 dd (9.6, 2.0)
2	37.4	2.35 ddd (13.0, 4.0, 2.0)	37.6	2.36 ddd (13.0, 4.0, 2.0)	37.5	2.34 ddd (13.0, 4.0, 2.0)	37.7	2.36 ddd (13.0, 4.0, 2.0)
		1.44 ddd (13.0, 9.6, 9.0)		1.45 ddd (13.0, 9.0, 9.0)		1.45 ddd (13.0, 9.0, 9.0)		1.45 ddd (13.0, 9.6, 9.0)
3	80.0	3.40 ddd (9.5, 9.0, 4.0)	80.2	3.41 ddd (9.5, 9.0, 4.0)	79.9	3.41 ddd (9.5, 9.0, 4.0)	80.4	3.41 ddd (9.5, 9.0, 4.0)
4	83.8	3.24 dd (9.5, 9.5)	84.2	3.24 dd (9.5, 9.5)	83.9	3.23 dd (9.5, 9.5)	84.3	3.23 dd (9.5, 9.5)
5	72.3	3.42 dq (9.5, 6.1)	72.3	3.41 dq (9.5, 6.2)	72.2	3.42 dq (9.5, 6.1)	72.6	3.42 dq (9.5, 6.1)
6	18.4	1.41 d (6.1)	18.5	1.40 d (6.2)	18.5	1.41 d (6.1)	18.8	1.40 d (6.1)
OMe	57.4	3.45 s	57.7	3.45 s	57.3	3.45	57.6	3.45 s
		β-D-The		β-D-The		β-D-The		β-D-The
1	104.1	4.46 d (7.9)	104.4	4.46 d (7.5)	104.4	4.47 d (7.5)	104.3	4.46 d (7.9)
2	75.4	3.24 dd (9.5, 7.9)	75.2	3.26 dd (9.5, 7.5)	75.1	3.27 dd (9.5, 7.5)	75.7	3.23 dd (9.5, 7.9)
3	87.6	3.06 m	86.4	3.21 dd (9.5, 9.5)	86.2	3.21 dd (9.5, 9.5)	87.9	3.06 m
4	76.6	3.06 m	82.8	3.38 dd (9.5, 9.5)	82.8	3.38 dd (9.5, 9.5)	76.5	3.05 m
5	72.9	3.31 m	72.5	3.46 m	72.5	3.47 m	72.9	3.30 m
6	18.1	1.30 d (6.1)	18.5	1.40 d (6.2)	18.5	1.41 d (6.1)	18.1	1.31 d (6.1)
OMe	60.8	3.65 s	61.1	3.66 s	61.1	3.66 s	61.1	3.65 s
				β-D-Glc		β-D-Glc		
1			104.4	4.44 d (7.5)	104.4	4.45 d (7.5)		
2			75.5	3.20 dd (9.0, 7.5)	75.5	3.20 dd (9.0, 7.5)		
3			78.0	3.37 dd (9.0, 9.0)	78.1	3.38 dd (9.0, 9.0)		
4			71.6	3.24 dd (9.0, 9.0)	71.6	3.24 dd (9.0, 9.0)		
5			78.2	3.28 ddd (9.0, 4.5, 2.0)	78.2	3.28 ddd (9.0, 4.5, 2.0)		
6			62.8	3.89 dd (12.0, 2.0)	62.8	3.88 dd (12.0, 2.0)		
				3.66 dd (12.0, 4.5)		3.66 dd (12.0, 4.5)		

linkage sites was obtained from the HMBC correlations which showed key correlation peaks between the proton signals at δ 4.68 (H-1_{oleI}) and the carbon resonances at δ 79.0 (C-3), 4.93 (H-1_{cym}) and 83.9 (C-4_{oleI}), 4.63 (H-1_{oleII}) and 83.6 (C-4_{cym}), and the proton signal at δ 4.46 (H-1_{the}) and the carbon resonance at δ 83.8 (C-4_{oleII}). Thus, the sugar sequence of compounds **1–3** was established as 3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside.

The HR-MALDI mass spectrum of **1** showed a major ion peak at *m/z* 961.5135 $[M+Na]^+$ ascribable to a molecular formula $C_{49}H_{78}O_{17}$ (calcd for $C_{49}H_{78}O_{17}Na$, 961.5137). The ^1H NMR spectrum of the aglycone portion showed signals for three methyl groups at δ 1.06 (3H, s), 1.20 (3H, s), and 1.65 (3H, s), one olefinic proton at δ 5.43 (1H, dd, $J=3.3$, 2.7 Hz), and one signal at δ 4.56 (1H, br m) corresponding to a secondary oxygenated carbon. In addition, the ^{13}C NMR spectrum showed for the aglycone moiety twenty-one signals suggesting the presence of a pregnane skeleton. The ^{13}C NMR chemical shifts of all the hydrogenated carbons could be assigned unambiguously by the HSQC spectrum. In particular, the analysis of the ^{13}C NMR spectrum on the basis of the HSQC correlations clearly showed the occurrence of one olefinic quaternary carbon (δ 141.3), one olefinic methine (δ 122.4), two secondary oxygenated carbons (δ 79.0 and 78.0), one primary oxygenated carbon (δ 71.9), and two ketal functions (δ 114.8 and 110.6) (see Table 2). The three six-membered rings of a pregnane skeleton were established from the analysis of the strong HMBC correlations of the protons of the angular methyls Me-18 and Me-19. Long-range correlations from the proton signal at δ 1.06 (Me-19) to the carbon resonances at δ 38.1 (C-1), 141.3 (C-5), 46.9 (C-9) and 37.3 (C-10), revealed the connectivity of the A and B rings. Similarly, the HMBC spectrum indicated correlations between the proton signals at δ 1.20 (Me-18) and the carbon resonances at δ 30.9 (C-12), δ 49.9 (C-13), δ 110.6 (C-14), and δ 58.9 (C-17) establishing the connectivity between the rings B and C. Finally, the ring D was deduced from the analysis of the HMBC and DQF-COSY spectra. In particular, the following key HMBC correlations were observed: δ_H 3.86 (H-15) and δ_C 78.0 (C-16), δ_C 58.9 (C-17), and δ_C 114.8 (C-20); δ_H 4.56 (H-16) and δ_C 71.9 (C-15) and δ_C 114.8 (C-20); and δ_H 1.65 (Me-21) and δ_C 58.9 (C-17) and δ_C 114.8 (C-20). On the other hand, the spin system H-15 (δ 3.86)/H-16 (δ 4.56)/H-17 (δ 2.52) was confirmed by the COSY spectrum. All this evidence allowed us to deduce that **1** was characterized by the opening of ring D between C-14 and C-15, and the occurrence of two ketal functions on C-14 and C-20 generating three five-membered rings with oxygenated functions in positions 16 and 15, respectively. Moreover, the structure of **1** was further confirmed by ESI-MS analysis in the following mode. The ESI mass spectrum of **1** acquired in MS^1 scanning mode gave the highest ion peak at *m/z* 961.4 $[M+Na]^+$ which was in accordance with the molecular formula $C_{49}H_{78}O_{17}$. The MS/MS analysis of this ion produced one intense peak at *m/z* 631.2 $[M+Na-330]^+$ due to the loss of the aglycone moiety. Another intense peak was observed at *m/z* 471.2 $[M+Na-330-160]^+$ corresponding, probably, to the loss of the oleandropyranosyl unit.

The relative stereochemistry of the aglycone portion of **1**

Table 2. ^{13}C and ^1H NMR data of the aglycone moieties of compounds **1–3** (600 MHz, CD_3OD)

Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.1	α 1.12 m	34.5	α 1.05 m	34.6	α 1.13 m
		β 1.93 m		β 2.06 m		β 2.10 m
2	30.4	α 1.94 m	30.7	α 1.91 m	30.4	α 1.96 m
		β 1.59 m		β 1.47 m		β 1.49 m
3	79.0	3.54 m	78.9	3.59 m	79.0	3.59 m
4	39.6	α 2.41 m	39.7	α 2.46 m	39.6	α 2.46 m
		β 2.21 m		β 2.24 m		β 2.25 m
5	141.3		136.0		135.5	
6	122.4	5.43 dd (3.3, 2.7)	126.1	5.69 dd (3.3, 2.7)	126.5	5.68 dd (3.3, 2.7)
7	25.6	α 2.01 m	25.2	α 2.06 m	25.1	α 2.07 m
		β 2.19 m		β 2.13 m		β 2.14 m
8	32.1	1.92 m	33.0	2.43 m	33.1	2.25 m
9	46.9	1.52 m	46.8	1.47 m	46.3	1.56 m
10	37.3		46.1		46.4	
11	20.4	α 1.68 m	21.1	α 1.68 m	20.9	α 1.74 m
		β 1.65 m		β 1.63 m		1.71 m
12	30.9	α 1.71 m	31.3	α 1.69 m	30.9	α 1.70 m
		β 1.56 m		β 1.57 m		β 1.59 m
13	49.9		49.4		49.4	
14	110.6		110.4		110.0	
15	71.9	3.86 m	71.9	3.86 m	71.8	3.87 m
		3.86 m		3.86 m		3.87 m
16	78.0	4.56 br m	78.1	4.56 br m	77.9	4.56 br m
17	58.9	2.52 d (2.2)	58.5	2.50 d (2.2)	58.7	2.53 d (2.2)
18	15.5	1.20 s	15.7	1.23 s	15.5	1.20 s
19	19.3	1.06 s	62.9	3.60 d (11.5)	64.9	4.02 d (11.8)
				3.89 d (11.5)		4.63 (11.8)
20	114.8		114.7		114.7	
21	23.5	1.65 s	23.5	1.65 s	23.3	1.65 s
*C*OMe					171.8	
CO*Me*					20.6	2.04 s

was deduced by analysis of the 2D-ROESY data. The observed ROESY correlations between H-16 (δ 4.56) and H-17 (δ 2.52), and between H-17 and Me-21 (1.65), and the lack of a ROESY correlation between Me-18 (δ 1.20) and H-16 suggested the α-orientation of H-16, H-17, and Me-21. A strong ROE cross peak between H-16 and H-12α (δ 1.71) revealed the *cis* CD ring junction. The strong ROE correlations between Me-18 and H-8 (δ 1.92), and between Me-19 (δ 1.06) and H-8, along with the dipolar correlations of H-1α (δ 1.12) with H-3 (δ 3.54) and H-9 (δ 1.52), allowed us to deduce the *trans* BC ring junction.

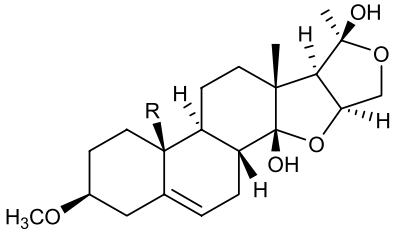
The HR-MALDI-MS of argeloside D (**2**) (*m/z* 977.5247 $[M+Na]^+$, calcd for $C_{49}H_{78}O_{18}Na$, 977.5086) and argeloside E (**3**) (*m/z* 1019.5142 $[M+Na]^+$, calcd for $C_{51}H_{80}O_{19}Na$, 1019.5192) supported a molecular formula of $C_{49}H_{78}O_{18}$ and $C_{51}H_{80}O_{19}$, respectively. The ESI mass spectrum of **2** gave the highest mass ion peak at *m/z* 977.5 which was assigned to the $[M+Na]^+$ ion. The MS/MS analysis of the ion at *m/z* 977.5 showed the most intense ion at *m/z* 947.5 $[M+Na-30]^+$ ascribable to the loss of a CH_2O molecule. A further fragmentation of this ion produced two intense ion peaks at *m/z* 631.2 $[M+Na-30-316]^+$ due to the loss of the aglycone moiety, and 471.0 *m/z* $[M+Na-30-316-160]^+$ corresponding, probably, to the loss of an oleandropyranosyl unit. The ^1H NMR spectrum of the aglycone portion of **2** in comparison to that of **1** showed in addition the presence of two signals at δ 3.60 (1H, d, $J=11.5$ Hz) and δ 3.89 (1H, d, $J=11.5$ Hz), and the absence of the methyl signal at δ 1.06 (3H, s, Me-19). Moreover, a detailed analysis of the ^{13}C NMR data and the HSQC and HMBC correlations, suggested that compound **2** differed from **1** only by the occurrence of a primary alcoholic function at C-19.

The ESI mass spectrum of **3** showed a major ion peak at *m/z* 1019.6 $[M+Na]^+$. Its MS/MS fragmentation showed an intense peak at *m/z* 959.4 $[M+Na-60]^+$ due to the loss of an acetate molecule. The MS^3 fragmentation of this ion showed two intense ions at *m/z* 631.2 $[M+Na-60-328]^+$ corresponding to the loss of the aglycone moiety, and *m/z* 471.1 $[M+Na-60-328-160]^+$ ascribable, probably, to the loss of an oleandropyranosyl unit. The NMR data (^1H, ^{13}C, HSQC, HMBC, DQF-COSY) of **3** closely resembled to that of **1** suggesting that compound **3** only differed from **1** by the presence of an acetoxy function at C-19.

It was observed that the ^1H and ^{13}C NMR chemical shifts corresponding to rings D and E of the aglycone of compound **1** were almost superimposable to those of the previously reported argelosides A and B.[10] In that previous report, from the NMR (^1H, ^{13}C, DQF-COSY, 2D-HOHAHA, HSQC, HMBC, 2D-ROESY) and MS data we were prompted to establish argelosides A (**9**) and B (**10**) as 14,15-secopregnane glycosides characterized by the occurrence of two hemiketalic functions on C-14 and C-20 generating two five-membered rings with oxygenated functions in positions 16 and 15, respectively.[10] In that case, we observed in the MS spectrum a major ion peak at *m/z* 1162.5765 corresponding to the molecular formula $C_{56}H_{90}O_{25}$ for argeloside A and a major ion peak at *m/z* 1278.6601 corresponding to the molecular formula $C_{62}H_{102}O_{27}$ for argeloside B. In addition, minor peaks at *m/z* 1144.5666 for argeloside A and at *m/z* 1260.6503 for argeloside B were attributed to the loss of a H_2O molecule. This interpretation, along with the absence of any literature report for this novel 14,15-secopregnane skeleton, led to the establishment of two hemiketalic functions in the molecule.

Since the structures of the aglycones of argelosides A (**9**) and B (**10**) were in disagreement to that proposed for **1**, we decided to reinvestigate their structures. The HR-MALDI-MS of argeloside A exhibited a major ion peak at *m/z* 1167.5568 $[M+Na]^+$ corresponding to the molecular formula $C_{56}H_{88}O_{24}$, and the HR-MALDI-MS of argeloside B exhibited a major ion peak at *m/z* 1283.6411 $[M+Na]^+$ corresponding to the molecular formula $C_{62}H_{100}O_{26}$. Thus, on the basis of MS and NMR data (^1H, ^{13}C, DQF-COSY, 2D-HOHAHA, HSQC, 2D-ROESY and HMBC) it was possible to deduce that argelosides A and B displayed two ketal functions on C-14 and C-20 instead of two hemiketalic functions as previously reported. Once again, from the DQF-COSY and HMBC correlations it was deduced that as in the case of **1**, the two ketal functions on C-14 and C-20 were involved in three five membered rings with oxygenated functions in positions 16 and 15, respectively. Thus, the structures of argelosides A and B were revised as **11** and **12**, respectively.

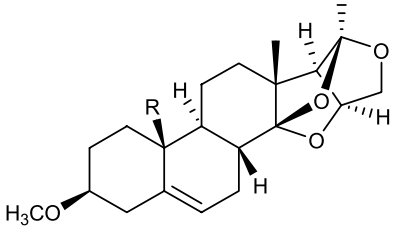
In order to confirm the structures of compounds **1**–**2**, and argeloside A (**11**), with particular regard to the presence of the two ketal functions on C-14 and C-20 instead of two hemiketalic functions previously reported, we performed quantum mechanical calculations of the ^{13}C chemical shifts[15] on the open (**1a**, **2a**, and **11a**, respectively) and closed (**1b**, **2b**, and **11b**) compounds taking into consideration only the aglycone moiety of compounds **1**–**2** and **11**, and replacing the sugar portion, not relevant for our analysis, with a methyl group.



1a R = Me

2a R = CH_2OH

11a R = CH_2OAc



1b R = Me

2b R = CH_2OH

11b R = CH_2OAc

In particular, a preliminar molecular mechanics and dynamics study of the six compounds afforded six minimum energy conformers that were further refined by a DFT geometry optimization, using the mPW1PW91 functional and the 6-31G(d) basis set. In accordance with a fast and efficient protocol recently reported in literature,[16] ^{13}C GIAO chemical shifts (c.s.) calculations at

Table 3. ^{13}C NMR data of the aglycone moieties of compounds **1**, **2**, and **11** (150 MHz, CD_3OD) and ^{13}C calculated (MPW1PW91/6-31G(d,p) level) chemical shifts for the open (**1a**, **2a**, and **11a**) and closed (**1b**, **2b**,and **11b**) simplified compounds

Position	**1** δ_C exp	**1a** δ_C calc	**1b** δ_C calc	**2** δ_C exp	**2a** δ_C calc	**2b** δ_C calc	**11** δ_C exp	**11a** δ_C calc	**11b** δ_C calc
1	38.1	37.5	38.2	34.5	35.8	34.5	34.6	31.8	31.8
2	30.4	26.3	30.2	30.7	30.5	26.8	30.4	30.0	30.1
3	79.0	77.9	77.7	78.9	77.8	77.5	78.5	77.0	76.9
4	39.6	40.9	37.9	39.7	37.5	40.6	39.6	38.5	38.6
5	141.3	134.7	135.9	136.0	130.8	132.2	135.5	131.6	132.1
6	122.4	123.2	122.3	126.1	127.3	128.6	126.5	126.9	126.8
7	25.6	27.1	26.9	25.2	26.9	26.3	25.1	26.9	26.9
8	32.1	**39.6**	33.0	33.0	**40.5**	34.3	33.1	**37.7**	33.2
9	46.9	45.4	45.5	46.8	45.5	45.3	46.3	45.4	45.5
10	37.3	38.7	38.9	46.1	42.6	43.2	46.4	42.8	42.8
11	20.4	23.2	22.2	21.1	23.1	22.6	20.9	22.9	22.9
12	30.9	**40.9**	30.9	31.3	**41.4**	31.2	30.9	**37.8**	30.9
13	49.9	48.2	49.6	49.4	48.6	50.0	49.4	50.8	49.5
14	110.6	107.4	106.4	110.4	107.5	106.5	110	104.8	106.0
15	71.9	79.4	75.8	71.9	79.6	75.9	71.8	80.3	70.2
16	78.0	72.1	70.2	78.1	72.0	70.0	77.9	67.1	75.8
17	58.9	**64.7**	58.4	58.5	**64.6**	58.7	58.7	61.3	58.8
18	15.5	16.2	16.6	15.7	16.1	16.9	15.5	16.5	16.6
19	19.3	21.1	20.9	62.9	64.7	61.8	64.9	65.6	65.8
20	114.8	**102.5**	111.4	114.7	**102.8**	111.5	114.7	**105.9**	111.5
21	23.5	28.4	24.1	23.5	28.4	24.2	23.3	25.5	24.1

The calculated values significantly different from the experimental are in bold.

mPW1PW91/6-31G(d,p) on the optimized structures were subsequently performed and the obtained calculated c.s. were then compared to the experimental. As it is shown in Table 3, at a first glance both the open and the closed compounds well represent the corresponding experimental data, due to the small differences for the two different structural hypotheses proposed for **1**, **2**, and **11**. Nevertheless, the Mean Absolute Errors (MAE), Figure 1, found for the calculated chemical shifts versus the experimental values, obtained for the open models and their closed counterparts, show very clearly a great accordance of **1b**, **2b** and **11b** with the corresponding experimental. In particularly, while MAE values of around 1.5 ppm were found for **1b**, **2b** and **11b**, compounds **1a**, **2a**, and **11a** displayed average errors of about 3 ppm. Moreover, the careful examination of selected crucial carbon atoms, mostly influenced by the presence of a third cyclization between C-14 and C-20, show high discrepancies for C-8, C-12, C-17, and C-20 of **1a** (see Table 1), and of **2a**, and for C-8, C-12, and C-20 for **11a**, finally confirming the two ketal

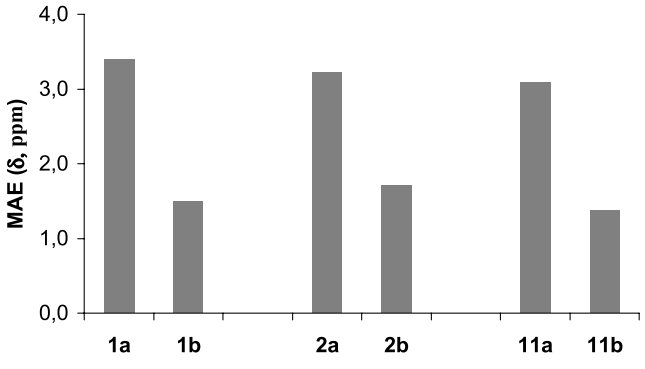


Figure 1. Mean absolute error (MAE) found for the ^{13}C NMR calculated chemical shifts of compounds **1a**, **1b**, **2a**, **2b**, **11a**, **11b** versus the ^{13}C experimental values. $MAE = \Sigma[|(\delta_{exp} - \delta_{calcd})|]/n$, summation through n of the absolute error values (difference of the absolute values between corresponding experimental and calculated ^{13}C chemical shifts), normalized to the number of the carbon atoms considered.

functions on C-14 and C-20 for the new compounds and for the previously described argelosides A and B. In particular, the experimental values for C-20, involved in the proposed ketal function, of 114.8, 114.7, and 114.7 for **1**, **2**, and **11**, respectively, is well reproduced for model compounds **1b**, **2b**, and **11b** (111.5, 111.4, and 111.4, respectively); the corresponding results for compounds **1a**, **2a**, and **11a** range between 102.5 and 105.9 (see Table 1), suggesting the exclusion of the hemiketalic function at C-20.

On the basis of these results, compound **1** was identified as the new (14*S*,16*S*,20*R*)-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside named argeloside C, compound **2** as the new (14*S*,16*S*,20*R*)-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3,19-diol-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside named argeloside D, and compound **3** as the new (14*S*,16*S*,20*R*)-19-acetoxy-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside named argeloside E. Furthermore, the structures of argelosides A and B were definitively determined as (14*S*,16*S*,20*R*)-19-acetoxy-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranoside (**11**) and (14*S*,16*S*,20*R*)-19-acetoxy-14,16-14,20-15,20-triepoxy-14,15-secopregnan-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside (**12**), respectively.

Compound **4** (Chain B). Its molecular formula was unequivocally established to be $C_{54}H_{86}O_{22}$ by HR-MALDI-MS (*m/z* 1109.5607 $[M+Na]^+$, calcd for

$C_{54}H_{86}O_{22}Na$, 1109.5508). The ^1H and ^{13}C NMR chemical shifts of the aglycone moieties of **4** and **1** were almost superimposable (see Section 3) suggesting the same aglycone portion. Additionally for **4**, resonances of anomeric protons were observed in the ^1H NMR spectrum at δ 4.81 (1H, dd, J=9.5, 2.0 Hz), 4.72 (1H, dd, J=9.2, 1.6 Hz), 4.64 (1H, dd, J=9.0, 2.0 Hz), 4.46 (1H, d, J=7.5 Hz) and 4.44 (1H, d, J=7.5 Hz). Complete assignments of the ^1H and ^{13}C NMR signals of the sugar portion were accomplished by HSQC, HMBC, DQF-COSY and 2D-HOHAHA experiments which led to the identification of one β-D-cymaropyranosyl unit (δ 4.81), one β-D-canaropyranosyl unit (δ 4.72), one β-D-oleandropyranosyl unit (δ 4.64), one β-D-thevetopyranosyl unit (δ 4.46) and one β-D-glucopyranosyl (δ 4.44) unit. Once again, direct evidence of the sugar sequence and the linkage sites was derived from HSQC and HMBC experiments. The absence of any ^{13}C glycosidation shift for the glucopyranose residue suggested that this sugar was the terminal unit, while the glycosidation shifts on C-4_{cym} (δ 83.3), C-4_{can} (δ 88.8), C-4_{ole} (δ 84.2) and C-4_{the} (δ 82.8) indicated the linkage sites. Key correlation peaks were observed in the HMBC spectrum of **4** between the proton signals at δ 4.72 (H-1_{can}) and the carbon resonances at δ 79.0 (C-3), 4.81 (H-1_{cym}) and 88.8 (C-4_{can}), 4.64 (H-1_{ole}) and 83.3 (C-4_{cym}), 4.46 (H-1_{the}) and 84.2 (C-4_{ole}) and the proton signal at δ 4.44 (H-1_{glc}) and the carbon resonance at δ 82.8 (C-4_{the}). Therefore, the sugar sequence of compound **4** was established as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranoside, and compound **4** as (14*S*,16*S*,20*R*)-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranoside named argeloside F.

Compounds **5** and **6** (Chain C). The molecular formula of compounds **5** and **6** was unequivocally established to be $C_{55}H_{88}O_{22}$ and $C_{57}H_{90}O_{24}$ by HR-MALDI-MS (*m/z* 1123.5660 $[M+Na]^+$, calcd for $C_{55}H_{88}O_{22}Na$, 1123.5665) and (*m/z* 1181.5751 $[M+Na]^+$, calcd for $C_{57}H_{90}O_{24}Na$, 1181.5720), respectively. It was apparent from their ^1H and ^{13}C NMR data that **5** possessed the same aglycone as **1**, while **6** was based on the same aglycone as **3** (see Section 3). Moreover, the ^1H and ^{13}C NMR spectra of the sugar portions of **5** and **6** were almost superimposable, suggesting that the sugar chain was identical in both of the compounds. In particular, compound **5** showed in the ^1H NMR spectrum resonances for five anomeric protons at δ 4.92 (1H, dd, J=9.2, 2.0 Hz), 4.68 (1H, dd, J=9.6, 1.8 Hz), 4.62 (1H, dd, J=9.0, 2.0 Hz), 4.47 (1H, d, J=7.5 Hz) and 4.45 (1H, d, J=7.5 Hz) (Table 1). A detailed analysis of the NMR data (^1H, ^{13}C, 2D-HOHAHA, DQF-COSY, HSQC) showed that **5** differed from **1** only in the occurrence of an additional β-D-glucopyranosyl terminal unit (δ 4.45). The linkage position of the β-D-glucopyranosyl unit was deduced from the HSQC and HMBC spectra. It was observed that the signal of C-4 of thevetose was significantly shifted to a downfield value of δ 82.8 when compared with compound **1** in which it resonated at δ 76.6. Finally, direct connectivity information was obtained from the HMBC spectrum, which showed the following key correlation peaks: δ_H 4.68 (H-1_{oleI}) and δ_C 79.0 (C-3), δ_H 4.92 (H-1_{cym}) and δ_C 83.9 (C-4_{oleI}), δ_H 4.62 (H-1_{oleII}) and δ_C 83.6 (C-4_{cym}), δ_H 4.47 (H-1_{the}) and δ_C 83.9 (C-4_{oleII}), and δ_H 4.45 (H-1_{glc}) and δ_C 82.8 (C-4_{the}). On the basis of all this evidence, the sugar chain of compounds **5** and **6** was deduced as 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside. Thus, the structures (14*S*,16*S*,20*R*)-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside and (14*S*,16*S*,20*R*)-19-acetoxy-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranoside were assigned to argeloside G (**5**) and argeloside H (**6**), respectively.

Compounds **7** and **8** (Chain D). The HR-MALDI-MS of compounds **7** (*m/z* 947.4948 $[M+Na]^+$, calcd for $C_{48}H_{76}O_{17}Na$, 947.4980) and **8** (*m/z* 1005.5039 $[M+Na]^+$, calcd for $C_{50}H_{78}O_{19}Na$, 1005.5035) supported a molecular formula of $C_{48}H_{76}O_{17}$ and $C_{50}H_{78}O_{19}$, respectively. The NMR data (^1H, ^{13}C, 2D-HOHAHA, DQF-COSY, HSQC, HMBC) of **7** and **8** in comparison to those of **1** and **3**, respectively, revealed that compounds **7** and **8** differed from **1** and **3**, only by replacement of the inner β-D-oleandropyranosyl unit with a β-D-canaropyranosyl unit. Therefore, compounds **7** and **8** were established as (14*S*,16*S*,20*R*)-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranoside named argeloside I, and (14*S*,16*S*,20*R*)-19-acetoxy-14,16-14,20-15,20-triepoxy-14,15-secopregn-5-en-3-ol-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranoside named argeloside J, respectively.

2.2. Anti-proliferative activity

KS cells produce and respond to various soluble mediators; in particular, IL-1, IL-6, VEGF, and GM-CSF, as well as Tat-protein are known to stimulate KS cell growth in vitro.[17–19] Furthermore, fibroblast growth factor (FGF), VEGF, and hepatocyte growth factor (HGF) have been postulated to be involved in angiogenesis.[20] In particular, the production of autocrine growth factors by KS, which includes VEGF, dictates the progression of the lesion and are likely to play a central role in the development and progression of KS.[21] In the present study, we modeled VEGF-induced KS cell proliferation to test the anti-proliferative properties of compounds **1**–**8**, **11**, and **12**.

Before studying the effect of these compounds on the cell proliferation we tested their cytotoxic concentration by the XTT and trypan blue dye assays. Results indicated that all the compounds did not show any cytotoxic activity up to a concentration of 20 μM. Therefore, a dose dependence study was performed to test the effect on the KS cell proliferation in a concentration range 0.1–20 μM. All of the compounds reduced the VEGF-induced KS cell proliferation in a dose-dependent manner and the highest reduction

Table 4. Effect of compounds **18**, **11**, and **12** on the VEGF induced cell proliferation

Compound	Cell proliferation inhibition (%)
Argeloside C (**1**)	55.3±0.6
Argeloside D (**2**)	38.3±1.3
Argeloside E (**3**)	41.2±0.7
Argeloside F (**4**)	38.5±1.1
Argeloside G (**5**)	53.4±0.5
Argeloside H (**6**)	52.5±0.8
Argeloside I (**7**)	45.3±0.6
Argeloside J (**8**)	44.5±0.9
Argeloside A (**11**)	35.4±0.3
Argeloside B (**12**)	33.5±1.5

KS cells were pre-treated with or without compounds **1–8**, **11**, and **12** (10 μM) followed by stimulation with VEGF (50 ng/mL). The cell proliferation was determined with XTT assay as described under Section 3. Data represent the means±SEM from at least three independent experiments in duplicates.

occurred at concentration of 10 μM. The results summarized in Table 4, clearly indicates that compound **1** showed the highest reduction of the VEGF-induced KS cell proliferation at 10 μM (55.3%). In particular, the effect of compound **1** on the VEGF-induced cell proliferation is shown in Figure 2.

It is worthwhile to note that in absence of VEGF the treated cells with compounds **1–8**, **11**, and **12** (10 μM) for 1 h followed by incubation for 48 h, scarcely reduced the cell proliferation.

3. Experimental

3.1. General

Optical rotations were measured on a Jasco DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. Exact masses were measured by a Voyager DE mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a 337 nm laser and delay extraction and operated in positive-ion reflector mode. Samples were analyzed by matrix assisted laser desorption ionization (MALDI) mass spectrometry. A mixture of analyte solution and α-ciano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18-39) at 2465.1989 Da and Angiotensin III at 931.5154 Da as internal standard. ESI-MS analyses were performed using a ThermoFinnigan (San Josè, CA, USA) LCQ Deca XP Max ion trap mass spectrometer equipped with Xcalibur software. Samples were dissolved in methanol and infused into the ES ionisation source using a syringe pump at a flow rate of 3 μl/min. The capillary voltage was at 40 V, the spray voltage was at 4.8 kV and the tube lens offset was at 35 V. The capillary temperature was 220 °C. Data were acquired in MS^1 and MS^n scanning mode operating in positive ion mode. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and Time Proportional Phase Increment (TPPI) used to achieve frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, 2D-TOCSY, HSQC, HMBC and ROESY spectra. The spectra were acquired at 600 MHz. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software. Column chromatography was performed over Sephadex LH-20 (Pharmacia), MPLC was carried out on a Büchi 688 chromatography pump and Büchi B-685 borosilicate glass column (230×26 mm). Silica gel 60 (0.040–0.063 mm; Carlo Erba) was used as column material. HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC_{18} column, and a U6K injector. TLC was performed on silica gel F254 (Merck) plates, and reagent grade chemicals (Carlo Erba) were used throughout.

3.2. Computational details

Molecular mechanics/dynamics calculations on each of the compounds under examination were performed on Silicon Graphics Indigo2 using the CVFF force field[22] and the INSIGHT II/Discover package.[23] MD calculations (500 K, 50 ps) were executed in order to allow a full exploration of the conformational space. The Verlet algorithm was used to integrate the equation of motions. The methanol solution phase was mimicked through the value of the corresponding dielectric constant. All the structures so obtained were minimized using the steepest descent and Newton-Raphson algorithms (maximum derivative less than 0.05 kcal/mol). This led to the selection of the lowest energy minimum conformers. The geometry of the above, as well as that of tetramethylsilane (TMS), was subsequently optimized at DFT level. QM calculations were carried out using the Gaussian03 software package.[24] Structures and energies of the considered species were optimized at mPW1PW91 level[25] using the 6-31G(d) basis set. Single point ^{13}C c.s. calculations,

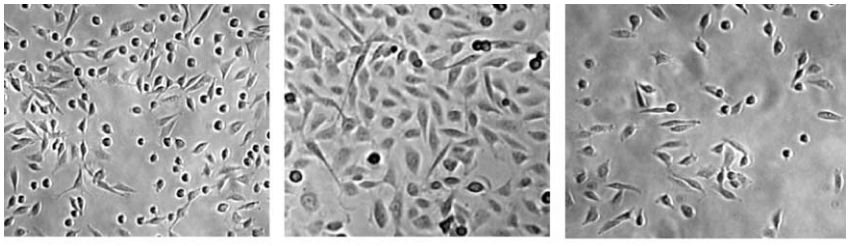


Figure 2. Effect of compound **1** on the VEGF-induced KS cell proliferation. Representatives photographs of (A) untreated cells (control), (B) cells treated with only VEGF (50 ng/mL), and (C) cells pre-treated with compound **1** (10 μM) followed by stimulation with VEGF (50 ng/mL). Data represent the means±SEM from at least three independent experiments in duplicates. Statistical analysis was performed by Student's *t*-test. Statistical significance was set at $P<0.05$.

carried out using as inputs the mPW1PW91/6-31G(d) optimized structures, were performed employing the same functional combined with the 6-31G(d,p) basis set. The calculated values of the chemical shifts were referred to the theoretical tetramethylsilane ^{13}C c.s. value, computed at the same level of theory.

3.3. Plant material

Fresh samples of *S. argel* hairy seeds were collected at Allaqi (South-East of Aswan, Egypt) in May 2002 and identified by one of the authors (A.I.H.).

3.4. Extraction and isolation

The hairy seeds (900 g) were extracted with EtOH 70% yielding 162 g of extract. Part of the extract (2.5×2 g) was fractionated on Sephadex LH-20 (100×5 cm) using MeOH as the mobile phase. Sixty-seven fractions (8 mL) were obtained. The fractions containing pregnane glycosides (frs. 14-18, 759.7 mg) were chromatographed by MPLC on Si gel with a gradient (flow rate 3.0 mL/min) of $CHCl_3$–MeOH (from 100:0 to 92:8, stepwise) as eluent to afford 1592 fractions (8 mL) monitored by TLC. Fractions 269-310 (13.7 mg) were chromatographed by RP-HPLC on a Waters (XTerra Prep MSC_{18}) column (300×7.8 mm) using MeOH–H_2O (4:1) as mobile phase (flow rate 2.5 mL/min) to yield compound **1** (1.6 mg, t_R=17.9 min), **3** (1.2 mg, t_R=10.2 min), and **7** (1.0 mg, t_R=15.3 min). Fractions 356–462 (18.0 mg) were chromatographed by RP-HPLC on a Waters (XTerra Prep MSC_{18}) column (300×7.8 mm) using MeOH–H_2O (73:27) as mobile phase (flow rate 2.5 mL/min) to yield compound **8** (2.8 mg, t_R=17.7 min). Fractions 545–634 (59 mg) were chromatographed by RP-HPLC on a Waters (XTerra Prep MSC_{18}) column (300×7.8 mm) using MeOH–H_2O (7:3) as mobile phase (flow rate 2.5 mL/min) to yield compound **2** (2.3 mg, t_R=16.3 min). Fractions 1000–1076 (38 mg) were chromatographed by RP-HPLC on a Waters (XTerra Prep MSC_{18}) column (300×7.8 mm) using MeOH–H_2O (3:2) as mobile phase (flow rate 2.5 mL/min) to yield compounds **4** (1.1 mg, t_R=60.9 min) and **6** (2.8 mg, t_R=32.0 min). Fractions 1077–1200 (34.1 mg) were chromatographed by RP-HPLC on a Waters (XTerra Prep MSC_{18}) column (300×7.8 mm) using MeOH–H_2O (7:3) as mobile phase (flow rate 2.5 mL/min) to yield compound **5** (1.0 mg, t_R=39.8 min).

3.4.1. Argeloside C (1). White amorphous powder, mp 140–143 °C; $[\alpha]_D^{24}$ −11.7 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3457, 2985, 1515; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety, see Table 2; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 961.4 $[M+Na]^+$; ESI-MS/MS *m/z* 631.2 $[M+Na-330]^+$, 471.2 $[M+Na-330-160]^+$; HR-MS (MALDI): $[M+Na]^+$, found 961.5135. $C_{49}H_{78}O_{17}Na$ requires 961.5137.

3.4.2. Argeloside D (2). White amorphous powder, mp 150–155 °C; $[\alpha]_D^{24}$ −20.1 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3465, 3025, 1505; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety, see Table 2; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 977.5 $[M+Na]^+$; ESI-MS/MS *m/z* 947.5 $[M+Na-30]^+$, 631.2 $[M+Na-30-316]^+$, 471.0 $[M+Na-30-316-160]^+$; HR-MS (MALDI): $[M+Na]^+$, found 977.5247. $C_{49}H_{78}O_{18}Na$ requires 977.5086.

3.4.3. Argeloside E (3). White amorphous powder, mp 140–142 °C; $[\alpha]_D^{24}$ −9.3 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3433, 2964, 1727, 1511; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety, see Table 2; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 1019.6 $[M+Na]^+$; ESI-MS/MS *m/z* 959.4 $[M+Na-60]^+$, 631.2 $[M+Na-60-328]^+$, 471.1 $[M+Na-60-328-160]^+$; HR−MS (MALDI): $[M+Na]^+$, found 1019.5142. $C_{51}H_{80}O_{19}Na$ requires 1019.5192.

3.4.4. Argeloside F (4). White amorphous powder, mp 171–174 °C; $[\alpha]_D^{24}$ −16.6 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3459, 2990, 1513; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety superimposable on those reported for compound **1**; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 1109.7 $[M+Na]^+$; ESI-MS/MS *m/z* 763.8 $[M+Na-346]^+$, 633.2 $[M+H-346-130]^+$; HR-MS (MALDI): $[M+Na]^+$, found 1109.5607. $C_{54}H_{86}O_{22}Na$ requires 1109.5508.

3.4.5. Argeloside G (5). White amorphous powder, mp 167–169 °C; $[0]_D^{24}$ −20.3 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3450, 2996, 1525; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety superimposable on those reported for compound **1**; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 1123.5 $[M+Na]^+$; ESI-MS/MS *m/z* 793.2 $[M+Na-330]^+$, 633.3 $[M+Na-330-160]^+$; HR-MS (MALDI): $[M+Na]^+$, found 1123.5660. $C_{55}H_{88}O_{22}Na$ requires 1123.5665.

3.4.6. Argeloside H (6). White amorphous powder, mp 165–169 °C; $[\alpha]_D^{24}$ −18.7 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3437, 2958, 1721, 1520; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety superimposable on those reported for compound **3**; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 1181.2 $[M+Na]^+$; ESI-MS/MS *m/z* 1121.4 $[M+Na-60]^+$, 793.2 $[M+Na-60-328]^+$, 633.2 $[M+Na-60-328-160]$; HR-MS (MALDI): $[M+Na]^+$, found 1181.5751. $C_{57}H_{90}O_{24}Na$ requires 1181.5720.

3.4.7. Argeloside I (7). White amorphous powder, mp 143–146 °C; $[\alpha]_D^{24}$ −15.8 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3463, 2978, 1516; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety superimposable on those reported for compound **1**; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 947.4 $[M+Na]^+$; ESI-MS/MS *m/z* 601.1 $[M+Na-346]^+$, 471.1 $[M+Na-346-130]^+$; HR-MS (MALDI): $[M+Na]^+$, found 947.4948. $C_{48}H_{76}O_{17}Na$ requires 947.4980.

3.4.8. Argeloside J (8). White amorphous powder, mp 145–149 °C; $[\alpha]_D^{24}$ −25.3 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3441,

2971, 1710, 1508; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the aglycone moiety superimposable on those reported for compound **3**; δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion, see Table 1; ESI-MS *m/z* 1005.5 $[M+Na]^+$; ESI-MS/MS *m/z* 945.4 $[M+Na-60]^+$, 601.2 $[M+Na-60-344]^+$, 471.1 $[M+Na-60-344-130]^+$; HR-MS (MALDI): $[M+Na]^+$, found 1005.5039. $C_{50}H_{78}O_{19}Na$ requires 1005.5035.

3.4.9. Argeloside A (11). White amorphous powder, mp 170–173 °C; $[\alpha]_D^{24}$ −16.9 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3438, 2961, 1720, 1521; δ_H (600 MHz, CD_3OD) data of the aglycone moiety 5.68 (1H, dd, $J=3.3$, 2.7 Hz, H-6), 4.60 (1H, dd, $J=11.8$ Hz, H-19b), 4.56 (1H, br m, H-16), 4.00 (1H, dd, $J=11.8$ Hz, H-19a), 3.86 (2H, m, H-15), 3.58 (1H, m, H-3), 2.53 (1H, d, $J=2.2$ Hz, H-17), 2.26 (1H, m, H-8), 2.11 (1H, m, H-1β), 2.04 (3H, s, CO*Me*), 1.64 (3H, s, Me-21), 1.56 (1H, m, H-9), 1.21 (3H, s, Me-18), 1.15 (1H, m, H-1α); δ_C (150 MHz, CD_3OD) data of the aglycone moiety 34.8 (C-1), 30.6 (C-2), 78.8 (C-3), 39.5 (C-4), 136.0 (C-5), 126.6 (C-6), 25.3 (C-7), 33.2 (C-8), 46.4 (C-9), 46.4 (C-10), 21.1 (C-11), 31.2 (C-12), 50.1(C-13), 110.4 (C-14), 72.0 (C-15), 78.3 (C-16), 58.5 (C-17), 15.5 (C-18), 65.0 (C-19), 114.6 (C-20), 23.4 (C-21), 172.1 (*C*OMe), 20.7 (CO*Me*); δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion superimposable on those reported for compound **4**; ESI-MS *m/z* 1167.5 $[M+Na]^+$; ESI-MS/MS *m/z* 1107.5 $[M+Na-60]^+$, 763.2 $[M+Na-60-344]^+$, 633.2 $[M+Na-60-344-130]^+$; HR-MS (MALDI): $[M+Na]^+$, found 1167.5568. $C_{56}H_{88}O_{24}Na$ requires 1167.5563.

3.4.10. Argeloside B (12). White amorphous powder, mp 193–197 °C; $[\alpha]_D^{24}$ −6.1 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3429, 2964, 1727; δ_H (600 MHz, CD_3OD) data of the aglycone moiety 4.56 (1H, br m, H-16), 4.41 (1H, dd, $J=11.8$ Hz, H-19b), 4.23 (1H, dd, $J=11.8$ Hz, H-19a), 3.86 (2H, m, H-15), 3.74 (1H, m, H-3), 2.49 (1H, d, $J=2.2$ Hz, H-17), 1.98 (1H, m, H-8), 2.11 (1H, m, H-1β), 2.09 (3H, s, CO*Me*), 1.63 (3H, s, Me-21), 1.53 (1H, m, H-9), 1.15 (3H, s, Me-18), 0.96 (1H, m, H-1α); δ_C (150 MHz, CD_3OD) data of the aglycone moiety δ 32.9 (C-1), 30.3 (C-2), 77.8 (C-3), 35.7 (C-4), 45.3 (C-5), 28.6 (C-6), 35.7 (C-7), 36.2 (C-8), 39.4 (C-9), 49.6 (C-10), 21.7 (C-11), 31.6 (C-12), 50.2 (C-13), 111.0 (C-14), 71.8 (C-15), 78.3 (C-16), 58.7 (C-17), 16.0 (C-18), 69.2 (C-19), 115.0 (C-20), 23.5 (C-21), 172.5 (*C*OMe), 20.8 (CO*Me*); δ_H (600 MHz, CD_3OD) and δ_C (150 MHz, CD_3OD) data of the sugar portion see Ref. 10. ESI-MS *m/z* 1283.3 $[M+Na]^+$; ESI-MS/MS *m/z* 1223.5 $[M+Na-60]^+$, 893.0 $[M+Na-60-330]^+$, 733.2 $[M+Na-60-330-160]^+$, 589.1 $[M+Na-60-330-160-144]^+$; HR-MS (MALDI): $[M+Na]^+$, found 1283.6411. $C_{56}H_{88}O_{24}Na$ requires 1283.6401.

3.5. Cell cultures and treatments

KS immortalized cells were cultured in 25-cm^2 flasks containing RPMI-1640 media supplemented with 10% fetal calf serum (Gibco BRL, Poland) at 37 °C in a humidified chamber with 5% CO_2. Cells were grown in 24 well-plates at a cell density of 5×10^3/well and were cultured for 12 h in RPMI-1640 media without 10% fetal calf serum before treatments. Stock solutions of compounds **1**–**8**, **11**, and **12** were prepared in DMSO at a concentration of 5 mM and afterward diluted to the required concentration. Successively, the cells were pre-treated for 1 h at 37 °C with compounds **1**–**8**, **11**, and **12** in serum-free RPMI-1640 media. The final DMSO concentration in the media was less than 0.1% and after treatments the cells viability was higher than 95%, as assessed by trypan blue dye exclusion. In the control cells, DMSO was added to the medium at the same concentration used to dissolve the tested compounds.

To stimulate proliferation, cells were washed twice with 2 mL of HBSS-10 mM Hepes and treated with Vascular Endothelial Growth Factor 50 ng/mL (VEGF, Peprotech, USA) in HBSS-10 mM Hepes for 48 h at 37 °C in 5% CO_2 and humidified air atmosphere.

Cell proliferation was determined by two methods, cell number count using trypan blue exclusion and XTT assay (XTT Kit I, Boehringer Mannheim Corp., Germany). Cell number count was expressed as cells/mL and was performed only on viable cells based on trypan dye test. For the XTT proliferation assay, 0.5 mL of XTT mixture was added to each well. After 1 h, only 0.2 mL of the solution was used to measure the spectrophotometrical absorbance at 490 nm. The XTT method was also used as assay to determine the cytotoxic effect of the tested compounds.

Data are expressed as means ± SEM from at least three independent experiments in duplicates. Statistical analysis was performed by Student's *t*-test. Statistical significance was set at $P<0.05$.

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Studies on the synthesis of DNA-damaging part of leinamycin: regioselectivity in Ti(O*i*Pr)$_4$ mediated opening of hydroxy epoxides with carboxylic acids

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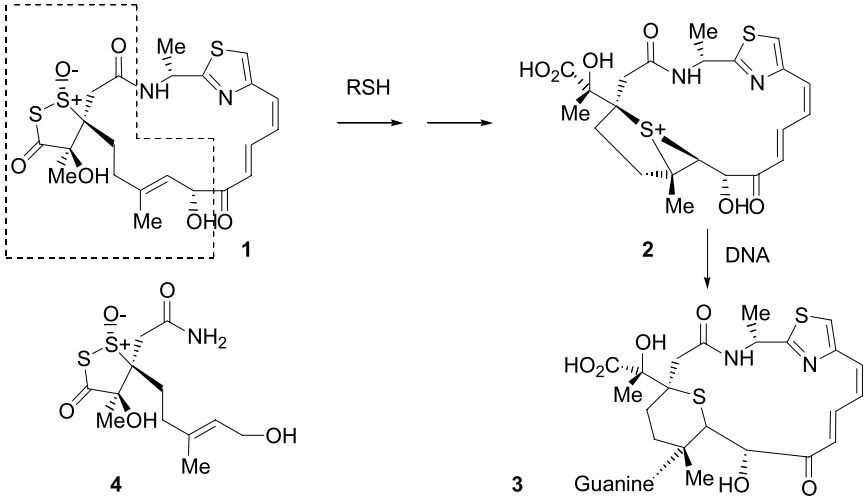
Abstract—The preparation of the key intermediate in the synthesis of the DNA damaging fragment of the anticancer antibiotic leinamycin starting from geraniol is described. The synthetic sequence involves the building of a quaternary asymmetric center through kinetic resolution through Sharpless epoxidation followed by the regioselective opening of the resultant enantiomerically pure hydroxyepoxide and intramolecular Wittig–Horner olefination.

1. Introduction

Leinamycin **1** is a highly potent macrocyclic anticancer antibiotic possessing a spiro-1, 3-dioxo-1, 2-dithiolane functionality (Scheme 1).[1] Leinamycin shows a high antiproliferative activity against a number of models such as human uterine carcinoma and murine leukemia.[2] It is unique among other DNA damaging compounds by having a dual mechanism of action working as a DNA alkylating agent[3] and also as a source of free radicals.

Reaction of intracellular thiols such as glutathione with leinamycin triggers a sequence of transformations resulting in the production of episulfonium cation of type **2** (Scheme 1). Cation **2** is capable of subsequent alkylation of guanine units of DNA resulting in a fast DNA strand



Scheme 1.

Keywords: Leinamycin; Sharpless epoxidation; Epoxide opening; Wittig–Horner olefination.
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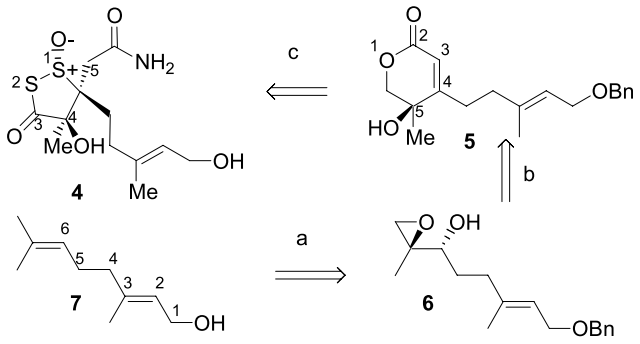


Scheme 2.

scission.[3] Formation of episulfonium cation **2** is accompanied by the formation of hydroxydisulfides that according to Gates[4] produce hydroxy radicals in the subsequent reaction with molecular oxygen hydroxy radicals that are capable of augmenting DNA damage from the above mentioned alkylation reaction.

From the chemical point of view it can be assumed that the DNA damaging activity of leinamycin is concentrated in the fragment of type **4**. The rest of the leinamycin molecule including the conjugated polyalkylidene chain and the thiazole cycle are most probably responsible for the binding with DNA.[5] This assumption may have a substantial importance for the design of new analogs of leinamycin possessing antineoplastic activity. These compounds will be of substantial practical interest since the use of leinamycin itself as an anticancer drug is seriously complicated by its low availability and stability in water solutions.[6] A number of semisynthetic leinamycin derivatives have been prepared in order to enhance its stability.[6] The radical solution, however, could involve the production of completely new leinamycin derivatives by the attachment of the DNA damaging fragment of type **4** to known DNA binding intercalators such as polycyclic heteroaromatic compounds.[7]

The synthesis of the DNA damaging part of leinamycin therefore constitutes a substantial interest. The only known synthesis of leinamycin[8] does not give a possibility for a separate synthesis of fragments of type **4**. A number of simple leinamycin mimetics have been synthesized.[9] However, these compounds contain only part of the necessary functions of the DNA damaging fragment **4** and therefore do not possess substantial DNA alkylating activity.

2. Results and discussion

Our approach toward the DNA damaging part of leinamycin **4** (Scheme 2) was based on commercially available geraniol **7** possesses all except two carbon atoms of fragment **4**. The synthetic plan for the preparation of **4** involved three main stages: (a) stereocontrolled formation of hydroxyepoxide **6**; (b) the attachment of two carbon units to the carbon skeleton of geraniol; (c) stereocontrolled introduction of sulfur atoms to C-4 of the key unsaturated lactone **5** followed by the formation of 1,3-dioxo-1,2-dithiolane cycle.

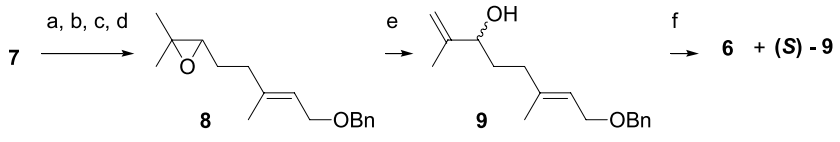
Two possible approaches towards the asymmetric functionalization of geraniol **7** at position 6 had been reported, both starting from the conversion of geraniol into monoepoxide of type **8** (Scheme 3). The first approach involved the hydrolysis of epoxide **8** to give the corresponding racemic diol which had been deracemized through the oxidation of the secondary hydroxy group followed by the baker yeast reduction.[10,11] The alternative approach[12] involved the isomerization of epoxides of type **8** into racemic allyl alcohols of type **9** followed by Sharpless epoxidation under kinetic resolution conditions.[13]

While the second approach is based on kinetic resolution and therefore should provide a lower theoretical yield, it is substantially more convenient than the microbiogical process. In line with this approach we converted geraniol **7** into racemic allyl alcohol **9** through sequential acetylation, hydroxy-bromination, epoxide ring closure, benzyl protection, and acid catalyzed rearrangement of the resultant epoxide **8** into racemic alcohol **9** (Scheme 3). While a number of protic and Lewis acids were found to catalyze the rearrangement of epoxide **8** (stage (e), Scheme 3), we found that the catalysis with aluminium isopropoxide[14] provided a much better yield than protic acids. Catalysis by titanium isopropoxide provided similarly high chemoselectivity but substantially lower reaction rates.

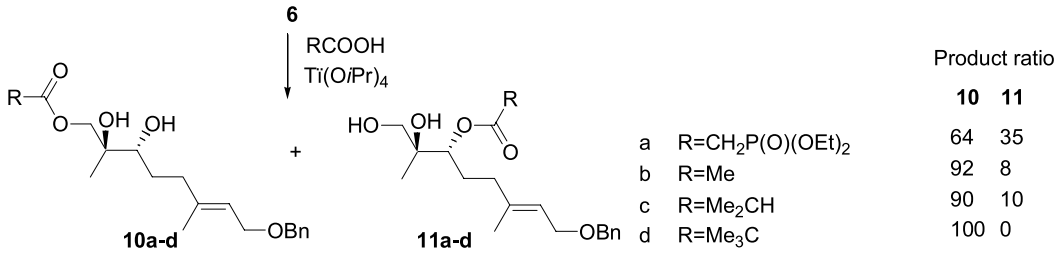
Sharpless epoxidation of racemic allyl alcohol **9** using (−)-DIPT and 0.45 equiv of *t*-BuOOH proceeded with a high enantio- (98%) and diastereoselectivity (>98%) thus providing hydroxyepoxide **6** with 45% yield and recovered predominantly (*S*)-allyl alcohol **9**.[15]

The conversion of hydroxyepoxide **6** into unsaturated lactone **5** was planned to involve a regioselective opening of the epoxide ring, oxidation of the resultant hydroxyepoxide function, and intramolecular Witting–Horner olefination.

The Ti(O*i*Pr)$_4$ mediated opening of hydroxyepoxides has been first reported by Sharpless.[16] Among other nucleophiles, carboxylic acids have been found to open hydroxyepoxides with a high regioselectivity. A number of preparative applications of this reaction are known, all proceeding with high regioselectivity.[17] We planned to use this reaction for regioselective opening of the epoxide ring with simultaneous introduction of diethylphosphonoacetate



Scheme 3. Reagents and conditions: (a) Ac_2O, DMAP, 99%; (b) NBS, dioxane–water, 98%; (c) NaOH, MeOH, 97%; (d) NaH, BnBr, 98%; (e) Al(O*i*Pr)$_3$, toluene, 110 °C, 95%; (f) (−)-DIPT (0.1 equiv), *t*-BuOOH (0.45 equiv) Ti(O*i*Pr)$_4$, molecular sieves, −20 °C, 45%.



Scheme 4.

residue. However, all our attempts to open the epoxide cycle using Ti(O*i*Pr)$_4$ resulted in the production of 64:35 mixture of isomers **10a** and **11a** (Scheme 4).

The formation of isomer **11a** can be attributed to the transesterification of the initially formed diol **10a** thus providing a thermodynamic mixture of isomers. The transesterification of diols of type **10** in neutral reaction conditions has not been reported previously. However, since titanium alkoxides catalyzed transesterifications are well documented,[18] it is reasonable to suggest that Ti(O*i*Pr)$_4$ indeed participate in the process. In order to verify this, pure isomer **10a** was treated with equimolar amounts of Ti(O*i*Pr)$_4$ in dichloromethane. Indeed this reaction provided a mixture of isomers **10a** and **11a** with the same ca. 2:1 composition.

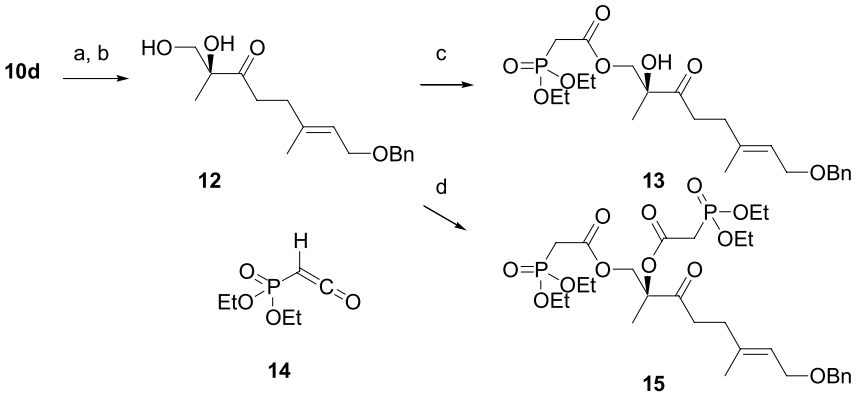
Our attempts to optimize the yield of diol **10a** using low temperatures and different reaction solvents were not successful as the equilibration between isomers **10a** and **11a** was found to proceed faster than the ring opening. Other methods of ring opening in hydroxyepoxide **6** in the absence of Ti(O*i*Pr)$_4$ such as reactions with cesium and potassium diethylphosphonoacetates[19] were also unsuccessful. Oxidation of hydroxyepoxide **6** into corresponding ketoepoxide followed by the treatment of cesium diethylphosphonoacetate up to 140 °C also did not provide desired products of epoxide ring opening.

It may be assumed that the observed transesterification is facilitated by the presence of the adjacent quaternary carbon atom. Such an arrangement should decrease the rate of nucleophilic attack on the epoxide ring due to steric effects, and in the same time increase the rate of transesterification due to Thorpe–Ingold-effect.

We have found, however, that the regioselectivity of Ti(O*i*Pr)$_4$ catalyzed ring opening in hydroxyepoxide **6** with different carboxylic acids is highly dependent on the structure of the carboxylic acids. Opening of hydroxyepoxide **6** with isobutyric and acetic acids proceeded with a substantial selectivity in favor of primary esters **10b**,**c**. The opening of hydroxyepoxide **6** with pivalic acid proceeded without any transesterification thus giving only desired ester **10d**.

Subsequent Swern oxidation of diol **10d** followed by the hydrolysis of pivaloyl group provided dihydroxy ketone **12** (Scheme 5). Initial attempts for acylating the primary hydroxy group of dihydroxy ketone **12** with diethylphosphonoacetyl chloride prepared from diethylphosphonoacetic acid (DEPA) by the treatment with triphosgene/collidine[20] failed, most probably due to the instability of diethylphosphonoacetyl chloride. Instead, the coupling was achieved by the treatment of a 1:1 mixture of DEPA and dihydroxy ketone **12** with 1 equiv of DCC without any acylation catalysts thus producing diethylphosphonoacetate ester **13**. These unusually mild reaction conditions that are usually not possible for conventional carbodiimide mediated esterifications went on in a high yield with DEPA since the acylation proceeded through the intermediacy of corresponding diethoxyphosphoryl ketene of type **14** (Scheme 5).[21] Ketenes with electron withdrawing substituents such as **14** possess a high reactivity and low sensitivity toward steric hindrance so that even the corresponding diacylation product (**15**) can be obtained if an excess of DEPA/DCC is used.

Our synthetic plan involved the cyclization of diethylphosphonoacetate ester **13** into the key unsaturated lactone of type **5** through intramolecular Wittig–Horner olefination.



Scheme 5. Reagents and conditions: (a) Oxalyl chloride, DMSO, Et$_3$N, 93%; (b) NaOH/MeOH, 97%; (c) DEPA (1 equiv), DCC (1 equiv), 93%; (d) DEPA (4 equiv), DCC (4 equiv), 95%.

Several examples of intramolecular Wittig–Horner olefination have been reported. Five- and six-membered unsaturated lactones have been prepared using NaH,[22] K_2CO_3,[23] and $LiClO_4$/DBU as a base.[24] The mildest reaction conditions involved the use of $LiClO_4$/iPr$_2$NEt.[25] In our attempts to obtain the intramolecular Wittig–Horner olefination products (Scheme 6), we tried the above mentioned reagents as well as potassium *tert*-butoxide and butyllithium. However, no traces of cyclization product were observed in all these reactions.

The total absence of cyclization products in these cases can be attributed to the presence of a hydroxy group in the α-position to the carbonyl group. Since pK_a of hydroxy groups in acyloins is about 14, the deprotonation of the tertiary hydroxy group in diethylphosphonoacetate ester **13** should proceed before the deprotonation of methylene group of the diethylphosphonoacetic moiety thus efficiently preventing the subsequent cyclization even if the deprotonation of the methylene group takes place.

To remove this obstacle the tertiary hydroxy group in diethylphosphonoacetate ester **13** was protected by silylation with an excess of chlorotrimethylsilane and hexamethyldisilazane. The subsequent treatment of *O*-silylated diethyl phosphonoacetate **16** with $LiClO_4$/DBU produced the desired lactone **17** although in a relatively low yield (up to 40%). Our attempts to optimize the reaction conditions as well as the use of other reagents mentioned above did not improve yields due to the formation of a number of byproducts. However, we were pleased to discover that a much higher yield (90%) of the intramolecular olefination can be achieved if *t*-BuOLi is used as a base for the reaction. The importance of lithium cation for the smooth deprotonation of phosphonoacetates is well documented[25] but to the best of our knowledge this is the first example of the use of *t*-BuOLi for Wittig–Horner olefination.

3. Conclusion

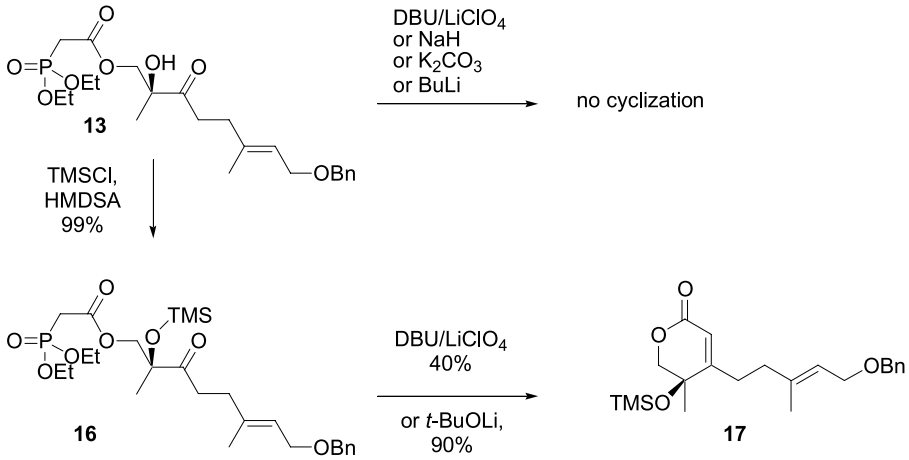
In conclusion, the key intermediate in the synthesis of DNA damaging fragment of leinamycin was synthesized from geraniol in 11 steps thus paving the road for a total synthesis of this fragment. The transesterification during titanium isopropoxide mediated epoxide ring opening with carboxylic acids was investigated and reaction conditions for achieving high regioselectivity were found. Intramolecular Wittig–Horner olefination was investigated and a new, more efficient reagent for the reaction was suggested.

4. Experimental

4.1. General information

Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck, Kieselgel 60 F-254), flash chromatographic separations were performed on silica gel (Merck, Kieselgel 60, 230–400 Mesh ASTM). Medium pressure liquid chromatography (MPLC) was performed on glass columns (Buchi, B-685, $d=26$ mm, $l=460$ mm) with LiChroprep™ 60 (particle size 15–25 μm). Flash chromatography and MPLC were performed using only ethyl acetate–40–60 petroleum ether mixtures as the eluent. IR spectra were obtained with a Bruker Tensor 27 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-300 spectrometer in $CDCl_3$ using the residual solvent peaks for calibration at rt. Optical rotation was measured by Jasco P-1010 polarimeter. Elementary analyses were performed by the microanalytical laboratory of the Hebrew University of Jerusalem, Jerusalem. High resolution mass-spectra were performed at the Technion on Bruker Daltonics APEX 3 instrument (CI). Unless otherwise stated, all reagents used are commercially available. Glassware was oven-dried before use and solvents were purified by conventional methods.

4.1.1. (3′*E*)-3-(5′-Benzyloxy-3′-methyl-pent-3′-enyl)-2,2-dimethyl-oxirane (8). To a solution of geraniol **7** (15.43 g, 100 mmol) in dichloromethane (50 mL) were added acetic anhydride (20 mL, 200 mmol) and DMAP (0.6 g, 5.0 mmol) at rt. The reaction mixture was stirred for 1 h, methanol (30 mL) was added, and additional 1 h stirring was carried out. The reaction mixture was dissolved in petrol ether, washed with water (2×30 mL) and saturated $NaHCO_3$ (2×30 mL), dried (Na_2SO_4) and evaporated to give the (2*E*)-Acetic acid 3,7-dimethyl-octa-2,6-dienyl ester as colorless oil (19.47 g, 99 mmol, 99%). ^{1}H NMR (300 MHz): 1.60 (s, 3H); 1.68 (s, 3H); 1.70 (s, 3H); 2.05 (s, 3H); 2.00–2.10 (m,



Scheme 6.

2H+2H); 4.59 (d, J=7 Hz, 2H); 5.08 (t, J=5 Hz, 1H); 5.42 (t, J=7 Hz, 1H). To a solution of the resultant (2*E*)-Acetic acid 3,7-dimethyl-octa-2,6-dienyl ester (36.31 g, 0.185 mol) in a mixture of dioxane (180 mL) and water (80 mL) was added dropwise a solution of *N*-bromosuccinimide (33.00 g, 0.185 mol) in dioxane (500 mL) at 0 °C for 2 h. The reaction mixture was stirred for additional 2 h at 0 °C, and 1 h at rt. The reaction mixture was concentrated to ca. 100 mL, dissolved in petroleum ether (100 mL), washed with brine (2×50 mL), dried (Na_2SO_4) and evaporated to give (2*E*)-Acetic acid 6-bromo-7-hydroxy-3,7-dimethyl-oct-2-enyl ester as pale yellow oil (53.09 g, 0.181 mol, 98%). ^{1}H NMR (300 MHz): 1.33 (s, 3H); 1.34 (s, 3H); 1.70 (s, 3H); 1.75–1.89 (m, 1H); 2.03 (m, 1H); 2.05 (s, 3H); 2.16 (m, 1H); 2.33–2.47 (m, 1H); 3.96 (dd, J_1=11 Hz, J_2=2 Hz, 1H); 4.58 (d, J=7 Hz, 2H); 5.42 (t, J=7 Hz, 1H). To a solution of the resultant (2*E*)-Acetic acid 6-bromo-7-hydroxy-3,7-dimethyl-oct-2-enyl ester (112.00 g, 0.38 mol) in methanol (1 L) at 0 °C was added a solution of NaOH (45.00 g, 1.10 mol) in methanol (350 mL). The reaction mixture was stirred for 1 h at 0 °C, and 3 h at rt. The reaction mixture was concentrated to ca. 100 mL, dissolved in petrol ether (100 mL), washed with water (2×50 mL), dried (Na_2SO_4) and evaporated to give (2*E*)-5-(3,3-Dimethyl-oxiranyl)-3-methyl-pent-2-en-1-ol as pale yellow oil (63.06 g, 0.37 mol, 97%). ^{1}H NMR (300 MHz): 1.26 (s, 3H); 1.30 (s, 3H); 1.62–1.67 (m, 2H); 1.70 (s, 3H); 2.05–2.29 (m, 2H); 2.71 (t, J=6 Hz, 1H); 4.17 (d, J=7 Hz, 2H); 5.42 (t, J=7 Hz, 1H). To a solution of the resultant (2*E*)-5-(3,3-Dimethyl-oxiranyl)-3-methyl-pent-2-en-1-ol (20.00 g, 0.117 mol) in anh. THF (60 mL) at −10 °C under inert atmosphere (N_2) were added a solution of NaH (60%, 6.40 g, 0.160 mol) in anh. THF (120 mL) and neat benzyl bromide (18.62 mL, 0.160 mol) subsequently. The reaction mixture was stirred for 5 h at −10 °C, and 1 h at rt. The reaction mixture was evaporated, dissolved in petrol ether (50 mL), washed with water (2×25 mL) and saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (29.98 g, 0.115 mol, 98%). IR (film): 2931, 2860, 1717, 1454, 1378, 1274, 1108, 747 cm^{-1}. ^{1}H NMR (300 MHz): 1.26 (s, 3H); 1.30 (s, 3H); 1.60–1.67 (m, 2H); 1.67 (s, 3H); 2.00–2.28 (m, 2H); 2.71 (t, J=6 Hz, 1H); 4.03 (d, J=7 Hz, 2H); 4.51 (s, 2H); 5.42 (t, J=7 Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.4 (CH_3), 24.8 (2×CH_3), 27.1 (CH_2), 36.1 (CH_2), 58.3 (C), 63.9 (CH), 66.4 (CH_2), 72.1 (CH_2), 121.3 (CH), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.3 (C, Bn), 139.3 (C). Anal. Calcd for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29; Found: C, 78.20; H, 9.31.

4.1.2. (6*E*)-8-Benzyloxy-2,6-dimethyl-octa-1,6-dien-3-ol (9).[14] To a refluxed (120 °C) solution of epoxide **8** (1.00 g, 3.84 mmol) in toluene (10 mL) was added aluminum isopropoxide (0.78 g, 3.84 mmol). The reaction mixture was stirred for 4 h. After cooling to rt, the reaction mixture is treated with 2 N hydrochloric acid (10 mL) in order to decompose the aluminum complex. The organic layer is separated, washed with water (2×25 mL) and saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (0.95 g, 3.65 mmol, 95%). IR (film): 3468, 2982, 2937, 2860, 1739, 1451, 1375, 1266, 1096, 903, 754 cm^{-1}. ^{1}H NMR (300 MHz): 1.65 (s, 3H); 1.58–1.72 (m, 2H); 1.72 (s, 3H); 1.95–2.15 (m, 2H); 4.03 (d, J=7 Hz, 2H); 4.05 (t, J=7 Hz, 1H); 4.51 (s, 2H); 4.84 (s, 1H); 4.94 (s, 1H); 5.42 (t, J=7 Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.4 (CH_3), 17.5 (CH_3), 32.8 (CH_2), 35.4 (CH_2), 66.4 (CH_2), 72.0 (CH_2), 75.5 (CH), 111.7 (C=CH_2), 121.0 (C=CH), 127.4 (CH, Bn), 127.7 (2×CH, Bn), 128.2 (2×CH, Bn), 138.4 (C, Bn), 140.0 (*C*=CH), 147.3 (*C*=CH_2).

4.1.3. (−)-(2′*S*,1*R*,4*E*)-6-Benzyloxy-4-methyl-1-(2′-methyl-oxiranyl)-hex-4-en-1-ol (6). To a solution of racemic alcohol **9** (5.00 g, 20 mmol) in anh. dichloromethane (80 mL) at −10 °C under inert atmosphere (N_2) were added (2*S*,3*S*)-(−)-diisopropyl tartrate (1.35 g, 6.6 mmol), molecular sieves (4 Å, powder activated particle size 5 μm, 4.00 g, 80% w/w of **9**) and titanium tetraisopropylate (1.10 mL, 4 mmol). The reaction mixture was stirred for 0.5 h at −10 °C, cooled down to −30 °C and *tert*-butyl hydroperoxide (TBHP, solution 5.5 M in decane, 1.64 mL, 9 mmol) was added. The reaction mixture was stirred for 6 h at −30 °C until a 1:1 ratio of two spots were observed in TLC. At −30 °C the reaction was quenched by addition of a 5% HCl solution (11 mL),warmed to rt, filtered through a sintered glass and extracted with chloroform (100 mL), washed with water (2×25 mL) and saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated. The residue was purified by MPLC (20–50% ethyl acetate–petrol ether [40–60]) to give the unreacted reactant alcohol **6** (2.60 g, 10 mmol, 50%), $[\alpha]_D^{22}$ −4.36 (*c* 2.43, $CHCl_3$) and the title compound as pale yellow oil (2.49 g, 9 mmol, 45%) $[\alpha]_D^{21}$ −2.42 (*c* 1.91, $CHCl_3$). IR (film): 3456, 3356, 2946, 2858, 1663, 1448, 1063, 756 cm^{-1}. ^{1}H NMR (300 MHz): 1.34 (s, 3H); 1.45–1.58 (m, 1H); 1.67 (s, 3H); 1.70–1.83 (m, 1H); 2.09 (s, 1H, ex); 2.09–2.20 (m, 1H); 2.23–2.33 (m, 1H); 2.61 (d, J=5 Hz, 1H); 2.90 (d, J=5 Hz, 1H); 3.63 (dd, J_1=9 Hz, J_2=3 Hz, 1H); 4.04 (d, J=7 Hz, 2H); 4.51 (s, 2H); 5.42 (t, J=7 Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.5 (CH_3), 18.0 (CH_3), 30.9 (CH_2), 35.3 (CH_2), 50.2 (CH_2), 58.9 (C), 66.5 (CH_2), 71.2 (CH_2), 72.0 (CH), 121.0 (CH), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.4 (C, Bn), 139.9 (C). Anal. Calcd for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 73.55; H, 8.77. The %ee of hydroxyepoxide **6** was determined by HPLC on Diacel Chiralpack® AD chiral column with 1 mL/min. 96:4 hexane/isopropanol as mobile phase. Retention times for both enantiomers were determined by separation of specially prepared racemic mixture.

4.1.4. (+)-(2*S*,3*R*,6*E*)-(Diethoxy-phosphoryl)-acetic acid-8-benzyloxy-2,3-dihydroxy-2,6-dimethyl-oct-6-enyl ester (10a) and (+)-(2*S*,3*R*,6*E*)-(diethoxy-phosphoryl)-acetic acid-6-benzyloxy-1(1,2-dihydroxy-1-methyl-ethyl)-4-methyl-hex-4-enyl ester (11a). To a solution of hydroxy epoxide **6** (0.0553 g, 0.20 mmol) in anh. dichloromethane (3.0 mL) at rt were added diethylphosphonoacetic acid (0.6 mL solution of 1.0 M, 0.60 mmol) and titanium tetraisopropylate (0.2 mL of 1.0 M solution in dichloromethane, 0.20 mmol). The reaction mixture was stirred for 72 h, filtered through a sintered glass and quenched by a 10% HCl solution (5.0 mL). The reaction mixture was extracted with chloroform (25 mL), washed with water (2×25 mL) and saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated. The residue had showed a 2:1 mixture of **10a**:**11a** products according to ^{1}H NMR of crude. The mixture was purified by MPLC (100% ethyl acetate) to give

the pure title compounds **10a** as pale yellow oil (0.0567 g, 0.128 mmol, 64%) and the more polar **11a** as pale yellow oil (0.0331 g, 0.070 mmol, 35%). Compound **10a**: $[\alpha]_D^{23}$ +11.84 (*c* 0.55, $CHCl_3$). IR (film): 3350 (br), 3002, 2935, 2859, 1738, 1451, 1393, 1259, 1158, 1024, 972, 756 cm^{-1}. ^1H NMR (300 MHz): 1.15 (s, 3H); 1.34 (q, $J=7$ Hz, 6H, $P[OCH_2CH_3]_2$); 1.40–1.52 (m, 1H); 1.66 (s, 3H); 1.68–1.82 (m, 1H); 2.05–2.18 (m, 1H); 2.34–2.47 (m, 1H); 2.95 (dd, $J_1=29$ Hz, $J_2=14$ Hz, 1H, CH_2-P); 3.02 (dd, $J_1=30$ Hz, $J_2=14$ Hz, 1H, CH_2-P); 3.26 (d, $J=7$ Hz, 1H, ex); 3.45 (t, $J=9$ Hz, 1H, ex); 3.73 (s, 1H, ex); 3.99 (d, $J=11$ Hz, 1H); 4.02 (d, $J=7$ Hz, 2H); 4.15 (app. sextet, $J=7$ Hz, 2×2H, $P[OCH_2CH_3]_2$); 4.42 (d, $J=11$ Hz, 1H); 4.51 (s, 2H); 5.42 (t, $J=7$ Hz, 1H); 7.20–7.32 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.2 ($P[OCH_2CH_3]_2$), 16.3 (CH_3), 16.6 (CH_3), 20.7 (CH_2), 29.2 (CH_2-P), 36.7 (CH_2), 63.1 ($P[OCH_2CH_3]_2$), 66.5 (CH_2), 69.9 (CH_2), 72.1 (CH_2), 72.9 (C), 76.1 (CH), 121.1 (C=*C*H), 127.5 (CH, Bn), 127.7 (2× CH, Bn), 128.3 (2×CH, Bn), 138.4 (C, Bn), 140.1 (*C*=CH), 165.5 ($PCH_2C{=}O$). HRMS calcd for $C_{23}H_{37}NaO_8P$ (MNa^+) 495.2124, found 495.2123.

Compound **11a**: $[\alpha]_D^{23}$ +5.41 (*c* 0.44, $CHCl_3$). IR (film): 3402 (br), 2994, 2932, 2862, 1731, 1448, 1395, 1261, 1112, 1029, 974, 752 cm^{-1}. ^1H NMR (300 MHz): 1.10 (s, 3H); 1.35 (t, $J=7$ Hz, 6H, $P[OCH_2CH_3]_2$); 1.64 (s, 3H); 1.65–1.77 (m, 1H); 1.89–2.15 (m, 3H); 2.95 (dd, $J_1=29$ Hz, $J_2=14$ Hz, 1H, CH_2-P); 3.02 (dd, $J_1=30$ Hz, $J_2=14$ Hz, 1H, CH_2-P); 3.32 (d, $J=12$ Hz, 1H); 3.65 (d, $J=12$ Hz, 1H); 4.01 (d, $J=7$ Hz, 2H); 4.17 (app. double sextet, $J=3$ Hz, 2×2H, $P[OCH_2CH_3]_2$); 4.51 (s, 2H); 4.88 (d, $J=9$ Hz, 1H); 5.42 (t, $J=7$ Hz, 1H); 7.20–7.32 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.2 ($P[OCH_2CH_3]_2$), 16.4 (CH_3), 18.8 (CH_3), 26.6 (CH_2), 29.6 (CH_2-P), 36.2 (CH_2), 63.1 ($P[OCH_2CH_3]_2$), 66.5 (CH_2), 72.1 (CH_2), 73.4 (C), 78.0 (CH), 121.6 (C=*C*H), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.4 (C, Bn), 139.1 (*C*=CH), 166.2 ($PCH_2C{=}O$). HRMS calcd for $C_{23}H_{37}NaO_8P$ (MNa^+) 495.2124, found 495.2122.

4.1.5. (+)-(2*S*,3*R*,6*E*)-2,2-Dimethyl-propionic acid 8-benzyloxy-2,3-dihydroxy-2,6-dimethyl-oct-6-enyl ester (10d). To a solution of hydroxyepoxide **6** (2.37 g, 8.58 mmol) in anh. dichloromethane (20 mL) at rt under inert atmosphere (N_2) were added pivalic acid (2.53 g, 24.7 mmol) and titanium tetraisopropylate (3.00 mL, 9.7 mmol). The reaction mixture was stirred overnight (ca. 12 h) at rt. The reaction mixture was quenched by a 5% HCl solution (50 mL), extracted with chloroform (2×100 mL), washed with water (2×25 mL) and saturated $NaHCO_3$ (2× 25 mL), dried (Na_2SO_4) and evaporated to give the title compound as pale yellow oil (2.99 g, 7.89 mmol, 92%). $[\alpha]_D^{21}$ +17.82 (*c* 2.96, $CHCl_3$). IR (film): 2970, 2933, 2862, 1727, 1462, 1369, 1286, 1167, 1073, 931, 742, 689 cm^{-1}. ^1H NMR (300 MHz): 1.17 (s, 3H); 1.22 (s, 9H); 1.40–1.55 (m, 1H); 1.65 (s, 3H); 1.67–1.78 (m, 1H); 2.06–2.17 (m, 1H); 2.27–2.51 (m, 1H+s, 1H, ex); 3.41 (d, $J=8$ Hz, 1H); 4.00 (d, $J=12$ Hz, 1H); 4.02 (d, $J=7$ Hz, 2H); 4.27 (d, $J=$ 12 Hz, 1H); 4.51 (s, 2H); 5.42 (t, $J=7$ Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.4 (CH_3), 20.5 (CH_3), 27.2 (3×CH_3, *t*-Bu), 28.8 (CH_2), 36.6 (CH_2), 38.9 (C), 66.4 (CH_2), 68.4 (CH_2), 72.1 (CH_2), 73.9 (C), 75.5 (CH), 121.4 (CH), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.4 (C, Bn), 139.9 (C), 178.9 (C=O). HRMS calcd for $C_{22}H_{34}NaO_5$ (MNa^+) 401.2304, found 401.2313.

4.1.6. (−)-(2*S*,6*E*)-8-Benzyloxy-1,2-dihydroxy-2,6-dimethyl-oct-6-en-3-one (12). To a solution of oxalyl chloride (1.20 mL, 13.9 mmol) in anh. dichloromethane (30 mL) at −78 °C under inert atmosphere (N_2) was added dropwise over period of 5 min a solution of DMSO (2.16 mL, 30.4 mmol) in anh. dichloromethane (6 mL). The reaction mixture was stirred for 10 min and a solution of diol **10d** (4.77 g, 12.60 mmol) in anh. dichloromethane (15 mL) was added dropwise over period of 5 min. The reaction mixture was stirred for 30 min and neat triethylamine (8.8 mL, 64.0 mmol) was added dropwise over period of 5 min. The reaction mixture was left to rt and quenched by a 1% HCl solution (40 mL) after TLC analysis showed total disappearance of diol **10d**. The reaction mixture was stirred for additional 10 min and the dimethylsulfide by-product was distilled off (38 °C). The residue was extracted with dichloromethane (2×50 mL), washed with water (2×25 mL) and saturated $NaHCO_3$ (2× 25 mL), dried (Na_2SO_4) and evaporated to give (+)-(2*R*)-(6*E*)-2,2-Dimethyl-propionic acid 8-benzyloxy-2-hydroxy-2,6-dimethyl-3-oxo-oct-6-enyl ester as pale yellow oil (4.41 g, 11.72 mmol, 93%). $[\alpha]_D^{22}$ +6.14 (*c* 1.52, $CHCl_3$). IR (film): 3420 (br), 2937, 2858, 1721, 1651, 1454, 1368, 1283, 1151, 1063, 753 cm^{-1}. ^1H NMR (300 MHz): 1.15 (s, 9H); 1.36 (s, 3H); 1.65 (s, 3H); 2.21–2.46 (m, 2H); 2.69 (t, $J=8$ Hz, 2H); 3.91 (s, 1H, ex); 4.02 (d, $J=7$ Hz, 2H); 4.50 (s, 2H); 5.42 (t, $J=7$ Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.4 (CH_3), 20.5 (CH_3), 27.2 (3×CH_3, *t*-Bu), 28.8 (CH_2), 36.6 (CH_2), 38.9 (C), 66.4 (CH_2), 68.4 (CH_2), 72.1 (CH_2), 79.9 (C), 121.6 (C=*C*H), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.4 (C, Bn), 138.5 (*C*=CH), 178.0 (C=O, ester) 210.9 (C=O, ketone). To a solution of the resultant (+)-(2*S*,6*E*)-2,2-Dimethyl-propionic acid 8-benzyloxy-2-hydroxy-2,6-dimethyl-3-oxo-oct-6-enyl ester (2.65 g, 7.04 mmol) in methanol (25 mL) at rt was added a solution of NaOH (7.04 mL of 1 N) in methanol. The reaction mixture was stirred for 12 h. at rt, neutralized to pH 6 by a few drops of 1% HCl solution, concentrated to ca.10 mL, dissolved in ethyl acetate (50 mL), washed with water (2×50 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (2.00 g, 6.83 mmol, 97%). $[\alpha]_D^{20}$ −0.82 (*c* 0.89, $CHCl_3$). IR (film): 3450 (br), 2923, 2859, 1711, 1452, 1370, 1060, 751 cm^{-1}. ^1H NMR (300 MHz): 1.27 (s, 3H); 1.66 (s, 3H); 2.35 (t, $J=8$ Hz, 2H); 2.72 (t, $J=8$ Hz, 2H); 3.62 (d, $J=$ 12 Hz, 1H); 3.85 (d, $J=12$ Hz, 1H); 4.01 (d, $J=7$ Hz, 2H); 4.50 (s, 2H); 5.42 (t, $J=7$ Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.7 (CH_3), 21.3 (CH_3), 32.6 (CH_2), 34.3 (CH_2), 66.4 (CH_2), 67.8 (CH_2), 72.2 (CH_2), 79.6 (C), 121.4 (C=*C*H), 127.6 (CH, Bn), 127.8 (2×CH, Bn), 128.3 (2×CH, Bn), 138.2 (C, Bn), 138.7 (*C*=CH), 212.5 (C=O). Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27. Found: C, 69.48; H, 8.23.

4.1.7. (−)-(2*S*,6*E*)-(Diethoxy-phosphoryl)-acetic acid 8-benzyloxy-2-hydroxy-2,6-dimethyl-3-oxo-oct-6-enyl ester (13). To a solution of keto diol **12** (2.70 g, 9.23 mmol) in anh. dichloromethane (35 mL) at rt were added neat diethyl phosphonoacetic acid (1.85 g, 9.23 mmol) and a solution of DCC (2.31 g, 9.23 mmol) in anh. dichloromethane (10 mL).

The reaction mixture was stirred for 30 min, filtered, dissolved in ethyl acetate, washed with saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (4.04 g, 8.58 mmol, 93%). $[\alpha]_D^{20}$ −4.49 (*c* 0.75, $CHCl_3$). IR (film): 3365, 2924, 1742, 1454, 1259, 1025, 739 cm^{-1}. ^1H NMR (300 MHz): 1.30 (s, 3H); 1.33 (dt, $J_1=7$ Hz, $J_2=0.5$ Hz, 6H, $P[OCH_2CH_3]_2$); 1.66 (s, 3H); 2.27–2.37 (m, 2H); 2.80–3.03 (dd, $J_1=29$ Hz, $J_2=14$ Hz, 1H, CH_2-P+dd, $J_1=30$ Hz, $J_2=14$ Hz, 1H, CH_2-P+m, 2H); 4.02 (d, $J=7$ Hz, 2H); 4.14 (m, 2×2H, $P[OCH_2CH_3]_2$); 4.34 (d, $J=11$ Hz, 1H); 4.38 (d, $J=11$ Hz, 1H); 4.50 (s, 2H); 4.72 (s, 1H, ex); 5.42 (t, $J=7$ Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.3 ($P[OCH_2CH_3]_2$), 16.7 (CH_3), 21.8 (CH_3), 32.5 (CH_2), 35.0 (CH_2-P), 35.3 (CH_2), 62.9 ($P[OCH_2CH_3]_2$), 66.4 (CH_2), 69.5 (CH_2), 72.1 (CH_2), 78.3 (C), 121.2 (C=*C*H), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.3 (C, Bn), 138.7 (*C*=CH), 165.2 (PCH_2C=*O*), 212.7 (C=O).

4.1.8. (−)-(2*S*,6*E*)-(Diethoxy-phosphoryl)-acetic acid-8-benzyloxy-2-[2-(diethoxy-phosphoryl) -acetoxy]-2,6-dimethyl-3-oxo-oct-6-enyl ester (15). To a solution of **10** (0.05 g, 0.17 mmol) in anh. dichloromethane (10 mL) at rt were added neat diethylphosphonoacetic acid (0.14 g, 0.68 mmol) and a solution of DCC (0.14 g, 0.68 mmol) in anh. dichloromethane (2 mL). The reaction mixture was stirred for 30 min, filtered, dissolved in ethyl acetate, washed with saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (0.09 g, 0.16 mmol, 95%) after flash chromatography (EtOAc:Methanol, 0–10%). $[\alpha]_D^{21}$ −3.86 (*c* 0.30, $CHCl_3$). IR (film): 2987, 2925, 2857, 1742, 1452, 1376, 1265, 1108, 1026, 966, 753 cm^{-1}. ^1H NMR (300 MHz): 1.33 (dt, $J_1=7$ Hz, $J_2=0.5$ Hz, 12H, 2×$P[OCH_2CH_3]_2$); 1.53 (s, 3H); 1.65 (s, 3H); 2.29 (t, $J=7$ Hz, 2H); 2.67 (t, $J=7$ Hz, 2H); 2.97 (dd, $J_1=29$ Hz, $J_2=14$ Hz, 1H, CH_2-P); 2.98 (d, $J=22$ Hz, 2H); 3.05 (dd, $J_1=30$ Hz, $J_2=14$ Hz, 1H, CH_2-P); 4.15 (quint., $J=7$ Hz, 8H, 2×$P[OCH_2CH_3]_2$); 4.44 (d, $J=12$ Hz, 1H); 4.48 (s, 2H); 4.62 (d, $J=12$ Hz, 1H); 5.42 (t, $J=7$ Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 16.2 2×($P[OCH_2CH_3]_2$), 16.3 (CH_3), 19.1 (CH_3), 22.6 (CH_2), 29.6 2×(CH_2-P), 32.7 (CH_2), 34.8 (CH_2), 66.5 (2×$P[OCH_2CH_3]_2$), 65.8 (CH_2), 72.1 (CH_2), 84.5 (C), 121.1 (C=*C*H), 127.51 (CH, Bn), 127.6 (2×CH, Bn), 128.4 (2×CH, Bn), 137.2 (C, Bn), 138.7 (*C*=CH), 165.3 (2×PCH_2C=*O*), 206.1 (C=O). HRMS calcd for $C_{29}H_{46}NaO_{12}P_2$ (MNa^+) 671.2363, found 671.2328.

4.1.9. (−)-(2*S*,6*E*)-(Diethoxy-phosphoryl)-acetic acid 8-benzyloxy-2,6-dimethyl-3-oxo-2-trimethylsilanyloxy-oct-6-enyl ester (16). To a stirred solution of diethylphosphono acetate ester **14** (2.20 g, 4.68 mmol) in DMF (10 mL) were added 1,1,1,3,3,3-hexamethyldisilazane (2.60 mL, 12.6 mmol) and trimethylsilyl chloride (1.30 mL, 10.3 mmol) at rt. The reaction mixture was stirred for 2 h, dissolved in ethyl acetate, washed with water (2×20 mL) and saturated $NaHCO_3$ (2×25 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (2.51 g, 4.63 mmol, 99%). $[\alpha]_D^{20}$ −2.55 (*c* 2.14, $CHCl_3$). IR (film): 2940, 1741, 1651, 1508, 1380, 1262, 1024, 755 cm^{-1}. ^1H NMR (300 MHz): 0.18 (s, 9H, $Si[CH_3]_3$); 1.30 (s, 3H); 1.34 (t, $J=7$ Hz, 6H, $P[OCH_2CH_3]_2$); 1.66 (s, 3H); 2.27 (t, $J=7$ Hz, 2H); 2.78 (t, $J=7$ Hz, 1H); 2.94 (d, $J=22$ Hz, 2H, CH_2-P); 4.02 (d, $J=7$ Hz, 2H); 4.11–4.20 (m, 2×2H, $P[OCH_2CH_3]_2$); 4.25 (d, $J=11$ Hz, 2H); 4.50 (s, 2H); 5.42 (t, $J=7$ Hz, 1H); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 1.3 ($Si[CH_3]_3$), 14.3 ($P[OCH_2CH_3]_2$), 16.7 (CH_3), 21.8 (CH_3), 25.8 (CH_2), 31.5 (CH_2), 35.0 (CH_2-P), 62.9 ($P[OCH_2CH_3]_2$), 66.4 (CH_2), 69.5 (CH_2), 72.1 (CH_2), 78.3 (C), 121.2 (C=*C*H), 127.5 (CH, Bn), 127.7 (2×CH, Bn), 128.3 (2×CH, Bn), 138.3 (C, Bn), 138.7 (*C*=CH), 165.3 (PCH_2C=*O*), 212.7 (C=O). HRMS calcd for $C_{23}H_{35}NaO_8P$ (MNa^+ − TMS) 493.1967, found 493.1977.

4.1.10. (+)-(5*R*,3′*E*,3*Z*)-4-(5′-Benzyloxy-3′-methyl-pent-3′-enyl)-5-methyl-5-trimethyl silyloxy-5,6-dihydro-pyran-2-one (17). To a stirred solution of silylated diethylphosphono acetate **16** (0.100 g, 0.18 mmol) in anh. THF (4 mL) under inert atmosphere (N_2) at 0 °C was added a solution of lithium *tert*-butylate in anh. THF (0.4 mL of 0.5 M solution prepared from *tert*-butanol and butyl lithium). The reaction mixture was stirred for 2 h, dissolved in ethyl acetate (20 mL), washed with water (2×20 mL) and saturated $NaHCO_3$ (2×20 mL), dried (Na_2SO_4) and evaporated to give the title product as pale yellow oil (0.063 g, 0.16 mmol, 90%) after flash chromatography (Petrol ether/EtOAc, 8:2→1:1→0:1). $[\alpha]_D^{21}$ +2.30 (*c* 0.89, $CHCl_3$). IR (film): 3013, 2951, 2858, 1729, 1453, 1378, 1251, 1147, 1069, 1020, 850, 755, 697 cm^{-1}. ^1H NMR (300 MHz): 0.16 (s, 9H, $Si[CH_3]_3$); 1.44 (s, 3H); 1.68 (s, 3H); 2.19–2.39 (m+m, 1H+2H); 2.43–2.56 (m, 1H); 4.04 (d, $J=10$ Hz, 1H); 4.07 (d, $J=10$ Hz, 2H); 4.18 (d, $J=10$ Hz, 1H); 4.51 (s, 2H); 5.42 (t, $J=7$ Hz, 1H); 5.66 (s, 1H, lactone); 7.27–7.37 (m, 5H, Ar). ^{13}C NMR (75 MHz): 1.9 ($Si[CH_3]_3$), 16.5 (CH_3), 24.4 (CH_3), 27.8 (CH_2), 36.4 (CH_2), 66.3 (CH_2), 70.8 (C), 72.5 (CH_2), 75.0 (CH_2), 113.6 (C=*C*H, lactone), 121.8 (C=*C*H), 127.5 (CH, Bn), 127.6 (2×CH, Bn), 128.4 (2×CH, Bn), 138.2 (C, Bn), 138.4 (*C*=*CH*), 163.6 (*C*=*CH*, lactone), 167.0 (C=O). Anal. Calcd for $C_{22}H_{32}O_4Si$: C, 68.00; H, 8.30. Found: C, 67.66; H, 8.31.

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Unexpected valence bond isomerization of [1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazines under flash vacuum pyrolytic (fvp) conditions

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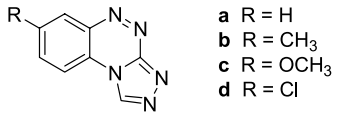
Abstract—Flash vacuum pyrolysis (fvp) of some substituted [1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazine derivatives (**1a–d**) has been studied between 450 and 600 °C. The only transformation observed up to 525 °C was the unexpected valence bond isomerization of the angularly fused starting compounds to the isomeric linearly fused [1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazine derivatives (**9a–d**), whereas at higher temperatures fragmentation products such as aromatic nitriles were also formed. Kinetic measurements revealed negative entropies of activation in the isomerization process, which suggest a concerted ring closure reaction to an intermediate antiaromatic diazirine. Reversibiblity of the title isomerization reaction was also proved by FVP experiments.

1. Introduction

Flash vacuum pyrolysis (fvp) of nitrogen containing heterocycles has been the focus of our studies for many years as reflected by a recently published review.[1] In the course of these investigations, heteroaromatic rings involving the N–N moiety in the ring seemed of special interest due to the possibility of nitrogen extrusion and formation of diradicals or vinylcarbenes.[1,2] From this point of view, interesting results have been obtained by comparison of the behavior of [1,2,3]- and [1,2,4]-benzotriazines: while most of [1,2,3]-benzotriazines afforded reaction products via benzazetes,[3] no evidence for these intermediates was found in fvp of 1-deoxy or 1-oxy derivatives of [1,2,4]-benzotriazines and, instead, benzonitriles and biphenylene were found as final products. Furthermore, in the case of 3-methylsulfanyl derivatives of these compounds, a competing radical reaction was also found in fvp due to the lability of the C–S bond.[4]

As a continuation of these studies we report here, on flash vacuum pyrolysis of some [1,2,4]triazolo[3,4-*c*]benzotriazines (**1a–d**, Scheme 1). These model compounds have the special structural feature that they contain two N–N parts in the ring and, in principle, two kinds of nitrogen elimination could occur under fvp conditions.



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Scheme 1.

Five-membered compounds with an N=N moiety in the ring can undergo nitrogen elimination under thermal and photochemical conditions. Thus, 1-arylbenzotriazoles are convenient precursors of carbazoles by thermal extrusion of nitrogen, which is known as the Graebe-Ullman synthesis. The reaction mechanism was confirmed to involve cyclisation of a diradical or iminocarbene to 4*H*-carbazole which isomerizes to substituted aromatic carbazoles by a hydrogen shift.[5]

Some [1,2,4]-triazoles with alkyl[6] or acyl[7] groups attached to a ring atom afforded migration of these substituents followed by further transformations. In the former case, isomeric 1,2,4-triazoles were formed, whereas in the second case nitrogen extrusion took place to result in a ring transformation of the starting compound (Scheme 2).

Keywords: Flash vacuum pyrolysis; Valence bond isomerisation; Sigmatropic rearrangement; Enthropy of activation; Azirine intermediate.
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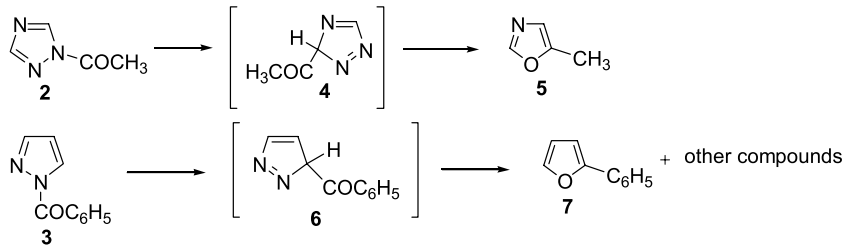


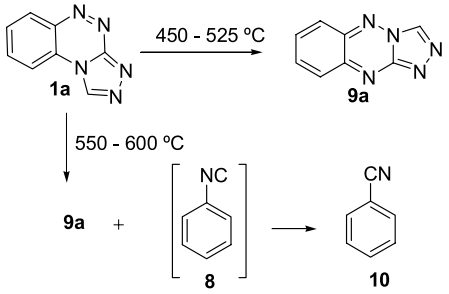
Scheme 2.

Thus, 1-acetyl-1*H*-1,2,4-triazole (**2**) underwent an N–C acyl migration, and the resulting intermediate (**4**) lost one molecule of nitrogen to yield 5-methyloxazole (**5**). A similar transformation was observed with 1-benzoyl-1*H*-pyrazole (**3**), where formation of 2-phenylfurane (**7**) via intermediate **6** was reported.[8] Unfortunately, no references on reactions of fused 1,2,4-triazoles were found.

2. Results and discussion

2.1. Fvp of [1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazine (1a)

Reactions of **1a** were carried out between 450 and 600 °C, with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. The reaction was clean affording one single product up to 525 °C identified as [1,2,4]triazolo[4,3-*b*][1,2,4] benzotriazine (**9a**) (Scheme 3). Over 550 °C small amounts of benzonitrile (**10**) and a carbonaceous residue were formed from decomposition (Scheme 3). The relative amounts of **1a** and **9a** calculated from the ^{1}H NMR spectra are summarized in Table 1.



Scheme 3.

Table 1. Fvp reactions of **1a** and **1b**

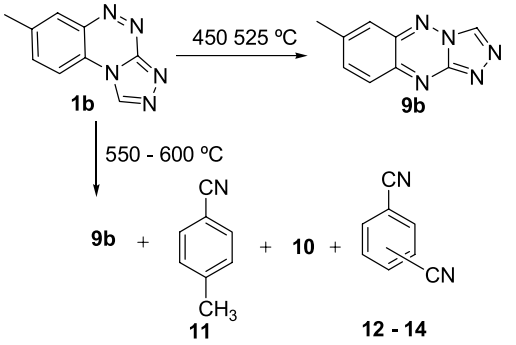
T (°C)		% **1**	% **9**	Other products
450	**1a**	95.3	4.7	—
	1b	94.2	5.8	—
475	**1a**	90.6	9.4	—
	1b	90.4	9.6	—
500	**1a**	82.5	17.5	—
	1b	82.4	17.6	—
525	**1a**	74.8	25.2	—
	1b	71.8	28.2	—
550	**1a**	72.7	27.3	**10**[a]
	1b	69.5	30.5	**11**[a]
575	**1a**	72.7	27.3	**10**[a]
	1b	62.5	37.5	**9**, **11**[a]
600	**1a**	79.2	20.8	**10**[a]
	1b	64.9	35.1	**10**, **12–14**[a]
625	**1a**	—	—	—
	1b	76.2	23.8	**10**, **12–14**[a]

[a] Detected by GC/MS, not quantified.

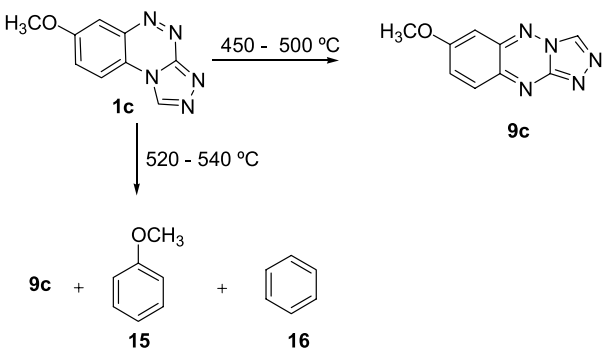
Compound **10** may be formed from **1a** or from **9a** by nitrogen extrusion or by fragmentation to benzeneisocyanide (**8**) and subsequent isomerization to **10** via a well known pathway.[9]

2.2. Fvp of 7-methyl-[1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazine (1b)

Reactions of **1b** were carried out between 450 and 625 °C, with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. The only product up to 525 °C was 7-methyl-[1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazine (**9b**); over this temperature **10**, *p*-tolunitrile (**11**) and a mixture of dicyanobenzenes (**12–14**) were also formed from decomposition of **1b** and/or **9b** (Scheme 4, Table 1). Relative quantification of **1b** and **9b** was carried out by the help of ^{1}H NMR spectra. It is important to note that 100% conversion could not be achieved and over 600 °C, coke was also formed. Compounds **10** and **12–14** can be formed by pyrolysis of **11** as already reported.[10]



Scheme 4.



Scheme 5.

2.3. Fvp of 7-methoxy [1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazine (1c)

Reactions of **1c** were carried out between 440 and 540 °C, with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. Up to 520 °C the only product was 7-methoxy-[1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazine (**9c**), over this temperature, anisole (**15**), benzene (**16**) and coke were also formed (Scheme 5 and Table 2). Relative quantification of **1c** and **9c** was performed by ^{1}H NMR.

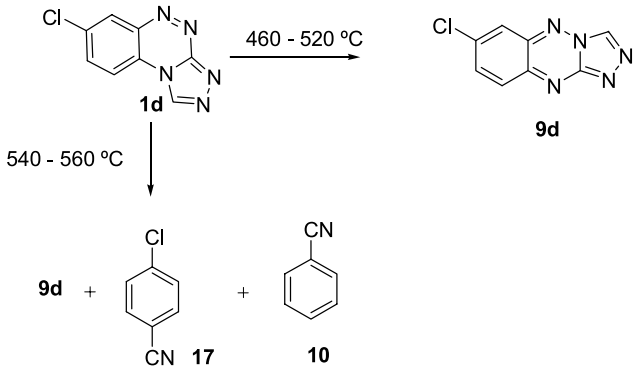
Table 2. Fvp reactions of **1c** and **1d**

T (°C)		% **1**	% **9**	Other products
440	**1c**	100	—	—
	1d	—	—	—
460	**1c**	97.7	12.3	—
	1d	85.6	14.4	—
480	**1c**	78.7	21.3	—
	1d	80.5	19.5	—
500	**1c**	69.2	30.8	—
	1d	74.7	25.3	—
520	**1c**	58.8	41.2	—
	1d	69.7	30.3	—
540	**1c**	61.1	38.9	**15**, **16**[a]
	1d	64.4	35.6	**17**[a]
560	**1c**	—	—	—
	1d	64.9	35.1	**10**, **17**[a]

[a] Detected by GC/MS, not quantified.

2.4. Fvp of 7-chloro [1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazine (1d)

Reactions of **1d** were carried out between 460 and 560 °C with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. As in reactions of **1a–c**, only one product was formed up to 520 °C, identified as 7-chloro[1,2,4]triazolo[4,3-*b*][1,2,4]-benzotriazine (**9d**), at higher temperatures, 4-chlorobenzonitrile (**17**) and benzonitrile (**10**) as well as coke were also formed (Scheme 6, Table 2). Relative quantification of **1d** and **9d** was performed by the help of ^{1}H NMR spectra.



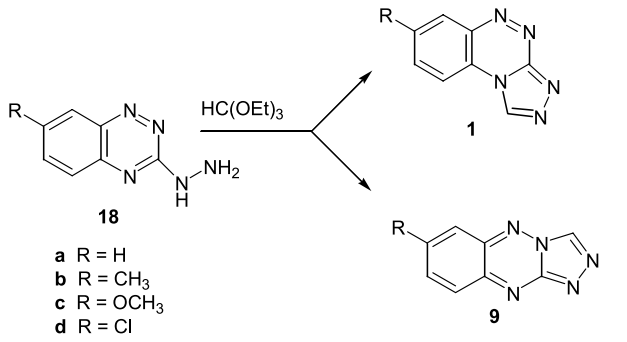
Scheme 6.

Although, spectral analyses of the main products of these FVP transformations suggested that derivatives of the the linearly fused ring system [1,2,4]triazolo[3,4-*c*]benzo[1,2,4] triazine (**9a–d**) were formed, preparative evidence for this asignment seemed also desirable. To this end, we repeated the ring closure reactions to **1a–d** according to the described literature procedure[27]—that is, treatment of substituted 3-hydrazinobenzo[1,2,4]triazines (**18**) with ethyl orthoformiate—and carried out thorough analyses of the mother liquors of these reaction mixtures to learn whether the isomeric linearly fused compounds **9a–d** had also been formed in small amounts in these transformations (Scheme 7).

TLC of all four reaction mixtures indicated that besides the intense spots for the main products (**1a–d**), a well defined other spot is also present. Separation of these fractions by column chromatography gave rise to the isolation of **9a–d** which proved to be entirely identical (mp, TLC, IR NMR-spectra) with the samples obtained by FVP. It is interesting to note that the **1/9** ratios in the reaction mixtures obtained from **18a–d** proved to be very different, the isolated yields are shown in Table 3 (Scheme 7).[27]

Table 3. Yields of **1a–d** and **9a–d** starting from **18a–d**

	1 (%)	**9** (%)
a	78.0	3.9
b	70.6	2.9
c	86.1	1.8
d	78.0	3.4

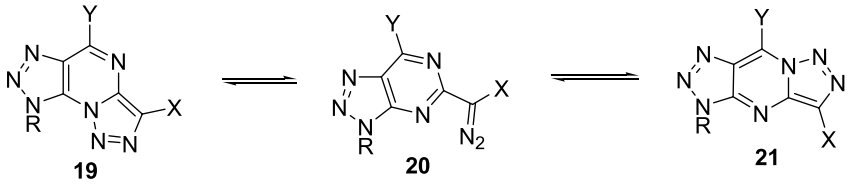


Scheme 7.

This finding that **9** can be formed from **1** under fvp conditions raises the interesting question of a possible mechanism for this transformation. Similar analoguous valence bond isomerizations have been reported in the literature. Four such cases for comparison should be recalled here.

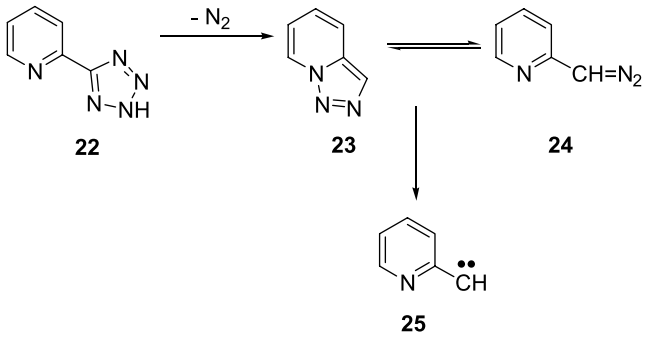
An example structurally related to the title compounds is the behavior of bis[1,2,3]triazolopyrimidines where an equilibrium between the angular (**19**) and the linear structure (**21**) is established through an open chain diazo form (**20**); this reaction is described to take place in the presence of a strong base. It is important to mention that the diazo form was detected by spectral methods and the equilibrium between the angular and the linear isomer is strongly dependent on the substituents X and Y (Scheme 8).[11] The introduction of an additional nitrogen atom into the fused azine ring facilitates triazole cleavage and potential rearrangements. Thus, [1,2,3]triazolo[5,1-*c*][1,2,4]triazine isomerized spontaneously to the thermodynamically more stable 1,2,3-triazolo[1,5-*b*]triazine during its preparation.[12]

A similar reaction was found in the pyrolysis of 2-(5-tetrazolyl)pyridine (**22**), where the first nitrogen extrusion



Scheme 8.

reaction afforded [1,2,3]triazolo[1,5-*a*]pyridine (**23**) in equilibrium with 2-pyridyldiazomethane (**24**). At higher temperatures [1,2,3]triazolo[1,5-*a*]pyridine (**23**) also afforded nitrogen loss to form pyridylcarbene (**25**) (Scheme 9).[13]

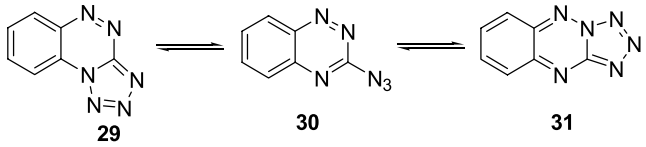


Scheme 9.

The same kind of isomerization, through an open chain compound, is described with several fused tetrazoles. Thus, the existence of an equilibrium between two isomeric tetrazolopyrimidones (**26** and **28**) through an azido intermediate (**27**) as well as Arrhenius parameters has been reported (Scheme 10).[14]

Angularly fused tetrazolo[5,1-*c*]benzo[1,2,4]triazines (**29**) and the linearly fused isomers tetrazolo[1,5-*b*]benzotriazines (**31**) were reported to be in equilibrium with an open chain azide (**30**) (Scheme 11) in DMSO, while **29** is stable in the crystalline form.[15] The relative amounts of **29**, **30** and **31** are strongly dependent on solvent and on substitution as was demonstrated in ^{1}H NMR experiments.[16]

It is important to note that in all these literature cases the isomerisation of the angularly fused ring to the linearly fused isomer (or the reverse reaction) proceeds via a well defined and relatively stable intermediate (i.e., via a heteroaromatic azide or diazomethane). An analysis of our present results described here, however, seemed more complicated because if the transformation of **1** to **9** proceeds in a pathway analogous to these cited reactions, an N–C bond cleavage would be anticipated to yield **32** and/or **33** as possible intermediates (Scheme 12).



Scheme 11.

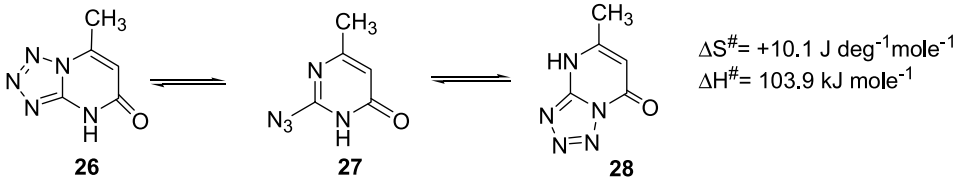
As formation of either **32** or **33** seemed rather unlikely, more detailed considerations concerning the acting mechanism involving reactive intermediates or concerted reactions seemed straightforward as depicted in Scheme 13.

Route a involves diradicals with an imine group, isomerization of these imines would lead to the final product by cyclization, which is a known stereoisomeric isomerization of imines.[17] Pathway b is a concerted [1,5] sigmatropic shift with rotation of a C–N bond partially doubly bonded and, finally, route c is a [1,3] sigmatropic shift with a ring contraction to form antiaromatic diazirine **34**, too unstable to be isolated, which by a concerted [1,3] shift (pathway d) or by ring opening affording **32** and/or **33** would lead to the final products.

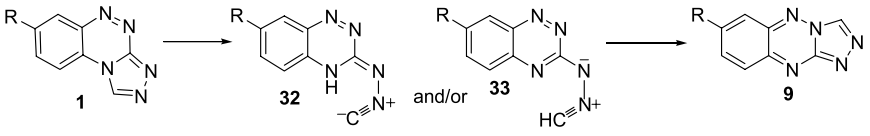
Isomerizations of fused tetrazoles through azides afforded positive entropies of activation ($\Delta S^{\#}$ of $+10.1$ J deg^{-1} mol^{-1} and $\Delta H^{\#}$ of $+103.9$ kJ mol^{-1}) (Scheme 10), with this reference for comparison, kinetic measurements of the reactions described here would give valuable information on the mechanism.

2.5. Kinetics

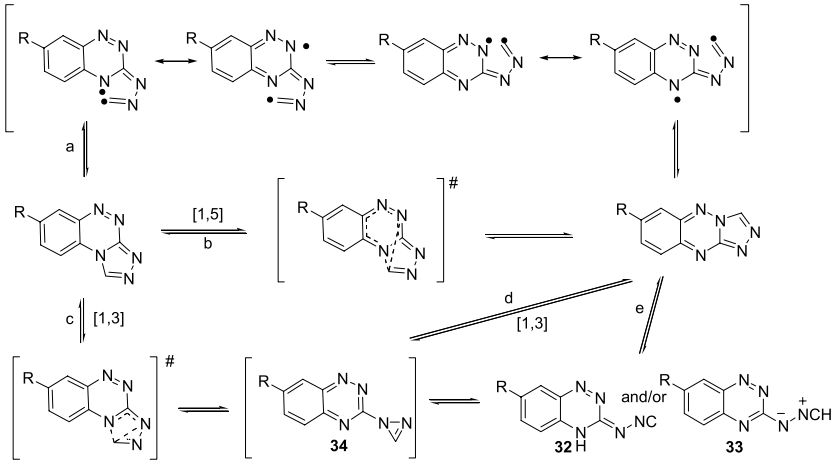
Reaction constants were measured at each temperature with the relative concentrations of starting materials and products determined by ^{1}H NMR and averaged over at least three determinations. Reaction times were calculated as V_0/μ being V_0 the volume of the reaction tube inside the hot zone and μ the carrier gas flow. Arrhenius parameters were calculated by the classical equation ($\ln k$ vs $1/T$) with data for at least four different temperatures. To check the system, kinetic parameters of ethylacetate pyrolysis were measured in the fvp system and compared with those reported for a static system, these results as well as a detailed description of the methodology have already been described.[18]



Scheme 10.



Scheme 12.



Scheme 13.

Table 4. Kinetics of fvp of **1a**

T (K)	C/C_0	t (10^{-2}) s	k (s^{-1})
723.15	0.953	2.2	2.2 ± 0.2^a
748.15	0.906	2.1	4.7 ± 0.2^a
773.15	0.825	2.0	9.2 ± 0.2^b
798.15	0.748	2.0	14.7 ± 0.6^b

[a] Average of three determinations.
[b] Average of four determinations.

Table 5. Kinetics of fvp of **1b**

T (K)	C/C_0	t (10^{-2}) s	k (s^{-1})
723.15	0.942	2.2	2.7 ± 0.4^a
748.15	0.907	2.1	4.7 ± 0.2^b
773.15	0.824	2.0	9.5 ± 0.9^b
798.15	0.718	2.0	16.8 ± 0.9^b

[a] Average of three determinations.
[b] Average of four determinations.

Table 6. Kinetics of fvp of **1c**

T (K)	C/C_0	t (10^{-2}) s	k (s^{-1})
733.15	0.888	2.2	5.4 ± 0.6^a
753.15	0.787	2.1	11.2 ± 0.9^a
773.15	0.692	2.1	17.8 ± 0.9^b
793.15	0.588	2.0	26.1 ± 0.9^b

[a] Averaged of three determinations.
[b] Averaged of four determinations.

Table 7. Kinetics of fvp of **1d**

T (K)	C/C_0	t (10^{-2}) s	k (s^{-1})
733.15	0.856	2.2	7.1 ± 0.9^a
753.15	0.805	2.2	10.1 ± 0.8^b
773.15	0.747	2.1	13.9 ± 0.9^b
793.15	0.697	2.0	17.9 ± 0.8^b
813.15	0.644	2.0	22.1 ± 0.9^b

[a] Averaged of three determinations.
[b] Averaged of four determinations.

Reactions are first order, rate constants for reactions of compounds **1a–d** are depicted in Tables 4–7 and Arrhenius parameters in Table 8.

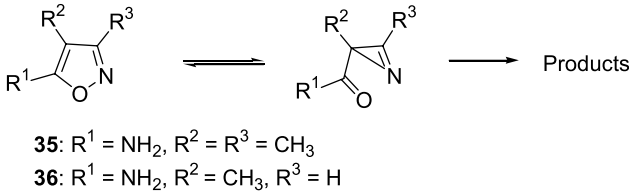
From all this information, the most important data are the negative entropies of activation, which preclude path a in Scheme 13: this is a ring opening reaction that should have a positive value and it is not expected for a ring closure reaction of a diradical to be the rate determining step. Paths b and c are concerted reactions where the better movement of the atoms is in the supra-supra mode as they are all bonded and a supra-antara situation should have steric impediments. Path b is a concerted [1,5] sigmatropic shift, although, this reaction is allowed by the Woodward-Hoffmann's rules,[19] it has strong steric impediments as the C=N bond (Fig. 1) of triazole ring should rotate 90° to allow a bonding interaction between all the atoms involved in the transition state.

The third possibility shown is Scheme 13 (path c) is a [1,3] sigmatropic shift; according to Woodward–Hoffmann′s rules it should be a supra-antara process that has strong steric impediment. But, it is possible to consider a supra-supra transition state, with lower steric requeriments, formally forbidden. This incompatibility can be over-compensated if the Migrating Group (MG) and the Migrating Framework (MF) differ in their electron releasing–electron withdrawing ability.[20] The difference I_D-A_A (I_D=ionization potential of the donor partner; A_A= electron affinity of the acceptor partner), affords an index of the activation energy of a sigmatropic shift with the reaction barrier becoming lower as I_D-A_A decreases.[21] These arguments, supported by molecular orbital calculations, were used in fvp isomerizations of isoxazoles,[22] which have similar entropies of activation values to the ones reported in this article suggesting a common reaction pathway. Recently, some theoretical calculations carried out by

Table 8. Arrhenius parameters of fvp of **1a–d** and of some isoxazoles*

	k (s^{-1}) (500 °C)	E_a (kJ mol^{-1})	log A (s^{-1})	$\Delta S^{\#}$ (eu)	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta G^{\#}$ (kJ mol^{-1})	r
1a	9.2±0.2	123±3	9.2±0.5	−18.2±0.4	116±3	176±4	−0.997
1b	9.5±0.9	118±3	9.0±0.3	−19.4±0.3	112±3	176±4	−0.998
1c	17.8±0.9	125±4	9.7±0.8	−16.0±0.8	119±4	172±8	−0.992
1d	13.9±0.9	71±2	5.9±0.2	−33.5±0.2	64±2	172±4	−0.997
35[a]		108±1	9.00				
36[b]		109±1	9.00				

[a] From Ref. 17.
[b] From Ref. 22.



Scheme 14.

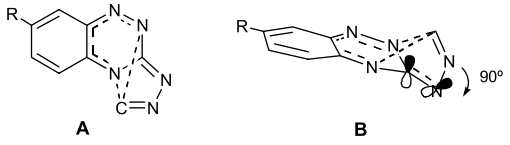


Figure 1. Steric representation of [1,5]sigmatropic shift of A via transition state B.

Davico[22] supported the mechanism proposed by some of us 20 years ago on a kinetic basis.[18,23] The author demonstrated that the rate limiting step is a concerted ring closure to the isomeric azirine in a similar reaction as described here. Arrhenius parameters for fvp isomerization reactions of 5-amino-3,4-dimethylisoxazole (**35**)[18] and 5-amino-4-methylisoxazole (**36**)[23] (Scheme 14) were compared with the ones of **1a–d** and reported in Table 8.

It should be mentioned that some of the azirines obtained from isoxazoles are stable enough to be isolated or detected by spectroscopy, which is not the case of the diazirines proposed as intermediates in the reactions here reported. These diazirines are antiaromatic and this is the reason why they are not detected nor isolated, similar antiaromatic compounds, such as the 1*H*-azirines were proposed as intermediates in reactions of *N*-substituted 1,2,3-triazoles and benzotriazoles[24] and in reactions of 1-phthalimido-1,2,3-triazoles.[25]

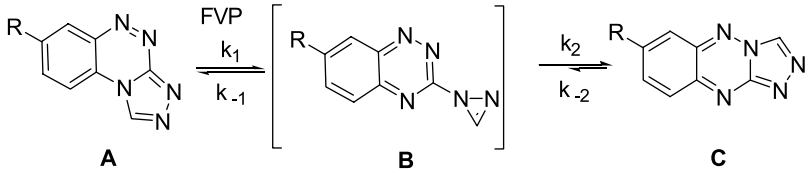
With the kinetic evidence reported here (large negative entropies of activation), it is possible to propose path c of Scheme 13 as the one taking place in these reactions. As formation of the diazirine is the rate determining step, with these results it is not possible to distinguish how the diazirine ring opens, as a concerted reaction (way d) or through an open chain isomer (way e).

The other point that was checked is the reversibility of the reaction: if the angular isomer is also formed from the linear one under the same fvp conditions. In order to study this possibility, fvp reactions of **9b** were carried out at 480 °C. As expected, **1b** (26.3%) was found to be the reaction product as well as unreacted **9b** (73.7%), confirming the reversibility of the reaction. These results also show that the system is not in equilibrium, probably because this is a flow system with short contact times, since the yields starting from **9a** and from **9b** are different. These results may bring clarity to the whole mechanism taking into account the microscopic reversibility principle. Thus, it was demonstrated that the angular compounds form the diazirine with negative entropies of activation. If the linear compounds also afford isomerization to the angular one, the reaction should proceed through the diazirine. If this is so, the open chain intermediates (**32** and **33**) postulated in path c–e (Scheme 13) cannot be present, as the angular compounds can be formed directly from these intermediates without cyclization to the diazirine. With this in mind, path c–d seems more possible (Scheme 15, Fig. 2) as the actual mechanism in accordance with the microscopic reversibility principle. As the linear isomer has an unfavorable *ortho* quinoid arrangement it is expected to be less stable than the linear isomer (as naphthalene and phenanthrene), so the qualitative energy profile of the reaction in Figure 2 could be a suitable representation of these reactions.

The kinetic expression for this reaction (assuming stationary state for B) is:

$$k = k_1 + (k_{-1}k_{-2}/k_2) \quad (1)$$

An analysis of the relative expected values of the different rate constants show that k_2 should be larger than k_{-1} as the reaction goes to products; k_{-2} should have a smaller value than k_2 but larger than k_1 as this reaction starts from the thermodynamically less stable isomer. These assumptions make the second term of the rate constant expression (Eq. 1) negligible, so the measured rate constant $k_{obs} \sim k_1$, which is



Scheme 15.

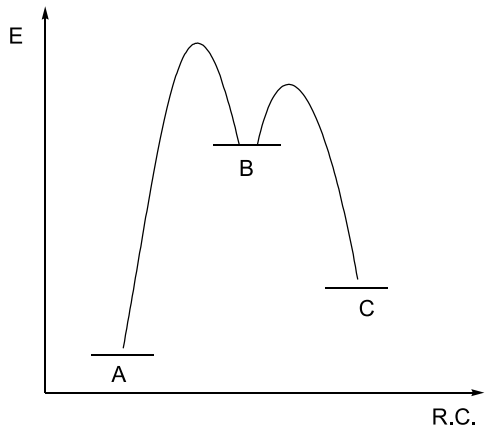


Figure 2. Qualitative representation of reaction coordinate for reactions of **1a–d**. A: initial angularly fused triazole **1**, B: azirine intermediate, C: linearly fused triazole product.

in accordance with the negative entropy of activation for a ring contraction reaction.

Valuable conclusions can be drawn by analysis of the effect of substituents reflected in the Arrhenius parameters. In NMR studies of the equilibrium between the angular and the linear structures of tetrazolo-benzo-*as*-triazines through the isomeric azide (Scheme 11) the authors demonstrated that substituents attached to C7 have a strong influence on the position of the ternary equilibrium.[16,26] Looking at the data reported in Table 8, it is clear that all the free energies of activation are almost the same, but, the entropic and enthalpic data are quite different, as well as reaction constants measured at the same temperature. The more electron donating the substituent (methoxy in this case) the faster the reaction. Chlorine has a lower rate constant than methoxy but faster than hydrogen and methyl. Hydrogen and methyl have almost the same values within the experimental errors. Thus, the rate constant values follow the electronic effect of the substituents while the entropies of activation have a different sequence. The large negative value for chlorine can be interpreted as a more concerted character of the reaction probably due to a combination of inductive (electron withdrawing) and electronic (electron donor) effects or an isokinetic relation, ΔH^* is very low, as all compounds have similar ΔG^* confirming that they react by the same mechanism.

Finally, with the above described results it can be concluded that these reactions could be included in types I or III AX of Epioti′s classification. Theoretical calculations should be made to determine, which are the donor and the acceptor partners with the lower energy difference for HOMO–LUMO interaction.

Some additional remarks on formation of aromatic compunds at higher temperatures should be made concerning the analysis of these reactions. As it was stated above, **1a** afforded benzonitrile (**10**) as well as **9a** over 550 °C; **1b** afforded *p*-methylbenzonitrile (**11**), dicyanobenzenes (**12–14**) and **9b** over 550 °C; **1c** afforded anisole (**15**), benzene (**16**) and **9c** over 520 °C and **1d** afforded *p*-chlorobenzonitrile (**17**), benzonitrile (**10**) and **9d**. In all of these reactions also, a carbonaceous residue was obtained at higher temperatures. These results are not surprising and were also found in reactions of 2-substituted 1,2,4-benzotriazines described in a previous paper.[4] It is common for aromatic hydrocarbons to lose a substituent by radical reactions, so products from radical combination are also formed as well as coke.

The striking point is that in reactions of **1b** and **1c** the cyano group is maintained while it is lost in reactions of **1c**. In order to rationalise this finding, some calculations on bond length have been carried out by using Hyperchem™ programme [A semi-empirical method (AM1) with algorithm Polak-Ribiere (Conjugate Gradient)]. Results showed that the C–CN bond is of 1.42 Å for **10**, **11**, **17**, and *p*-methoxybenzonitrile while the C–CH_3 in **11** bond is of 1.48 Å, the C–Cl bond in **17** is of 1.70 Å and the C–OCH_3 in *p*-methoxybenzonitrile is of 1.38 Å. These data show that C–CN is the weaker bond in *p*-methoxybenzonitrile and this may be the reason why anisole is the product formed by the radical scission reaction.

3. Conclusions

Reactions described here support that the isomerization equilibrium between [1,2,4]triazolo[3,4-*c*][1,2,4]benzotriazines (**1**) and [1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazines (**9**) goes through antiaromatic diazirines. Formation of these intermediates is the rate-limiting step with large negative entropies of activation. It was proved that electron-donating substituents diminish the concerted character reflected in a less negative entropy of activation and larger reaction rate constant. All the studied compounds have almost the same free energy of activation values, which suggests that all of them react by the same mechanism. Reactions described here show that the mechanism of isomerization is different from those described for similar compounds with 1,2,3-triazoles and tetrazoles where open chain intermediates were isolated or detected by spectroscopy.

4. Experimental

4.1. General

Flash vacuum pyrolysis reactions were carried out in a vycor glass reactor using a Thermolyne 21100 tube furnace with a temperature controller device. Oxygen-free dry nitrogen was used as carrier gas. Samples to be pyrolyzed were of ~40 mg. Contact times were around 10^{-2} s and pressures of 0.02 Torr were used. Products were trapped at the liquid air temperature, extracted with solvent and submitted to different analysis or separation techniques. Gas chromatography/mass spectrometry (GC/MS) analysis were performed with a SE-30 column, using helium as eluent at a flow rate of 1 mL/min, the heating rate was different for each compound or mixture of compounds. Mass spectra were obtained in the electron impact mode (EI) using 70 eV as ionization energy. ^{1}H NMR and ^{13}C NMR spectra were carried out in $CDCl_3$ with a Bruker 200 FT spectrometer (at 200 MHz) and in DMSO with Varian UNITY INOVA spectrometer (200 and 400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are reported in ppm

downfield from TMS. The IR spectra were recorded with a Thermo Nicolet AVATAR 320 FT-IR spectrophotometer. Column and thin layer chromatographies were performed on silica gel. Solvents were analytical grade. Recovery of material was >90% in all fvp experiments. Melting Points were determined by a Büchi apparatus and are uncorrected.

4.2. Synthesis of starting materials

Compounds **1a** and **1d** were prepared by a previously described procedure.[27]

4.2.1. 7-Methyl[1,2,4]triazolo[3,4-*c*]benzo[1,2,4]triazine (1b). This compound was prepared by the same procedure as described for **1a** and **1d** starting from 3-hydrazino-7-methyl[1,2,4]benzotriazine[26] (1.0 g, 5.7 mmol). It was mixed with triethyl orthoformate (10.0 g, 11.2 mL, 67.5 mmol) and refluxed for 10 h (oil bath temperature 130–140 °C). The reaction mixture was cooled down to room temperature and the precipitated solid was filtered off. The crude product was recrystallized from DMF to give the product (0.746 g, 70.6%): mp 289–290 °C; IR (KBr) ν_{max} 3107, 1579, 1524, 1477, 1360, 1316, 1216, 1227, 1191, 1124, 1030, 957, 825, 772, 662, 636, 600, 552, 424 cm^{-1}; ^1H NMR (DMSO-d_6 400 MHz) δ 2.6 (s, 3H, H–CH_3), 7.95 (dd, 1H, $J=2.0$, 8.4 Hz, H-8), 8.34 (d, 1H, $J=8.4$ Hz, H-9), 8.48 (d, 1H, $J=2.0$ Hz, H-6), 10.5 (s, 1H, H-1); ^{13}C NMR (DMSO-d_6) δ 21.3 (C–CH_3), 117.0 (C-9), 120.8 (C-9a), 131.4 (C-6), 135.7 (C-1), 138.0 (C-8), 138.4 (C-5a), 139.2 (C-7), 153.5 (C-3a). Anal. Calcd for $C_9H_7N_5$ (185.19): C, 58.37; H, 3.81; N, 37.82. Found: C, 58.36; H, 3.67; N, 37.52.

4.2.2. 7-Methoxy[1,2,4]triazolo[3,4-*c*]benzo[1,2,4]triazine (1c). This compound was prepared by the same procedure as described for **1a** and **1d** starting from 3-hydrazino-7-methoxy[1,2,4]benzotriazine[26] (1.0 g, 5.2 mmol). It was mixed with triethyl orthoformate (10.0 g, 11.2 mL, 67.5 mmol) and refluxed for 10 h (oil bath temperature 130–140 °C). The reaction mixture was cooled down to room temperature and the precipitated solid was filtered off. The crude product was recrystallized from DMF to give the product (0.905 g, 86.1%): mp 298–299 °C; IR (KBr) ν_{max} 3111, 1616, 1582, 1520, 1458, 1389, 1364, 1327, 1253, 1156, 1116, 1038, 1009, 945, 847, 770, 650, 631, 529 cm^{-1}; ^1H NMR (DMSO-d_6 400 MHz) δ 4.0 (s, 3H, H–OCH_3), 7.77 (dd, 1H, $J=9.0$, 2.8 Hz, H-8), 8.18 (d, 1H, $J=2.8$ Hz, H-6), 8.42 (d, 1H, $J=9.0$ Hz, H-9), 10.06 (s, 1H, H-1); ^{13}C NMR (DMSO-d_6) δ 57.1 (C–MeO), 111.9 (C-6), 117.7 (C-9a), 118 (C-9), 126.2 (C-8), 136.2 (C-1), 139.3 (C-5a), 153.7 (C-3a), 159.6 (C-7). Anal. Calcd for $C_9H_7N_5O$ (201.18): C, 53.73; H, 3.51; N, 34.81. Found: C, 53.69; H, 3.27; N, 34.41.

4.3. General procedure for isolation of [1,2,4]triazolo-[4,3-*b*]benzo[1,2,4]triazines (9a–d)

Reaction mixtures obtained with the synthesis of **1a–d** starting from 3-hydrazinobenzo[1,2,4]triazines (**18a–d**, 5 mmol) were worked up as follows. The main product (**1**) was removed by filtration, the mother liquor was evaporated and the residue was subjected to column chromatography on silica. Besides the main product a well defined yellow fraction with a higher R_f value appeared in all cases which was separated, evaporated, and the residue recrystallized from the given solvent.

4.3.1. [1,2,4]Triazolo[4,3-*b*]benzo[1,2,4]triazine (9a). Synthesis of **1a**[27] was carried out starting from 3-hydrazinobenzo[1,2,4]triazine[26] (**18a**, 0.806 g, 5 mmol). Work up of the reaction mixture (column chromatography with eluent hexane/ethyl acetate 3:7) yielded, besides **1a**, **9a** as brown crystals (0.033 g, 3.86%): mp 207–209 °C; IR (KBr) ν_{max} 3091, 1505, 1381, 1290, 1140, 1023, 932, 783, 760, 729, 708, 607, 421 cm^{-1}; ^1H NMR (DMSO-d_6 400 MHz) δ 7.74–7.86 (m, 2H, H-7, H-8), 7.86 (d, 1H, $J=9.5$ Hz, H-9), 7.92 (d, 1H, $J=9.5$ Hz, H-6), 10.08 (s, 1H, H-3); ^{13}C NMR (DMSO-d_6) δ 127.4 (C-9), 129.6 (C-8), 133.7 (C-7), 136.0 (C-6), 137.3 (C-3), 142.0 (C-5a), 145.2 (C-9a), 147.4 (C-10a); HRMS calcd for $C_8H_5N_5$ 171.0545, found 171.0544. Anal. Calcd for $C_8H_5N_5$ (171.1625): C, 56.14; H, 2.94; N, 40.92. Found C, 56.50; H, 2.76; N, 40.62.

4.3.2. 7-Methyl[1,2,4]triazolo[4,3-*b*]benzo[1,2,4]triazine (9b). Synthesis of **1b** was carried out starting from 7-methyl-3-hydrazinobenzo[1,2,4]triazine[26] (**18b**, 0.876 g, 5 mmol). Work up of the reaction mixture yielded, besides **1b**, **9b** as ocher crystals (0.026 g, 2.89%): mp 240–241 °C; UV λ (nm) log ε in acetonitrile: 352 (3.645), 444 (3.379); IR (KBr) ν_{max} 3109, 1540, 1373, 1296, 1150, 1029, 1009, 939, 814, 728, 709, 659, 567, 424 cm^{-1}; ^1H NMR ($CDCl_3$ 200 MHz) δ 2.58 (s, 3H, H–CH_3), 7.60 (s, 1H, H-6), 7.59 (d, 1H, $J=9.5$ Hz, H-8), 7.84 (d, 1H, $J=9.5$ Hz, H-9), 9.43 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 22.52 (C–CH_3), 123.7 (C-6), 129.2 (C-9), 135.9 (C-8), 138.5 (C-7), 138.9 (C-3), 142.0 (C-9a), 144.3 (C-5a), 147.2 (C-10a); HRMS calcd for $C_9H_7N_5$ 185.0701, found 185.0696. Anal. Calcd for $C_9H_7N_5$ (185.19): C, 58.37; H, 3.81; N, 37.82. Found C, 58.26; H, 3.75; N, 37.48.

4.3.3. 7-Methoxy[1,2,4]triazolo[4,3-*b*]benzo[1,2,4]triazine (9c). Synthesis of **1c** was carried out starting from 7-methoxy-3-hydrazinobenzo[1,2,4]triazine[26] (**18c**, 0.956 g, 5 mmol). Work up of the reaction mixture yielded, besides **1c**, **9c** as yellow crystals (0.0179 g, 1.77%): mp 216–218 °C; UV λ (nm) log ε in acetonitrile: 368 (3.564), 439 (2.992); IR (KBr) ν_{max} 3094, 1626, 1548, 1535, 1450, 1428, 1383, 1300, 1249, 1213, 1180, 1132, 1034, 998, 944, 828, 738, 709, 660, 622, 557, 406 cm^{-1}; ^1H NMR ($CDCl_3$ 400 MHz) δ 4.00 (s, 3H, H–OCH_3), 6.90 (s, 1H, H-6), 7.44 (d, 1H, $J=9.9$ Hz, H-8), 7.80 (d, 1H, $J=9.9$ Hz, H-9), 9.34 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 56.6 (C–OCH_3), 99.2 (C-6), 130.78 (C-9), 132.8 (C-8), 135.9 (C-3), 143.4 (C-9a), 143.5 (C-5a), 147.2 (C-7), 162.6 (C-10a); HRMS calcd for $C_9H_7N_5O$ 201.065, found 201.0642. Anal. Calcd for $C_9H_7N_5O$ (201.18): C, 53.73; H, 3.51; N, 34.81. Found C, 53.47; H, 3.35; N, 34.70.

4.3.4. 7-Chloro[1,2,4]triazolo[4,3-*b*]benzo[1,2,4]triazine (9d). Synthesis of **1d** was carried out starting from 7-chloro-3-hydrazinobenzo[1,2,4]triazine[26] (**18d**, 0.978 g, 5 mmol). Work up of the reaction mixture yielded, besides **1d**, **9d** as yellow crystals (0.035 g, 3.40%): mp 295–299 °C; UV λ (nm) log ε in acetonitrile: 354 (3.672), 444 (3.352); IR (KBr) ν_{max} 3122, 3066, 1611, 1505, 1435, 1296, 1248, 1149, 1056, 1022, 935, 877, 834, 799, 712, 615, 579, 522, 423 cm^{-1}; ^1H NMR ($CDCl_3$ 200 MHz) δ 7.68 (d, 1H, $J=$

9.7 Hz, H-8), 7.91 (s, 1H, H-6), 7.94 (d, 1H, $J=9.7$ Hz, H-9), 9.49 (s, 1H, H-3); ^{13}C NMR ($CDCl_3$) δ 118.3 (C-6), 124.4 (C-9), 131.1 (C-8), 131.7 (C-7), 136.2 (C-9a), 136.6 (C-5a), 140.0 (C-3), 141.6 (C-10a); HRMS calcd for $C_8H_4N_5Cl$ (^{35}Cl) 205.01552, found 205.01550. Anal. Calcd for $C_8H_4N_5Cl$ (205.60): C, 46.73; H, 1.96, N, 34.06. Found C, 46.76, H, 1.96, N, 33.76.

4.4. Flash vacuum pyrolysis of 1a

Reactions of **1a** were carried out between 450 and 600 °C, with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. The reaction was clean affording a single product up to 525 °C identified as [1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazine (**9a**) (Scheme 6). Over 550 °C small amounts of **10** and a carbonaceous residue were formed from decomposition. The relative amounts of **1a** and **9a** were calculated by ^{1}H NMR. The structure of **9a** was confirmed by comparison with authentic sample prepared from **18a**. Data for at least three reactions were averaged for the kinetic measurements.

Benzonitrile (**10**). Mass Spectrum: more than 90% match with NIST database. CAS No. 100-47-0.

4.5. Flash vacuum pyrolysis of 1b

Reactions of **1b** were carried out between 450 and 600 °C, with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. The reaction was clean affording a single product up to 525 °C identified as [1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazine (**9b**) (Scheme 8). Over 550 °C small amounts of **10**, *p*-methylbenzonitrile (**11**) and a mixture of dicyanobenzenes (**12–14**) were also formed from decomposition of **1b** and/or **9b** (Scheme 8). The structure of **9b** was confirmed by comparison with authentic sample prepared from **18b**. Relative quantification of **1b** and **9b** was performed by ^{1}H NMR. It is important to mention that 100% conversion could not be achieved and that over 600 °C coke was also formed. Compounds **10** and **12–14** are formed from pyrolysis of **11**. Data for at least three reactions were averaged for the kinetic measurements.

p-Methylbenzonitrile (**11**). Mass Spectrum: more than 90% match with NIST database. CAS No. 100-47-0.

Dicyanobenzenes (**12–14**). Mass Spectrum: (1,2) More than 90% match with NIST database CAS No. 91-15-6. Mass Spectrum: (1,3) More than 90% match with NIST database. CAS No. 626-17-5. Mass Spectrum: (1,4) more than 90% match with NIST database. CAS No. 623-26-7.

4.6. Flash vacuum pyrolysis of 1c

Reactions of **1c** were carried out between 440 and 540 °C, with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. Up to 520 °C the only product was 7-methoxy-[1,2,4]triazolo[4,3-*b*][1,2,4]benzotriazine (**9c**), over this temperature anisole (**15**), benzene (**18**) and coke were also formed (Scheme 9). The structure of **9c** was confirmed by comparison with authentic sample prepared from **18c**. Relative quantification of **1c** and **9c** were performed by ^{1}H NMR. Data for at least three reactions were averaged for the kinetic measurements.

Methoxybenzene (anisole) (**15**). Mass Spectrum: more than 90% match with NIST database. CAS No. 100-66-3.

Benzene (**16**). Mass Spectrum: more than 90% match with NIST database. CAS No. 71-43-2.

4.7. Flash vacuum pyrolysis of 1d

Reactions of **1d** were carried out between 460 and 560 °C with pressures of $\sim 10^{-2}$ Torr and contact times of $\sim 10^{-2}$ s. As in reactions of **1a–c** only one product was formed up to 520 °C, identified as 7-chloro[1,2,4]triazolo[4,3-*b*][1,2,4]-benzotriazine (**9d**), at higher temperatures 4-chlorobenzonitrile (**17**) and **10** as well as coke were also formed (Scheme 10). The structure of **9d** was confirmed by comparison with authentic sample prepared from **18d**. Relative quantification of **1d** and **9d** was performed by ^{1}H NMR. Data for at least three reactions were averaged for the kinetic measurements.

4-*Chlorobenzonitrile* (**17**). Mass Spectrum: more than 90% match with NIST database. CAS No. 623-03-0.

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Molecular structure and hydrolytic stability amidinium salts derived from triazatricyclo[5.2.1.0^{4,10}]decane

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Abstract—Five amidinium salts have been prepared from triazatricyclo[5.2.1.0^{4,10}]decane (tacnoa) and characterised by mass spectrometry, NMR spectroscopy and X-ray crystallography. The X-ray structures revealed a long distance between the methine carbon and the ammonium nitrogen, viz., C–N distance 1.64–1.70 Å, cf. other C–N distances of 1.40–1.50 Å. An NMR study of 1-ethyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}]decane and 1-benzyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}]decane, confirmed that these amidinium salts hydrolyse in aqueous solution, the latter 60 times faster than the former. Tacnoa, which has C–N distances typical of single bonds, showed no evidence of hydrolysis after several days at 80 °C. Molecular modeling calculations indicate that the preferred gas phase structure of the salts is one where the positive charge is delocalised over the two secondary amines and the methine carbon. The calculated distance between this carbon and the ammonium nitrogen is 0.15–0.4 Å longer than in the crystal structure. The energy difference between the preferred gas phase and solid state conformations is 2 kJ mol^{-1} and presents little barrier to nucleophilic attack of the methine carbon. Further analysis of the methine carbon geometry (C(7)) reveals that the bond angles in the benzyl salt are closer to those expected for an sp^2 centre than in the ethyl salt and that this could be the origin of the faster hydrolysis rate.

1. Introduction

Triazatricyclo[5.2.1.0^{4,10}]decane, tacnorthoamide (tacnoa) has been extensively used as a synthon to N-substituted derivatives of 1,4,7-triazacyclononane (tacn).[1–18] A reaction sequence summarising aspects of this efficient chemistry is shown in Scheme 1. In aprotic solvents, tacnoa reacts readily with a variety of electrophiles forming amidinium salts, which are readily hydrolysed in water to give formyl derivatives. These formyl derivatives have a single secondary nitrogen at which further functionalisation can be achieved by reaction with a second electrophile. Hydrolysis of the formyl group in acid yields a di-substituted tacn derivative, which can be reacted with a third electrophile to yield the asymmetric tri-substituted tacn derivative.[7]

The tacnoa synthon has also proved extremely useful in the development of syntheses to ligands that incorporate two, three or four tacn macrocycles within the same framework.[19–30] Reaction of bis-, tris- or tetrakis-electrophiles with tacnoa yields the corresponding bis-, tris- or tetrakis-amidinium salts, which can be hydrolysed to ligands comprising two to four macrocycles held together by various aliphatic and aromatic groups. In principle, asymmetric poly(tacn) assemblies can also be prepared since, as was the case for the mono-amidinium salts, hydrolysis of the poly-amidinium salts gives poly-formyl derivatives that can be functionalised at each exposed secondary amine. Hydrolysis of the formyl groups generates derivatives in which each macrocycle is mono-functionalised and has a single secondary amine on which further functionalisation can be carried out.

The synthetic utility of tacnoa and amidinium derivatives thereof has led us to explore the structure and hydrolytic stability of amidinium derivatives of tacnoa (such as **1**–**5** below). In particular, we report herein the X-ray crystal structure of four such amidinium salts (**2**–**5**), some of which have been reported by other workers[4,7,31] but not subjected to X-ray structure determination, together with measurements of the rate of hydrolysis of two of these compounds. To aid in the elucidation of the origin of differences in hydrolytic reactivity, the X-ray structures have been complemented by molecular modeling calculations and measurements of the rate of hydrolysis, in neutral aqueous solution, on two amidinium salts and tacnoa.

Keywords: 1,4,7-Triazacyclononane; Amidinium salts; X-ray structure; Hydrolysis rate; Molecular modeling.
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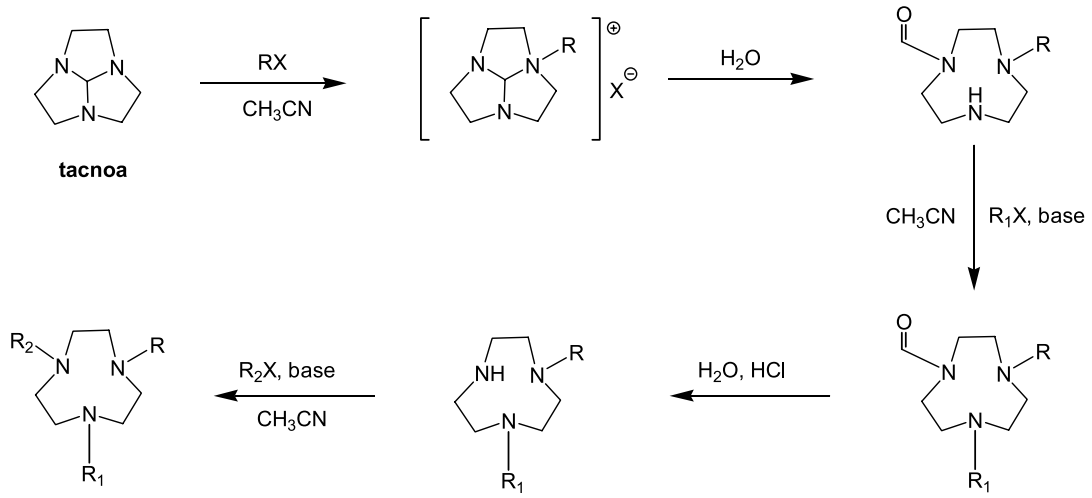
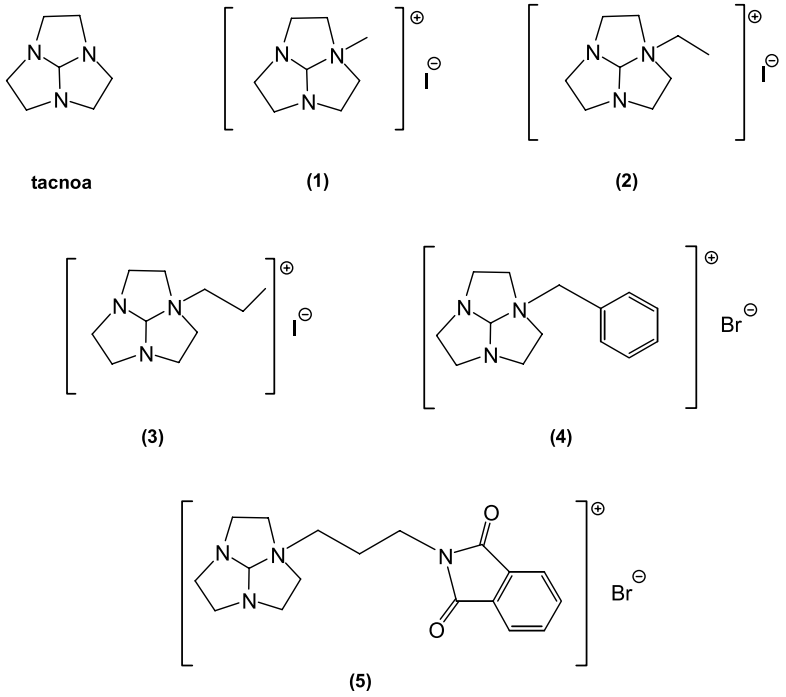


Scheme 1.



2. Results and discussion

All compounds were obtained as white microcrystalline solids in excellent yields ($\geq$90%). They gave clean ^{1}H and ^{13}C NMR spectra and ESI-MS signal at the appropriate *m*/*z* values. They are moderately stable to moisture but dissolve readily in H_2O, whereupon hydrolysis occurs. The rates of hydrolysis of two compounds, **2** and **4**, were found to differ (see below) but both were faster than the rate of hydrolysis of triazatricyclo[5.2.1.0^{4,10}]decane (tacnoa).

2.1. Description of structures

Details of crystal structure refinements are given in Table 1. A single X-ray crystal analysis confirmed the structure of **2** to consist of discrete tacnoaet cations and iodide anions

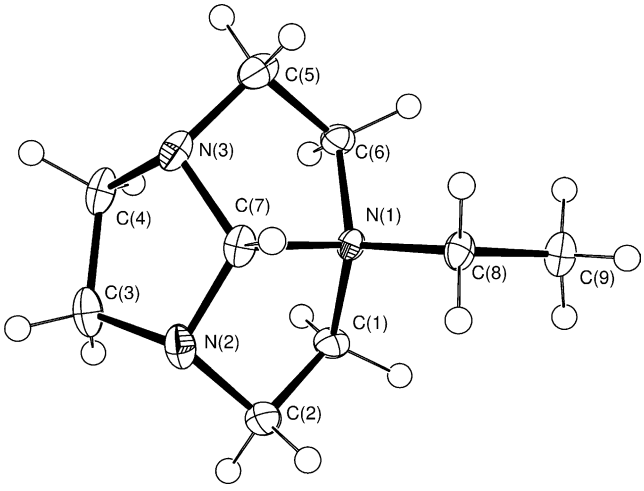


Figure 1. ORTEP representation of the cation in **2** (probability ellipsoids drawn at 50%).

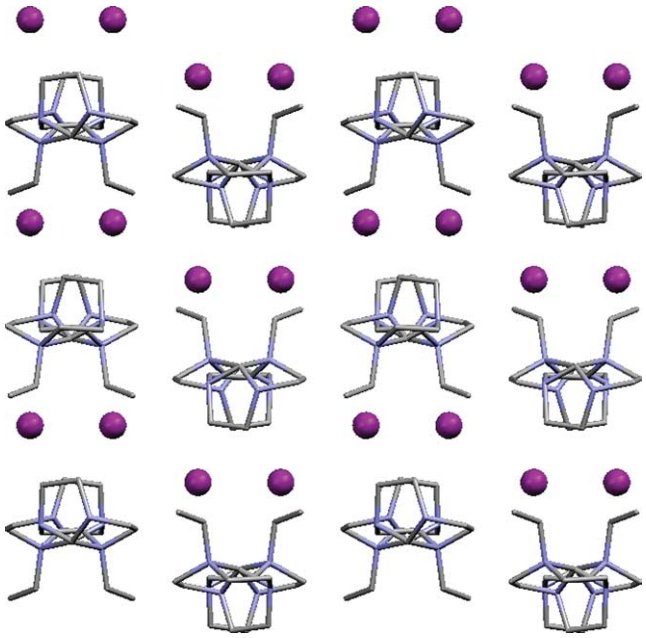


Figure 2. Mercury representation of the packing in **2**.

(Fig. 1), the cations themselves forming channels within the crystal lattice (Fig. 2), with the space between them occupied by the iodide anions. Data refinement ($R=2.3\%$) was such that the hydrogen atoms were located in the difference map. Weak interactions occur in the structure, and the molecules are oriented so as to maximize the spacing between each layer, the ethyl chain on each cation being directed into the cavities between the iodide anions (Table 1).

Table 2 summarises the bond length and angles for **2** while Table 3 details close contacts between the iodide ion and cation unit below 3.2 Å. Other contacts between cations units include N(3)···H(1) $(-x+1/2,\ +y+1/2,\ +z)$ 2.663(1) Å and N(2)···H(11) $(x-1/2,-y+1/2,-z+1)$ 2.668(1) Å. The angles around the central methine bridge are an interesting feature, particularly that between H(18)–C(7)–N(1) (103(1)°), which is lower than the ideal tetrahedral angle. The closest contact between the methine group and I(1) is 3.45 Å $(x+0.5,\ -y+0.5,\ -z+1)$ indicating that steric factors do not contribute to the deviation from tetrahedral geometry around this carbon.

Table 2. Selected bond lengths (Å) and angles (°) in **2–5**

	2	**3**	**4**	**5**
N(1)–C(1)	1.505(3)	1.511(5)	1.511(3)	1.501(4)
N(1)–C(6)	1.504(3)	1.505(4)	1.494(3)	1.504(5)
N(1)–C(7)	1.658(3)	1.638(4)	1.661(3)	1.700(5)
N(1)–C(8)	1.509(3)	1.508(4)	1.513(3)	1.496(4)
N(2)–C(2)	1.476(3)	1.474(5)	1.463(3)	1.465(5)
N(2)–C(3)	1.488(3)	1.478(5)	1.478(3)	1.487(5)
N(2)–C(7)	1.417(3)	1.428(5)	1.413(3)	1.410(5)
N(3)–C(4)	1.484(3)	1.494(4)	1.488(3)	1.491(5)
N(3)–C(5)	1.461(3)	1.468(4)	1.457(3)	1.455(5)
N(3)–C(7)	1.413(3)	1.421(5)	1.411(3)	1.402(5)
C(1)–C(2)	1.516(3)	1.518(5)	1.517(3)	1.531(6)
C(4)–C(3)	1.516(4)	1.514(6)	1.472(4)	1.501(6)
C(5)–C(6)	1.539(3)	1.541(5)	1.518(3)	1.542(6)
N(1)–C(1)–C(2)	103.6(2)	104.5(3)	103.8(2)	103.3(3)
N(1)–C(6)–C(5)	103.6(2)	103.1(3)	104.2(2)	103.8(3)
N(1)–C(8)–C(9)	114.3(2)	115.5(3)	115.2(2)	115.2(3)
N(2)–C(2)–C(1)	105.4(2)	105.1(3)	105.7(2)	105.6(3)
N(2)–C(3)–C(4)	105.9(2)	102.9(3)	106.8(2)	105.8(3)
N(2)–C(7)–N(1)	106.8(2)	105.9(3)	106.4(2)	105.5(3)
N(3)–C(4)–C(3)	104.2(2)	105.3(3)	106.4(2)	104.4(3)
N(3)–C(7)–N(1)	106.1(2)	107.1(3)	106.0(2)	105.4(3)
N(3)–C(7)–N(2)	110.7(2)	110.3(3)	111.0(2)	110.7(3)
N(3)–C(5)–C(6)	105.3(2)	105.8(3)	105.8(2)	105.2(3)
C(1)–N(1)–C(7)	101.9(2)	103.2(3)	102.0(2)	101.7(3)
C(1)–N(1)–C(8)	112.5(2)	112.6(3)	112.4(2)	112.8(3)
C(2)–N(2)–C(3)	115.7(2)	115.4(3)	115.8(2)	116.1(3)
C(5)–N(3)–C(4)	115.1(2)	115.3(3)	114.6(2)	116.1(3)
C(6)–N(1)–C(1)	116.9(2)	116.2(3)	116.0(2)	116.8(3)
C(6)–N(1)–C(7)	102.6(2)	102.1(3)	102.4(2)	101.4(3)
C(6)–N(1)–C(8)	112.6(2)	112.6(3)	113.9(2)	113.9(3)
C(7)–N(2)–C(2)	106.1(2)	105.1(3)	106.4(2)	107.0(3)
C(7)–N(2)–C(3)	106.9(2)	105.3(3)	106.8(2)	107.5(3)
C(7)–N(3)–C(4)	106.4(2)	106.1(3)	105.9(2)	106.9(3)
C(7)–N(3)–C(5)	105.1(2)	106.7(3)	106.2(2)	105.5(3)
C(8)–N(1)–C(7)	108.9(2)	108.9(3)	108.7(2)	108.2(3)

This deviation may be due, in part, to the effect imposed by the long N(1)–C(7) bond length of 1.658(3) Å, and partial amidinium character imposed on N(2) and N(3), as evidenced by the shorter N(2)–C(7) and N(3)–C(7) bond lengths of 1.417(3) and 1.413(3) Å, respectively, compared with the other N–C distances in the molecule, which are typically 1.48–1.51 Å. Figure 2 shows a representation of the packing in the molecule revealing that the ethyl arms are pointed towards the cavities between the molecules and that the iodo anions occupy the spaces between the cations.

An ORTEP representation of the cation **3** is shown in

Table 1. Crystal structure refinement data for **2–5**

	2	**3**	**4**	**5**
Formula	$C_9H_{18}N_3I$	$C_{10}H_{20}N_3I$	$C_{14}H_{20}N_3Br$	$C_{18}H_{23}N_4O_2Br$
Formula weight	295.16	309.19	310.24	407.31
Crystal system	Orthorhombic	Monoclinic	Orthorhombic	Monoclinic
Space group	*Pbca*	$P2_1/n$	*Pbca*	$P2_1/c$
a (Å)	12.254(3)	7.009(1)	9.865(2)	13.458(3)
b (Å)	12.678(3)	14.038(3)	9.286(2)	32.440(7)
c (Å)	14.789(3)	12.731(3)	29.966(6)	8.263(2)
Volume (Å^3)	2297.7(8)	1212.1(4)	2745.0(10)	3572(13)
β (°)		104.61(3)		98.03(3)
ρ_c (g cm^{-3})	1.707 ($Z=8$)	1.694 ($Z=4$)	1.501 ($Z=8$)	1.515 ($Z=8$)
μ_{Mo} (mm^{-1})	2.752	2.612	2.982	2.321
$T_{min/max}$	0.2169/0.8523	0.6953/0.9027	0.5599/0.8652	0.7370/0.9129
Reflections collected	15,782	9369	23,129	32,592
Independent reflections	2729 [$R_{int}=0.0459$]	2872 [$R_{int}=0.0824$]	3283 [$R_{int}=0.0787$]	8244 [$R_{int}=0.1266$]
R	0.023	0.0372	0.0372	0.0574
R_w	0.0392	0.0872	0.0853	0.1445

Table 3. Hydrogen bonding contacts in **2** and **4**

2			**4**		
	X···H Distance (Å)	X···H–Y Angle (°)		X···H Distance (Å)	X···H–Y Angle (°)
I(1)···H(2)–C(1)[a]	3.09	169.6	C(8)–H(8A)···Br(1)[b]	2.80	172.2
I(1)···H(7)–C(4)[c]	3.15	154.5	C(5)–H(5A)···Br(1)[b]	2.81	155.4
I(1)···H(13)–C(7)[d]	3.17	157.1	C(2)–H(2A)···Br(1)[e]	2.84	168.9
I(1)···H(3)–C(2)[f]	3.18	81.3	C(2)–H(2B)···Br(1)[f]	2.87	160.9
			C(4)–H(4A)···N(2)[g]	2.52	141.4

[a] $(x+0.5, +y, -z+0.5)$.
[b] $(x, +y+1, +z)$.
[c] $(-x+1, +y-0.5, -z+0.5)$.
[d] $(x+0.5, -y+0.5, -z+1)$.
[e] $(-x+1.5, +y+0.5, +z)$.
[f] $(-x+1, -y, -z+1)$
[g] $(x+0.5, +y, -z+0.5)$.

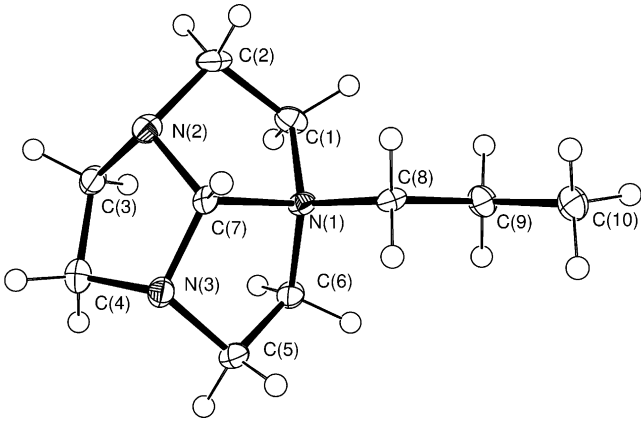


Figure 3. ORTEP representation of the cation in **3** (probability ellipsoids drawn at 50%).

Figure 3. The bond lengths and angles for the orthoamidinium ring are shown in Table 2. Again, this compound forms channels of cation and anion, the cation units being inverted in each column, so that the propyl chains of one row face those in the next row (i.e., in a head to head manner, Fig. 4), presumably in order to minimize contacts between neighbouring rings. The iodide anion sits almost equidistant between two propyl chains, the distance to the nearest hydrogen H(10C) (x, y, z) being 3.11 Å, while the distance to the nearest (symmetry related) propyl chain is 3.05 Å (I(1)···H(10B) $(x+1, +y, +z)$). This atom position is almost perfectly equidistant from H(1A) of another symmetry related cation unit, the distance being also 3.05 Å (I(1)···H(1A) $(-x-0.5, +y+0.5, -z+0.5)$). Indeed, this contact may be representative of a weak hydrogen bond, the angle between C1–H(1A)···I(1) being 170.9°. The other closest contact occurs between N(3)···H(1B) $(x+1, +y, +z)$, the distance being 2.78 Å. The propyl chain adopts the expected staggered conformation, the torsion angle between N(1)–C(8)–C(9)–C(10) being −176.3(3)°.

The crystal structure of **4** is shown in Figure 5, the bond lengths and angles around the tacn orthoamidinium ring being detailed in Table 2. As is the case for the other compounds, the N(1)–C(7) distance is much longer than the other C(7)–N bonds, again indicating that this cation exhibits significant amidinium character. No π-stacking occurs in the molecules, the closest aromatic C···C interactions being around 4.1 Å. The cations stack in columns, each row of cations interspaced in a head to head manner, so that the aromatic rings are spaced perpendicular to each other (Fig. 6). The anion, in this instance bromide, occupies a cavity between neighbouring orthoamidinium rings. A hydrogen bonding network is present with the structure (Fig. 7) which is defined by close interactions between: (i) Br(1) and H(8B) $(-x+1.5,$

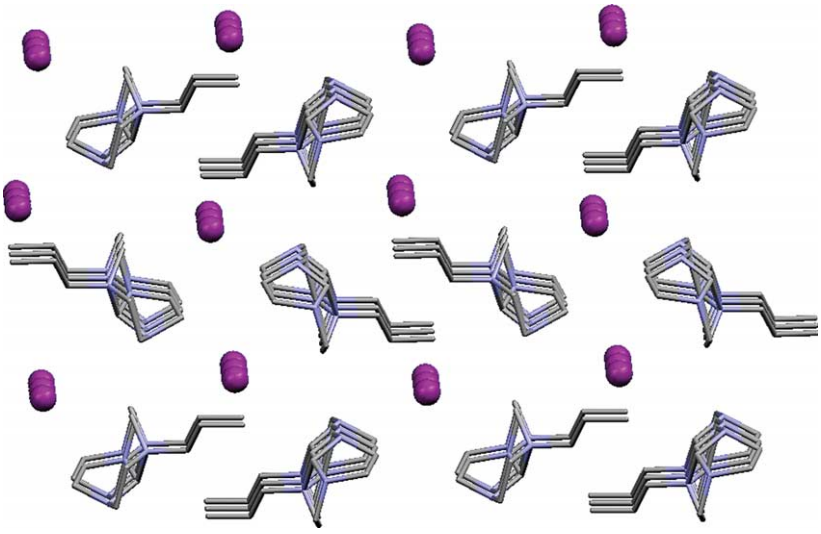


Figure 4. Mercury representation of the packing in **3**.

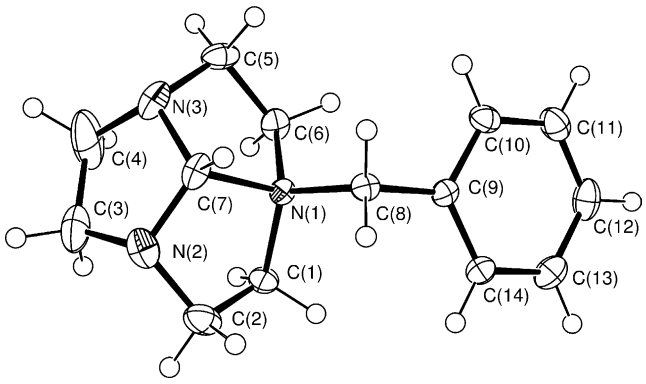


Figure 5. ORTEP representation of the cation **4** (probability ellipsoids drawn at 50%).

$+y+0.5$, $+z$) on the CH_2 bridge, Br···H distance 2.84 Å, and C(8)–H(8B)···Br(1) angle 168.9°; (ii) H(8B) on the CH_2 bridge and a symmetry related bromide ion (H(8A)Br(1) (x, $+y+1$, $+z$; distance 2.80 Å); and (iii) a weak hydrogen bond between C(4)–H(4A) and N(2) (distance 2.52 Å). The bromide also forms an interaction (2.87 Å) with H(2B) (tacn C–H) ($-x+1.5$, $+y+0.5$, $+z$) on the same cation.

Two molecules comprise the asymmetric unit in the crystal structure of **5**, an ORTEP representation of one being shown in Figure 8. The main difference between the two molecules arises primarily from the different twists of the phthalimido group about the propyl C–N bonds, as indicated by the torsion angles N(phth)–C(a)–C(b)–C(c) (propyl) of −171.4° and 178.0°. Table 2 details selected bond lengths and angles around the orthoamidinium ring in one molecule. The orthoamidinium rings form rows, the spaces between the rings being once again occupied by the bromide anion. The molecules are oriented so that the phthalamide rings are arranged in a head to head fashion but do not stack directly above one another and so only form weak π-stacking interactions. Several hydrogen bonds are found in this structure and of particular note is the hydrogen bond occurring between C(36)···H(36) and O(2) on the second cation in the asymmetric unit (distance 2.35 Å). A weaker intermolecular interaction occurs between C(29)–H(29) and O(4) on the same molecule (distance 2.53 Å). In the other cation this interaction is weaker, the distance being 0.1 Å longer, as a consequence of a less acute twist of the phthalamide ring.

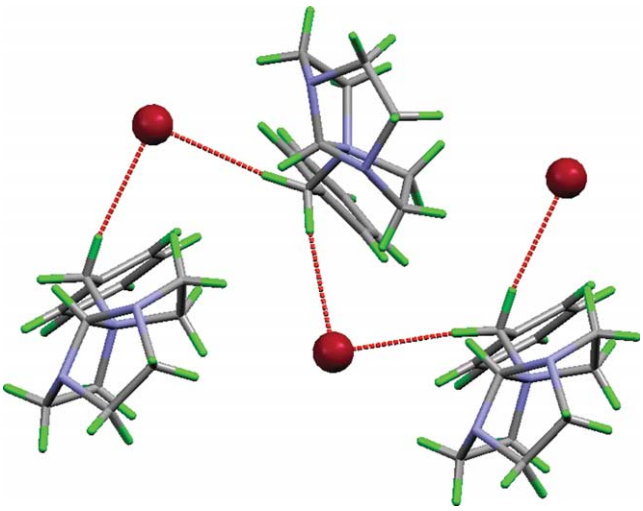


Figure 7. Mercury representation of the hydrogen-bonding network in **4**.

In summary, the structures of the amidinium salts presented herein exhibit long quaternary amine–methine carbon distances (1.64–1.70 Å) coupled with shorter methine–N bonds to the other nitrogen atoms (1.40–1.43 Å), which are indicative of double bond character within the N(2)–C(7)–N(3) unit (see Table 4). Farrugia et al.[32] have noted similar deviations in N′-(3-phenoxypropyl)4-7diaza-1-azoniatricyclo[5.2.1.$0^{4,10}$]decane bromide hydrate. In contrast, in the crystal structure of triazatricyclo[5.2.1.$0^{4,10}$]decane, determined by Blake et al.[33] a symmetric conformation was observed, with an average methine–N distance of 1.474 Å.

2.2. Molecular modelling studies

Density functional calculations were undertaken on **2**, **4** and tacnoa using the crystal structures as the starting point for the optimizations. B3LYP optimization of **2** and **4** indicate that the low energy conformation is the amidinium ion in which the positive charge is delocalized over the two secondary amines instead of the tertiary amine N(1) (Figs. 9

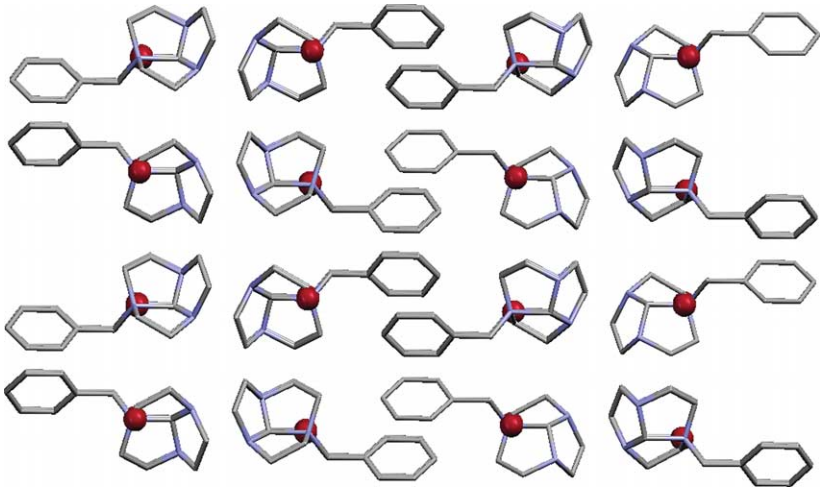


Figure 6. Mercury representation of the packing in **4**.

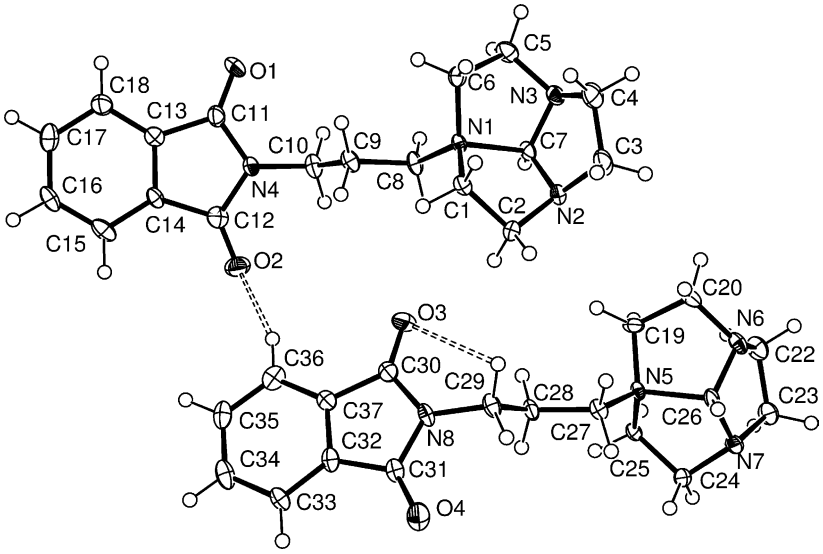


Figure 8. ORTEP representation of the cation **5** (probability ellipsoids drawn at 50%).

Table 4. C–N bond lengths (measured and calculated) for **2**, **4** and tacnoa

Bond	**2**		**4**		Tacnoa	
	Crystal (Å)	Model (Å)	Crystal (Å)	Model (Å)	Crystal (Å)	Model (Å)
N(1)–C(7)	1.658(3)	2.047	1.661(3)	1.803	1.472(3)	1.501
N(2)–C(7)	1.417(3)	1.366	1.413(3)	1.399	1.478(3)	1.469
N(3)–C(7)	1.418(3)	1.366	1.411(3)	1.395	1.473(3)	1.462

and 10). This is highlighted by a shortening of the N(2)–C(7) and N(3)–C(7) distance in both compounds, and a longer N(1)–C(7) bond, which is the site of cleavage that ultimately forms the tertiary amine. The relevant bond distances are shown in Table 4. In order to determine the energy difference between the conformation found in the crystal structure and energy minimized structure, a minimization was preformed in which the C–N bonds on the rings were constrained at their crystal structure values and this was then compared to the energy of structures determined without constraint. In each case, the lower energy amidinium conformations were found to be only slightly more stable than the other but the energy differences were quite small, 2.4 and 1.9 kJ mol^{-1} for the ethyl and benzyl derivatives, respectively. This means that minor crystal packing or solvation effects could easily result in a change in the preferred conformation in the solid state or solution.

By comparison, the energy minimized model of triazatricyclo[5.2.1.0^{4,10}]decane[33] is in good agreement with the crystal structure in bond lengths (Table 4), with an even atomic charge distribution over all three nitrogen atoms, all three atoms having a near neutral charge. Very little bond length or conformational changes occur in the other bonds, indicating that the charge and the methine bridge itself accounts for the instability of the amidinium salts. In both compounds the DFT calculations predicted that the positive charge on the tertiary N(1) migrates to N(2) and N(3). While

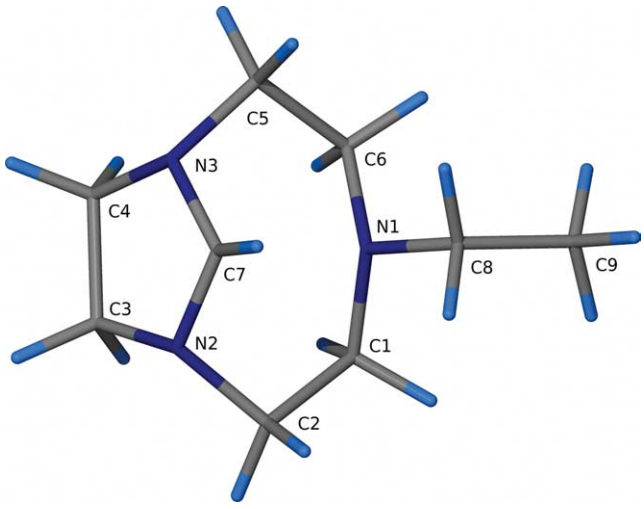


Figure 9. POVRAY representation of the energy minimized structure of compound **2**.

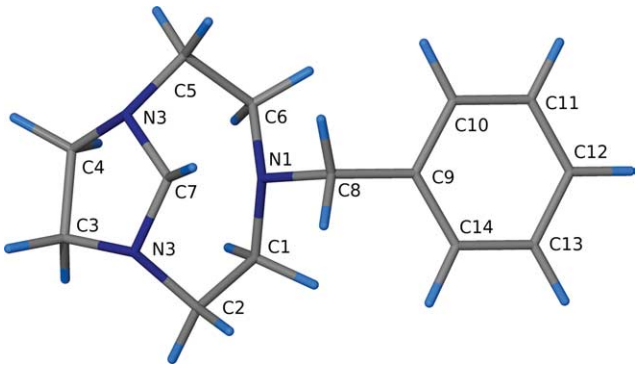


Figure 10. POVRAY representation of the energy minimized structure of compound **4**.

this is feasible, it is likely that this charge is distributed over the N(2)–C(7)–N(3) bond, facilitating nucleophilic attack of the methine carbon by water.

A noteworthy feature of these systems is the difference in electron density on the CH_2 of the appended ethyl and benzyl groups. For the benzyl derivative, the energy minimized model shows a large negative atomic charge on C(8), while in the ethyl derivative this charge is typical of sp^3 hybridised carbon alkyl chains. This increase in negativity on the benzyl derivative is supported by both experimental and crystallographic evidence. The crystal structure of **4** shows hydrogen bonding between the C(8) and two adjacent bromine molecules. Conversely, the three other crystal structures do not show the halide anions participating in hydrogen bonding with the equivalent CH_2 groups. We have also found (unpublished data) that benzyl derivatives of tacn undergo some cleavage of the benzyl group in acidic solution with up to 10% loss being observed. No such loss was noted for the corresponding ethyl or propyl derivatives.

2.3. Kinetic studies

The hydrolysis of tacnorthoamide and two amidinium derivatives, tacnoet (**2**) and tacnobz (**4**) to the corresponding formyl derivatives, 1-formyl-1,4,7-triazacyclononane, 1-ethyl-4-formyl-1,4,7-triazacyclononane and 1-benzyl-4-formyl-1,4,7-triazacyclononane was followed at 80 °C by ^{1}H NMR spectroscopy and rates of hydrolysis determined as described in Section 3. This experiment revealed that the non-ionic tacnorthoamide does not hydrolyse under the conditions used whilst compounds **2** and **4** do hydrolyse. Moreover, the rate of hydrolysis of **4** ($k=6.28(\pm0.20)\times10^{-5}\,s^{-1}$) was found to be 60 times faster than that measured for **2** ($k=1.10(\pm0.03)\times10^{-6}\,s^{-1}$). The slower rate of hydrolysis of tacnoa is not unexpected given the absence of a long N–C (methine) in this molecule (all bonds 1.474(3) Å) whilst in **2** and **4**, the C(methine)–N(quaternary) is much longer (1.660(3) Å) than the other bonds (1.41–1.42 Å). A difference in ground state stability could account for the difference in reaction rates observed for **2** and **4**. However, as the bond distances in **2** and **4**, determined by X-ray structure analysis, were identical it would be anticipated that the two amidinium salts would hydrolyse at the same rate. The conformation of the amidinium ion may be an important determinant of the relative rates of hydrolysis. As described above, molecular modelling calculations show a preference for this form, in which the positive charge is delocalized over the atoms N(2), C(7) and N(3). The effect this charge delocalisation has on the puckering of the three five membered rings is shown in Table 5, which details the RMS deviation from the least squares plane defined by those rings. The ring defined by N(3)–C(7)–N(2)–C(3)–C(4) shows the least deviation from the plane in both **2** and **4** but no such flattening of this ring is seen in the tacnorthamide structure. Indeed, deviation of the plane by all three rings in this structure is almost uniform. In the case of **2** and **4**, the latter shows least deviation from the plane, and hydrolyses 60 times faster than **2**, while under the above conditions there is no noted hydrolysis of the non-ionic tacnorthoamide. It can therefore be argued that the flatter conformation in **4** is indicative of more amidinium ion character than in **2** and that this contributes to faster nucleophilic attack at C(7) by water and hence a higher hydrolysis rate.

Table 5. RMS deviation from the least squares planes in **2**, **4** and tacnoa

Compound	Ring	Deviation (Å)
Ethyl (**2**)	N(3)–C(7)–N(2)–C(3)–C(4)	0.1003
	N(1)–C(1)–C(2)–N(2)–C(7)	0.1703
	N(3)–C(5)–C(6)–N(1)–C(7)	0.1748
Benzyl (**4**)	N(3)–C(7)–N(2)–C(3)–C(4)	0.0723
	N(1)–C(1)–C(2)–N(2)–C(7)	0.1644
	N(3)–C(5)–C(6)–N(1)–C(7)	0.1660
Tacnorthoamide	N(3)–C(7)–N(2)–C(3)–C(4)	0.1542
	N(1)–C(1)–C(2)–N(2)–C(7)	0.1523
	N(3)–C(5)–C(6)–N(1)–C(7)	0.1523

3. Experimental

3.1. Materials

Reagents and solvents were obtained from commercial suppliers and used without further purification. Distilled water was used throughout and acetonitrile was pre-dried over sieves prior to use. 1,4,7-Triazacyclonane trihydrochloride was prepared by the Richman–Atkins method.[34] Triazatricyclo[5.2.1.0^{4,10}]decane was synthesized according to a published method.[21]

3.2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury AM300 300 MHz spectrometer. The chemical shifts, δ, are reported in ppm (parts per million) using the high frequency positive convention, relative to an internal standard of tetramethylsilane (TMS) for non-aqueous solvents and sodium (2,2,3,3-d4-3-(trimethylsilyl))propionate (TMSP-D) for D_2O. Microanalyses were performed by the Campbell Microanalytical Laboratory, University of Otago, New Zealand. Mass spectra were obtained using a Micromass Platform Quadrupole Mass Spectrometer fitted with an electrospray source.

3.3. Preparation of amidinium compounds

Compounds **1** and **2**,[31] **4**[4] and **5**[7] have been reported previously. In this study, however, these and compound **3** were prepared using the procedure described below for 1-methyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}]decane iodide (**1**). Typically triazatricyclo[5.2.1.0^{4,10}]decane (0.41 g, 2.9 mmol) was dissolved in 10 mL MeCN and the solution was stirred. To this solution was added iodomethane (0.489 g, 3.45 mmol) in 5 mL MeCN and the solution was stirred at room temperature. A white solid slowly formed, and the solution was allowed to stir at room temperature for 18 h. The white solid was collected by vacuum filtration, and washed with ether and air dried, the filtrate producing more precipitate on washing with ether. This solid was also collected by filtration and was found to be of equal purity to the first crop of product.

3.3.1. 1-Methyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}] decane (1). Yield, 0.72 g, 90%. Anal. Calcd for **1**·1/2H_2O

($C_8H_{17}N_3O_{1/2}I$): C 33.1, H 5.9, N 14.5%. Found: C 32.7, H 5.9, N 14.2%. NMR spectra (d_3-MeCN): ^{1}H δ 5.43 (s, 1H, CH bridge), 3.72–3.47 (m, 8H, CH_2 ring), 3.25–3.15 (m, 4H, CH_2 ring), 3.11 (s, 3H, NCH_3); ^{13}C δ 126.3, (1C, CH bridge), 61.74, (2C, $CH_2N(Me)CH_2$), 57.40 (2C, CH_2 ring), 53.21 (2C, CH_2 ring), 48.53 (1C, NCH_3). ESI mass spectrum (MeCN): M^+154.2.

3.3.2. 1-Ethyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}] decane (2). Yield, 95%. Anal. Calcd for $C_9H_{18}N_3I$: C 36.6, H 6.2, N 14.2%. Found: 36.7, H 6.0, N 14.5%. NMR spectra (d_3-MeCN): ^{1}H, δ 5.58 (s, 1H, CH bridge), 3.70 (m, 3H, CH_2 tacn ring), 3.46 (m, 4H, NCH_2CH_3 and CH_2 tacn ring), 3.24 (m, 4H CH_2 tacn ring), 1.48 (t, 3H, NCH_2CH_3); ^{13}C δ 142.2 (1C, CH bridge), 57.5 (2C, CH_2 tacn ring), 56.5 (2C, CH_2 tacn ring), 54.3 (1C, NCH_2CH_3), 52.6 (2C, CH_2 tacn ring), 10.6 (1C, NCH_2CH_3). ESI mass spectrum (MeCN): M^+168.2. Crystals for X-ray crystallography were grown by vapour diffusion of ether into a MeCN solution of **2**.

3.3.3. 1-*n*-Propyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}] decane (3). Yield, 95%. Anal. Calcd for $C_{10}H_{20}N_3I$: C 38.9, H 6.5, N 13.6%. Found: C 39.1, H 6.5, N 13.6%. NMR Spectra (d_3-MeCN): ^{1}H, δ 5.49 (s, 1H, CH bridge), 3.67–3.57 (m, 6H, CH_2 tacn ring), 3.42–3.12 (m, 8H, 6H from CH_2 tacn ring and 2H $NCH_2CH_2CH_3$), 1.87–1.76, (sextet, 2H, $NCH_2CH_2CH_3$), 0.97, (t, 3H, $NCH_2CH_2CH_3$); ^{13}C δ 124.9, (1C, CH bridge), 60.53, (1C, $NCH_2CH_2CH_3$), 58.07 (2C, CH_2 tacn ring), 56.70, (2C, CH_2 tacn ring), 52.70 (2C, CH_2 tacn ring), 18.97 (1C, $NCH_2CH_2CH_3$), 11.00 (1C, $NCH_2CH_2CH_3$). ESI mass spectrum (MeCN): M^+182.3. Crystals for X-ray crystallography were grown by vapour diffusion of ether into a MeCN solution of **3**.

3.3.4. 1-Benzyl-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}] decane (4). Yield, 92%. Anal. Calcd for **4**·1/2H_2O ($C_{14}H_{21}N_3O_{1/2}Br$): C 52.7, H 6.6, N 13.2%. Found: C 52.3, H 6.4, N 13.1%. NMR spectra (d_3-MeCN): ^{1}H, δ 7.63–7.61 (m, 2H, CH ar), 7.51–7.49 (m, 3H, CH ar), 5.79, (s, 1H, CH bridge), 3.77–3.71 (m, 2H, NCH_2-C(ar)), 3.56–3.51 (m, 4H, CH_2 tacn ring), 3.30–3.10 (m, 8H CH_2 tacn ring); ^{13}C δ 133.6, (2C CH(ar)), 131.7 (1C, CH(ar)), 131.1 (1C, C(ar)–CH_2), 130.7 (2C, CH(ar), 125.62 (1C, CH bridge), 62.46 (1C, NCH_2–C(ar), 58.37 (2C, CH_2 tacn ring), 57.26 (2C, CH_2 tacn ring), 53.16 (2C, CH_2 tacn ring). ESI mass spectrum (MeCN): M^+230.3. Trace hydrolysis (~1%) of the compound occurred due to trace amounts of H_2O in the d_3-MeCN. Crystals for X-ray crystallography were grown by vapour diffusion of ether into a MeCN solution of **4**.

3.3.5. 1-Propylphthalimido-4,7-diaza-1-azoniatricyclo[5.2.1.0^{4,10}]decane (5). Yield, 93%. Anal. Calcd for **5**·1/2H_2O ($C_{18}H_{24}N_4O_{5/2}Br$): C 51.9, H 5.8, N 13.5%. Found C 52.2, H 5.7, N 13.6%. NMR Spectra (d_3-MeCN): ^{1}H δ 7.84–7.78 (m, 4H, CH(ar)), 5.46 (s, 1H, CH bridge), 3.73 (t, 2H, $NCH_2CH_2CH_2$-phth), 3.63–3.51 (m, 6H CH_2 tacn ring), 3.31–3.08 (m, 8H, 6H from CH_2 tacn ring, and 2H from $NCH_2CH_2CH_2$-phth), ($NCH_2CH_2CH_2$-phth under H_2O peak), ^{13}C δ 169.75 (2C, C=O), 135.72 (2C, CH(ar), 133.69(2C, C(ar) 127.05 (1C, CH bridge), 124.37 (2C, CH(ar), 58.20 (2C, CH_2 tacn ring), 57.41 (2C, CH_2 tacn ring), 56.89 (1C, $NCH_2CH_2CH_2$-phth), 53.09 (2C, CH_2 tacn ring), 36.29 (1C, $NCH_2CH_2CH_2$-phth), 25.59 (1C, NCH_2-CH_2CH_2-phth). ESI mass spectrum (MeCN): M^+328.4. Crystals for X-ray crystallography were grown by vapour diffusion of ether into a MeCN solution of **5**.

3.4. X-ray crystallography

All structures were collected on an Enraf-Nonius CAD4 diffractometer with monochromated Mo Kα radiation (λ= 0.71073 Å) at 123(2) K using phi and/or omega scans. Data were corrected for Lorentz and polarization effects and absorption corrections were applied. The structures were solved by the direct methods and refined using full matrix least-squares within the programs SHELXS-97 and SHELXL-97[35] respectively. The program X-Seed[36] was used as an interface to the SHELX[35] programs, and to prepare the figures. Crystallographic data (excluding structure factors) for the structures presented in this paper have been deposited with the Cambridge Crystallography Data Centre as supplementary publication numbers CCDC 258759–258762. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ [fax: +44 1223 336 033 or e-mail: deposit@ccdc.cam.ac.uk]. Crystal data for the compounds are given in Table 1.

3.5. Molecular modelling calculations

All theoretical calculations were carried out using Gaussian 98[37] running SuSe linux 9.1 on a Dell Optiplex 4700 PC. B3LYP optimisations[38,39] using the 6-31G* basis[40,41] were employed for both compounds.

3.6. Kinetic studies

The hydrolysis of tacnoa, tacnoaet (**2**) and tacnoabz (**4**) was studied in aqueous solution at pH ~7 using ^{1}H NMR spectroscopy. A 0.1 M solution of each compound was prepared by dissolving a sample of each compound in 5 mL of D_2O. The ^{1}H NMR spectrum of each solution was recorded immediately after preparation and then at various times after heating to 80 °C after quenching the reaction on ice. The fraction of each compound converted into the corresponding formyl derivative was determined from the ratio of the integration of the formyl proton signal on the product to that of the single methine proton on the reactant. In the case of tacnoa, there was little evidence of hydrolysis, after several days heating at 80 °C. For the other two compounds, the rate of conversion of starting materials to the formyl derivatives was obtained by fitting the variation in the fraction of formyl product formed with time to the simple exponential function, $F = m\exp(kt)$, where F is the fraction of reactant converted to formyl product, m is a pre-exponential term, k is the rate constant and t is the time elapsed.

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Reaction of methyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)-propenoate with ureas: facile entry into the polycyclic meridianin analogues with uracil structural unit

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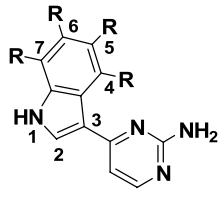
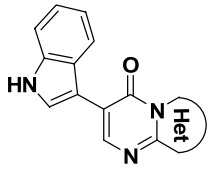
Abstract—Methyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)-propenoate was prepared simply and efficiently in two steps from 3-indoleacetic acid employing *N*,*N*-dimethylformamide dimethylacetal (DMFDMA). Upon treatment of (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)-propenoate with various (thio)ureas in the presence of an acid 2-(1*H*-indol-3-yl)-3-(3-substituted(thio)ureido)propenoates were obtained in high yields. A base promoted cyclization of these (thio)ureidopropenoate derivatives afforded 5-(indol-3-yl)-3-substituted-pyrimidine-2,4-diones which represent a new family of meridianine analogues.

1. Introduction

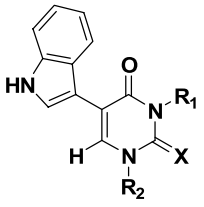
Interest in indole alkaloids originates from their broad spectrum of biological activities.[1] Moreover, many marine natural products with potent pharmacological activity contain an indole nucleus.[2] The meridianins are a family of biologically active marine alkaloids produced by a tunicate *Aplidium meridianum*. Structurally, the meridianins comprise a brominated and/or hydroxylated indole nucleus which bears a 2-aminopyrimidine substituent at 3-position (Fig. 1).[3] They show cytotoxicity towards murine tumor cell lines and are therefore interesting synthetic targets. Recently, it was shown that meridianins are potent inhibitors of several protein kinases. It was suggested that meridianins constitute a promising scaffold from which more potent and selective protein kinase inhibitors could be designed.[4] The synthesis of meridianins A–E was reported by Molina and co-workers in 2000,[5] while Jiang et al.[6] reported the synthesis of meridianin D at the same time using another approach. Quite recently, we developed a



	4	5	6	7
1 Meridianin A	OH	H	H	H
2 Meridianin B	OH	H	Br	H
3 Meridianin C	H	Br	H	H
4 Meridianin D	H	H	Br	H
5 Meridianin E	OH	H	H	Br
6 Meridianin F	H	Br	Br	H
7 Meridianin G	H	H	H	H



condensed indolylpyrimidones



X = O, S

indolyluracils

Figure 1. Structure of meridianins, condensed indolylpyrimidones and indolyluracils.

Keywords: Alkaloids; Enaminones; Heterocycles; Meridianines; Natural products; Urea derivatives; Uracil; Cyclizations.
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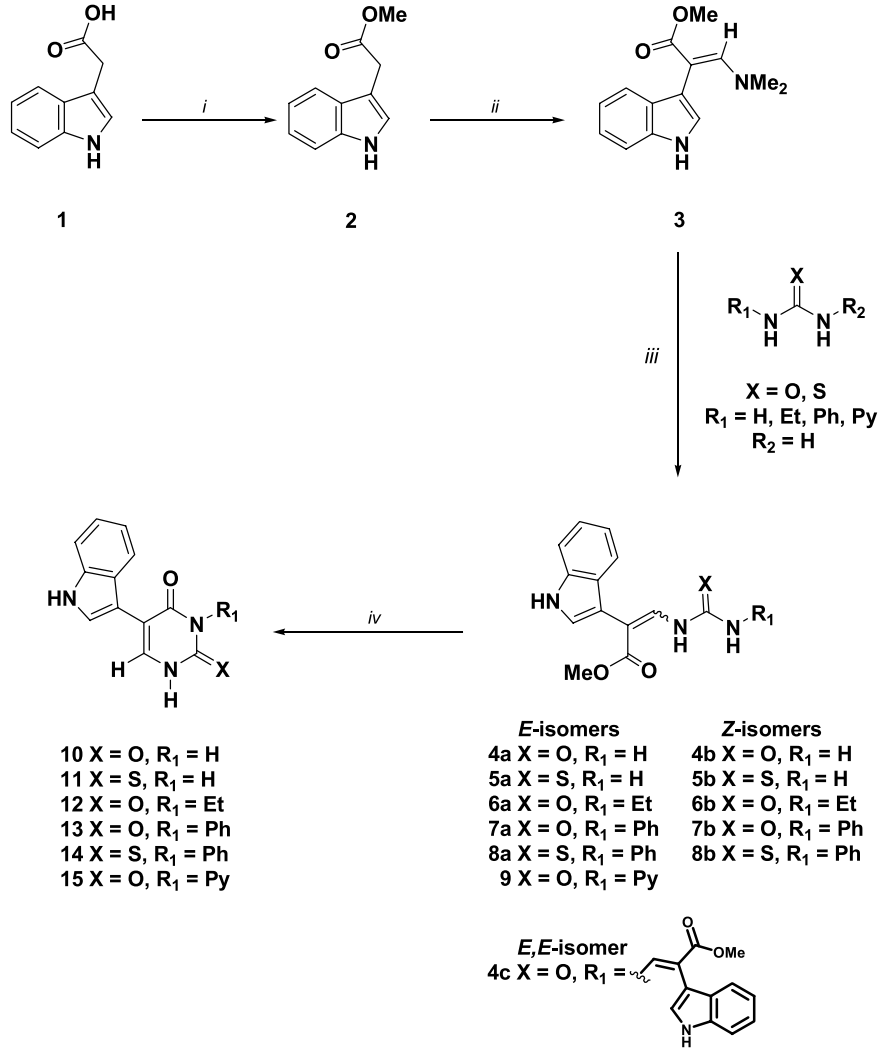
method for the facile synthesis of condensed indolylpyrimidones from alkyl 3-dimethylamino-2-(1*H*-indol-3-yl)propenoates with ambident nucleophiles such as α-heteroarylamines (Fig. 1).[7] These derivatives can be considered as condensed meridianin analogues. In the light of these results and the recently revealed inhibition of several protein kinases by meridianins, we reasoned that it would be of interest to develop a methodology for the synthesis of non-condensed analogues of meridianins. In particular, the pyrimidine-2,4-dione (uracil) structural unit at 3-position of indole ring would be appealing since the uracil nucleus is a constituent of nucleosides which play an important role in biological systems. Indeed, substituted uracils and their nucleosides are of biological significance. For example, 5-fluorouracil (FU) and 5-fluoro-2′-deoxyuridine (FUDR) have been used in the chemotherapy of cancer,[8] while trifluorothymidine (F_3TDR), *E*-5-(2-bromovinyl-2′-deoxyuridine) (BVDU), 3′-azido-3′-deoxythymidine (AZT) and 5-(2-chloroethyl)-2′-deoxyuridine (CEDU) have been applied for viral diseases.[9–13] Both BVDU and CEDU effectively inhibit herpes simplex type 1 virus (HSV-1) and varicella zoster virus (VZU) replication in vitro[9,14–16] and AZT, CNT are anti-AIDS compounds.[17] Also, many 5-substituted uracils have been developed as enzyme inhibitors[18] and have been used in the synthesis of modified nucleotides.[19] Thus, the combined presence of an indole and uracil motif, which feature prominently in bioactive natural products, in the same molecule presents an attractive family of meridianin analogues with immense synthetic appeal. In order to extend the utility of alkyl 3-(dimethylamino)propenoates in the field of the indole natural product synthesis,[20] we describe herein the reaction of methyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoate with amido *N*-nucleophiles (ureas) which enables the synthesis of meridianin analogues with uracil structural unit—indolyluracils (Fig. 1).

2. Results and discussion

Recently, we reported the synthesis of alkyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoates in one step from alkyl 3-indoleacetates and *tert*-butoxy-bis(dimethylamino)methane (Bredereck's reagent). These propenoates proved to be versatile reagents for the synthesis of various indole alkaloids.[7] Thus, we decided firstly to develop a more



Scheme 1. Reaction conditions: (i) conc. H_2SO_4, MeOH, rt, 1 h, 98%; (ii) DMFDMA, DMF, reflux, 14 h, 85%; (iii) conc. HCl or CF_3COOH, DMAA, 50 °C, 3 h; (iv) MeONa or *t*-BuOK, DMAA, rt, 3–9 h.

convenient synthetic approach to (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoates. We examined the reaction of *N*,*N*-dimethylaformamide dimethylacetal (DMFDMA) with methyl 3-indoleacetate **2**[21] which was prepared simply in excellent 98% yield from 3-indoleacetic acid **1** in the presence of H_2SO_4 in methanolic solution (Scheme 1).

It is noteworthy that the commercially available ethyl 3-indoleacetate was avoided since initial experiments showed that transesterification took place in the presence of DMFDMA and a mixture of ethyl and methyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoates was obtained, which rendered purification of product by crystallization difficult. Moreover, it was found that the best yield of product **3** is obtained when methyl 3-indoleacetate **2** and DMFDMA (2 equiv) were heated under reflux in DMF for 7 h, formed product is then removed and the remaining mixture treated with another portion of DMFDMA (2 equiv) for 7 h. This procedure enabled us to isolate methyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoate **3** in 85% yield on multigram-scale (60 mmol) which surpasses previous methods regarding the yield and significantly in the quantity of product, using cheap starting materials.

In our synthetic strategy towards 5-(indol-3-yl)-pyrimidine-2,4-diones we envisaged that the reaction of 3-dimethylamino-2-(1*H*-indol-3-yl)propenoate **3** with ureas would give ureidopropenoate intermediates, which could subsequently cyclize into indolyluracils. Surprisingly, until now, only one example of acid catalyzed dimethylamine substitution reaction of 3-(dimethylamino)propenoates with amido *N*-nucleophiles such as ureas has been described in the literature probably due to the very low nucleophilic reactivity of amide nitrogen atoms.[22] This is in sharp contrast with existing literature on the acid catalyzed dimethylamine substitution with amino *N*-nucleophiles where numerous examples are known. Indeed, until now substitution products have been mainly obtained with primary and secondary aliphatic, aromatic and hetero-aromatic amines as reactive *N*-nucleophiles.[20] Thus, the initial experiments were focused on exploring the feasibility of the acid-catalyzed dimethylamine substitution in (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoate **3** with amido *N*-nucleophiles such as ureas. The model reactions were carried out using propenoate **3** as a substrate and urea as an amido *N*-nucleophile and the following reaction variables were examined: the stoichiometry, the acid catalyst, the solvent, and the reaction temperature, in order to find the best method for the substitution reaction.

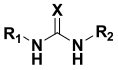
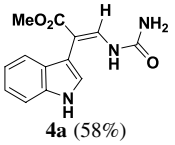
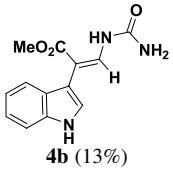
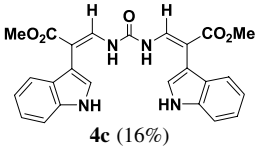
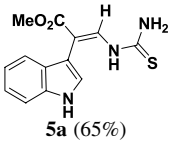
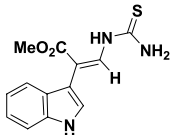
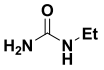
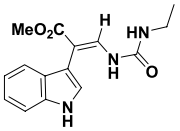
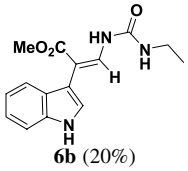
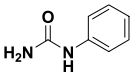
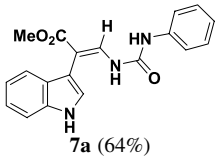
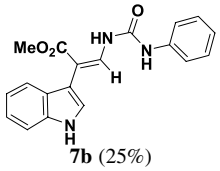
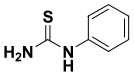
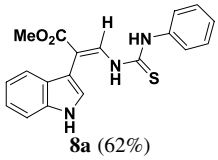
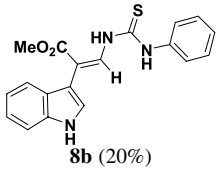
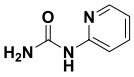
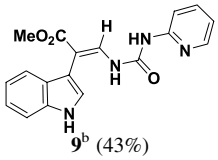
In the first attempt equimolar mixture of propenoate **3** and urea in boiling AcOH produced a complex mixture of products, which made the isolation of a desired product impossible. When the equimolar mixture of propenoate **3** and urea was heated for 6 h in boiling EtOH in the presence of 37% HCl (2 equiv), (*E*)-methyl 2-(1*H*-indol-3-yl)-3-ureidopropenoate **4a** isolated as a single isomer in yields up to 37%. Moreover, we observed that the ethyl ester of ureidopropenoate **4a** was also formed, providing evidence that the transesterification also took place under these conditions. The yield of ureidopropenoate **4a** increased to 46% when propenoate **3** (1 equiv) and urea (3 equiv) were heated for 2 h in boiling MeOH in the presence of 37% HCl (2 equiv). It should be noted that significant amount of yellow colored unidentifiable side products were formed under these conditions. In order to eliminate these side products the use of a polar, aprotic solvent such as *N*,*N*-dimethylacetamide (DMAA) was considered. Heating the mixture of propenoate **3** (1 equiv) and urea (3 equiv) in DMAA at 50 °C in the presence of 37% HCl (9 equiv) for 3 h brought the reaction to completion. In this case ureidopropenoate **4a** was isolated in 58% yield. In addition, this reaction enabled us to isolate two other products: (*Z*)-methyl 2-(1*H*-indol-3-yl)-3-ureidopropenoate **4b** (13%) and bis-ureidopropenoate **4c** (16%) (Scheme 1, Table 1). In order to minimize the formation of bis-ureidopropenoate **4c**, we also performed a slow dropwise addition (1 h) of DMAA solutions of enaminone **3** to a urea solution using triple the amount of solvent. Nevertheless, **4a**, **4b** and **4c** were still formed in the ratio 1:0.32:0.36 as evidenced by ^{1}H NMR analysis of the crude product.

From these studies, it appears that the best conditions for the substitution reactions were met when urea (3 equiv) and propenoate **3** (1 equiv) are reacted in DMAA in the presence of an excess of conc. HCl (9 equiv) at 50 °C. Therefore, these conditions were applied for dimethylamino substitution reaction with various (thio)ureas in order to establish it as a general procedure. In the reaction of **3** with thiourea an *E*/*Z* mixture of methyl 2-(1*H*-indol-3-yl)-3-thioureidopropenoate **5** was formed, however, without the bis-thioureidopropenoate analogous to **4c**. We were able to isolate pure (*E*)-isomer **5a** in 65% yield and (*Z*)-isomer **5b** in 9% yield by column chromatography (Scheme 1, Table 1).

Extension of the method to monosubstituted ureas was then attempted, since they could react either with the substituted or the unsubstituted nitrogen atom. Furthermore, 3-(3-substituted-(thio)ureido)-2-(1*H*-indol-3-yl)propenoates could subsequently be cyclized to N^3-substituted uracil derivatives, which are of interest due to their inherent bioactivity[23] as well as their use in the synthesis of oligonucleotides[24] and non-nucleoside reverse transcriptase inhibitors.[25] In this way introduction of N^3-substituent on uracil ring would not be needed in the final step to form N^3-substituted derivatives. In particular, because chemoselective alkylations are the critical step and mixtures of N^1 and N^1+N^3 alkyl derivatives with some O^4-substituted by products are formed.[26]

In our case, we found that monosubstituted ureas, such as *N*-ethylurea, *N*-phenylurea, and *N*-phenylthiourea reacted smoothly and regioselectively with propenoate **3** in DMMA at 50 °C in the presence of conc. HCl (9 equiv) to form *E*/*Z* mixtures of methyl 3-(3-substituted-(thio)ureido)-2-(1*H*-indol-3-yl)propenoates **6**, **7**, **8**. Purification with column chromatography afforded pure (*E*)-isomers (**6a**, **7a** and **8a**) and (*Z*)-isomers (**6b**, **7b** and **8b**) in high overall yields (**6a**+**6b**: 88%, **7a**+**7b**: 89%, **8a**+**8b**: 82%) (Scheme 1, Table 1). The reason for the regioselectivity of these reactions became evident after the reaction was performed with *N*,*N*′-diphenylurea. In this case no reaction took place even when rigorous reaction conditions were applied (heating up to 150 °C in DMAA in the presence of 9 equiv of HCl). This demonstrates clearly that

Table 1. Reaction of 3-dimethylamino-2-(indol-3-yl)propenoate **3** with ureas

Urea	Product (yield[a])	
		
	 **4a** (58%)	 **4b** (13%)
		 **4c** (16%)
	 **5a** (65%)	
		 **5b** (9%)
	 **6a** (68%)	
		 **6b** (20%)
	 **7a** (64%)	
		 **7b** (25%)
	 **8a** (62%)	
		 **8b** (20%)
	 **9**[b] (43%)	

[a] Yields refer to chromatographically pure isolated products.
[b] CF_3CO_2H was used.

N-substituted amido *N*-nucleophiles do not undergo a substitution reaction with propenoate **3**.

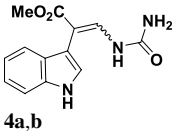
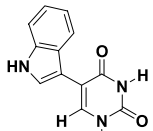
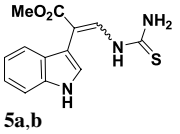
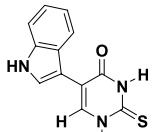
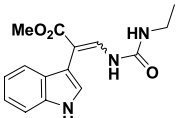
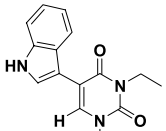
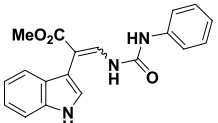
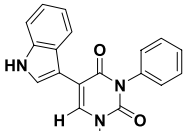
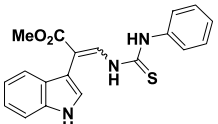
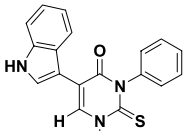
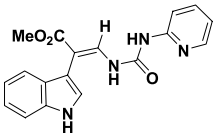
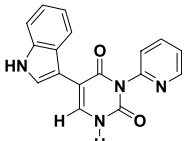
With the intention of exploring the scope of the above method we extended our studies to heterocyclic urea derivative. When propenoate **3** was treated with *N*-(2-pyridyl)urea in DMAA at 50 °C for 3 h using conc. HCl (9 equiv) rather insoluble *N*-(2-pyridyl)urea hydrochloride was formed which precipitated from the reaction mixture. Consequently, the (*E*)-methyl 2-(1*H*-indol-3-yl)-3-[3-(pyridin-2-yl)ureido]propenoate **9** was formed in a very low yield (4%). Thus, we focused our attention on an acid catalyst which would give a soluble salt of *N*-(2-pyridyl)urea at the applied reaction conditions. We found that using CF_3CO_2H kept the formed *N*-(2-pyridyl)urea salt completely dissolved in DMAA at 50 °C. In this case propenoate **3** was consumed after stirring for 5 h giving the (*E*)-ureidopropenoate **9** in 43% yield as a single isomer (Scheme 1, Table 1).

With the key precursors **4–9** in hands, we next turned attention to the cyclization step. We anticipated that the N^3–C^4 intramolecular ring closure in (thio)ureidopropenoates **4–9** could be promoted by a base.[27] Indeed, ureidopropenoates **4–8** underwent a smooth cyclisation in 3–9 h when treated with NaOMe (5 equiv) in DMAA at room temperature giving the 5-(indol-3-yl)pyrimidine-2,4-diones **10–14** in very good yields (Scheme 1, Table 2). Surprisingly, when ureidopropenoate **9** was subjected to the same reaction conditions, reaction did not take place and **9** was recovered quantitatively after work up. Therefore, a stronger base was employed in order to promote the ring closure. Thus, when **9** was treated with *t*-BuOK in DMAA at room temperature for 3.5 h, it reacted completely and the ring closed product **15** could be isolated in 46% yield (Scheme 1, Table 2).

3. Structure determination

The structures of substitution products **4–9** were determined by spectroscopic methods (IR, ^{1}H and ^{13}C NMR, 2D NMR,

Table 2. Base promoted cyclization of methyl 2-(1*H*-indol-3-yl)-3-(thio) ureidopropenoates **4**–**9** into 5-(indol-3-yl)-pyrimidine-2,4-diones **10**–**15**

Ureidopropenoate **4**–**9**	5-(Indol-3-yl)-pyrimidine-2,4-diones **10**–**15**	Yield[a] %
 **4a**,**b**	 **10**	58
 **5a**,**b**	 **11**	83
 **6a**,**b**	 **12**	83
 **7a**,**b**	 **13**	83
 **8a**,**b**	 **14**	81
 **9**	 **15**[b]	46

[a] Yields refer to pure isolated products.
[b] *t*-BuOK was used.

NOESY spectroscopy) and mass spectrometry. Compounds **4**–**8a**, **4**–**8b** and **9** were characterised as pure (*E*)- or (*Z*)-isomers. The configuration around the C=C double bond in compounds **4a**, **4b**, **4c** and **9** was determined by NMR on the basis of long-range coupling constants ($^3J_{C-H}$) between the methylidene proton (*H*–C(3)) and the carbonyl carbon atom (O=*C*(1)), measured from the antiphase splitting of cross peaks in the HMBC spectrum. Generally, the magnitude of coupling constant, $^3J_{C-H}$ for nuclei with *cis*-configuration around the C=C double bond are smaller (2–6 Hz) than for the *trans*-oriented nuclei (8–12 Hz).[20,28–38] In compound **4a**, the magnitude of the coupling constant ($^3J_{C-H}=3$ Hz) showed an (*E*)-configuration around the exocyclic C=C double bond. Similarly, the (*Z*)-configuration was established for compound **4b** ($^3J_{C-H}=13$ Hz), (*E*,*E*)-configuration for bis-substituted product **4c** ($^3J_{C-H}=5$ Hz) and the (*E*)-configuration for compound **9** ($^3J_{C-H}=5$ Hz) (Fig. 2). The configuration around the exocyclic C=C double bond in the major isomers **4a** and **5a** and the minor isomers **4b**, **5b**, **6b** and **8b** was confirmed by NOESY spectroscopy. In compounds **4a** and **5a**, the (*E*)-configuration was established on the basis of NOE between N–*H* and *H*–C(2′). Accordingly, the (*E*,*E*)-configuration for compound **4c** was additionally confirmed. On the other hand, NOE between *H*–C(2′) and *H*–C(3) indicated a (*Z*)-configuration in compounds **4b**, **5b**, **6b** and **8b** (Fig. 2).

In major isomers **4**–**8a**, minor isomers **4**–**8b**, compounds **4c** and **9** the configuration around the exocyclic C=C double bond were correlated with the chemical shifts δ for *H*–C(3) and N*H*. In the case of the (*Z*)-isomers, signals for *H*–C(3) appeared at higher field (7.55–8.39 ppm) than in the case of the (*E*)-isomers (8.18–8.87 ppm). Signals for N*H* exhibited an even stronger dependence of chemical shift on the configuration. Typically, chemical shifts for the N*H* protons of the (*Z*)-isomers were 9.93–11.19 ppm and, in the case of the (*E*)-isomers, 8.18–9.26 ppm. In this manner, the configuration of compounds **4**–**8a** and **4**–**8b** were additionally determined. In compounds **4c** and **9** chemical shifts δ for *H*–C(3) and N*H* correspond to those observed for (*E*)-isomers. The downfield shift of the non-terminal N*H* protons in (*Z*)-isomers can be rationalised by the intramolecular hydrogen bonding to the methoxycarbonyl group, N–H···O=C(1). Similarly, the downfield shift of *H*–C(3) signals in the case of the (*E*)-isomers, can be attributed to the diamagnetic anisotropy of the carbonyl group. In addition, it is interesting to note that sulfur substitution in thioureidopropenoate derivatives **5a**,**b** and **8a**,**b** affects the chemical shifts δ for *H*–C(3) and N*H* notably. Indeed, in the case of the thioureidopropenoates **5a** and **8a** signals for *H*–C(3) appeared at lower field (8.70 and 8.87 ppm) than in the case of the ureidopropenoates **4a** and **7a** (8.18 and 8.24 ppm). Signals for N*H* have been influenced in an even more pronounced manner. Thus, in the case of **5a** and **8a**, signals for N*H* appeared at perceivably lower field (9.12 and 9.26 ppm) compared to the **4a** and **7a** (8.18 and 8.39 ppm). Similar behaviour has been observed for (*Z*)-isomers **5b** and **8b** compared to **4b** and **7b** (Table 3).

Uracil derivatives **10**–**15** may appear in various tautomeric forms differing by the position of the protons either in the vicinity of ring nitrogen atoms or exocyclic sulfur/oxygen atoms. The prototropic tautomerism in uracils and thiouracils attracted a widespread interest few years ago since the relative stability of tautomers of the pyrimidine bases is of fundamental importance to the structure and functioning of nucleic acids.[39] Therefore, informations on the physicochemical properties of possible tautomeric forms are clearly essential for understanding many important biochemical processes. The identification of tautomeric forms in uracils **10**–**15** was accomplished by means of spectroscopic methods. The data were obtained by ^{1}H and ^{13}C NMR measurements in DMSO-d_6. ^{1}H NMR experiments revealed that coupling between *H*–C(6) and *H*–N(1) exists in derivatives **10** ($J=4.9$ Hz), **12** ($J=5.7$ Hz) and **13** ($J=5.7$ Hz), while signals for *H*–C(6) and *H*–N(1) in **11**, **14** and **15** appeared as singlets (Table 4). This suggested that **10**, **12** and **13** exist in the oxo form. However, the structure of **11**, **14** and **15** was less clear. Some times ago, ^{13}C NMR spectroscopy proved to be a powerful tool for the assignment of the uracil structure.[40] Therefore, we compared acquired ^{13}C NMR data with known literature values for

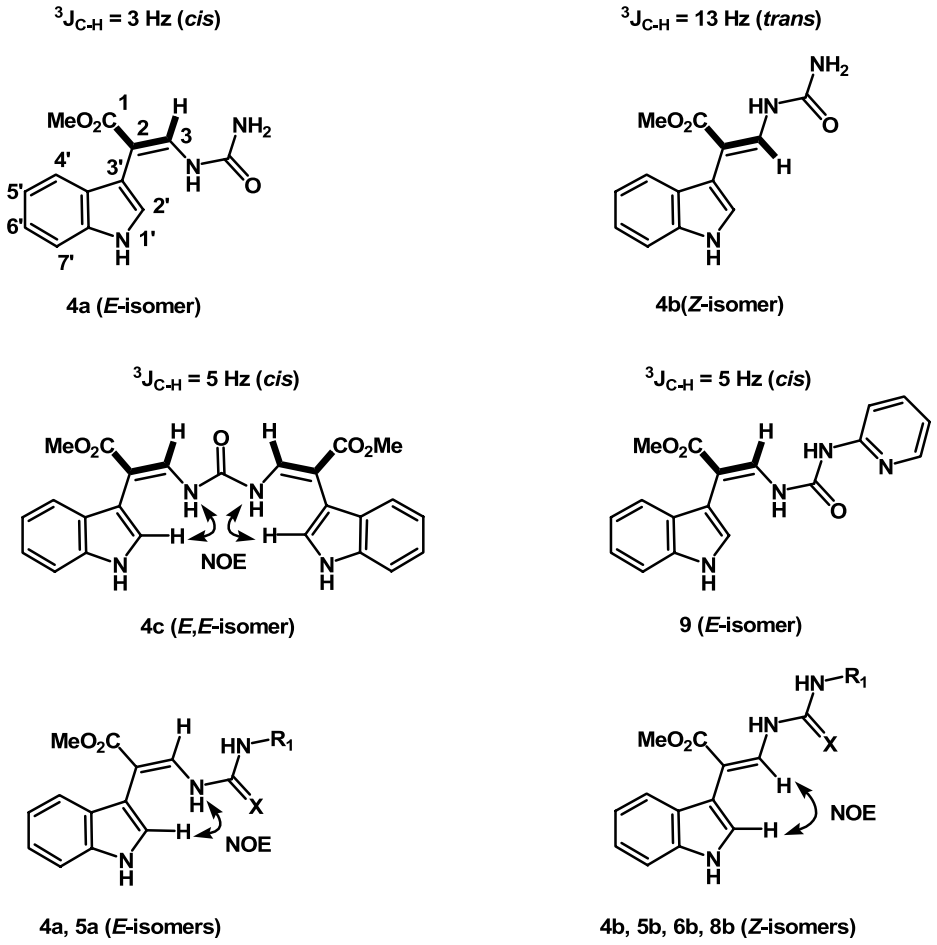


Figure 2. Determination of configuration around C=C double bond by NMR.

uracil and thiouracil derivatives (Table 4). Chemical shifts of C(2), C(4) and C(6) in compounds **10**, **12**, **13** and **15** are close to those observed in uracil and its *N*-alkylated derivatives. Similarly, chemical shifts of C(2), C(4) and C(6) in compounds **11** and **14** are close to those in parent compound and its *N*-alkylated derivatives. Thus, (thio)uracil derivatives **10**–**15** exist in the oxo(thione) form in polar solution (DMSO-d_6) as suggested by ^{1}H and ^{13}C NMR spectroscopy.

4. Conclusion

In summary, a new efficient and simple method for the synthesis of 5-(indol-3-yl)-3-substituted-pyrimidine-2,4-dione derivatives has been developed. The synthetic strategy is based on the base-promoted intramolecular N^3–C^4 cyclization of (thio)ureidopropenoate derivatives which are prepared in high yield from methyl 3-dimethylamino-2-(1*H*-indol-3-yl)propenoate employing

Table 3. Correlation between the chemical shift δ of *H*–C(3) and *H*–N protons and configuration around the C=C double bond in compounds **4**–**8a**, **4**–**8b**, **4c** and **9**

E-isomer (major)	δ [ppm][a]		*Z*-isomer (minor)	δ [ppm][a]	
	H–C(3)	*H*–N		*H*–C(3)	*H*–N
4a	8.18[b]	8.18[b]	**4b**	7.55	9.93
5a	8.70	9.12	**5b**	8.19	10.85
6a	8.19	8.09	**6b**	7.58	9.94
7a	8.24	8.39	**7b**	7.63	10.29
8a	8.87	9.26	**8b**	8.39	11.19
9	8.27[c,d]	10.78[c,d]			
4c	8.14	9.04			

[a] All spectra were recorded at 29 °C in DMSO-d_6 as solvent if not otherwise stated.
[b] Overlap of two signals.
[c] Spectrum recorded at 83 °C since at 29 °C *H*–N signal was not observable.
[d] Weak dependence of δ on temperature was observed for compound **9**. ^{1}H NMR data for **9** at 29 °C: 3.67 (3H, s, CH_3), 6.81–7.80 (9H, m), 8.26 (1H, d, J= 11.7 Hz, *H*–C(3)), 10.00 (1H, s, N*H*), 11.38 (1H, br s, N*H*).

Table 4. Selected NMR data of uracil derivatives

Compound	^{1}H NMR δ [ppm][a,b]		^{13}C NMR δ [ppm][a]		
	H–C(6)	*H*–N(1)	*H*–C(2)	*H*–C(4)	*H*–C(6)
10	—	10.87 (d, J=4.9 Hz)	151.6	164.4	137.3
11	7.64 (s)	—	173.7	161.2	137.0
12	—	11.12 (d, J=5.7 Hz)	150.8	162.5	136.4
13	7.73 (d, J=5.7 Hz)	11.31 (d, J=5.7 Hz)	151.0	163.0	136.4
14	7.77 (s)	12.77 (br s)	174.7	160.3	139.9
15	7.77 (s)	11.39 (br s)	151.3	163.3	137.0
(U)[c]			152.7	165.2	143.0
(2TU)[d]			175.9	160.4	142.0

[a] All spectra were recorded at 29 °C in DMSO-d_6 as solvent.
[b] Data for *H*–C(6) in **10**, **12** and for *H*–N(1) in **11** are not presented due to the overlap with other signals.
[c] (U) stands for uracil (Ref. 40).
[d] (2TU) stands for 2-thiouracil (Ref. 40).

acid-catalyzed dimethyl-amine substitution reaction with various (thio)ureas. Our strategy disclosed herein constitutes a new effective general synthetic approach to heterocyclic substituted uracil nucleus. This approach allowed us to prepare various highly substituted pyrimidine-2,4-dione derivatives. Furthermore, N^3 installation of the alkyl, aryl or even heteroaryl substituent of the pyrimidine-2,4-dione core can be achieved using this method. The N^1 atom in 5-(indol-3-yl)-3-substituted-pyrimidine-2,4-dione derivatives could be attached to sugar moieties in order to form new nucleosides.

5. Experimental

5.1. General

Melting points were taken on a Kofler micro hot stage. The ^{1}H NMR, ^{13}C NMR and 2D NMR HMBC, NOESY spectra were obtained on a Bruker Avance DPX 300 (300 MHz) spectrometer with DMSO-d_6 as solvent and TMS as internal standard (δ in ppm, J in Hz). IR spectra were recorded with Perkin–Elmer Spectrum BX FTIR and BIO RAD Excalibur Series FTS 3000 MX FTIR spectrophotometers (KBr discs for solids or thin film on NaCl plates for liquid, ν in cm^{-1}). MS spectra were obtained on an Autospeck Q spectrometer. The microanalyses for C, H, and N were obtained on a Perkin Elmer CHN Analyser 2400 and Perkin Elmer Series II CHN Analyser 2400.

3-Indolylacetic acid **1**, DMFDMA and used ureas are commercially available (Fluka AG) except of *N*-(2-pyridyl)urea which was prepared according to the procedure described in the literature.[41] Methyl 3-indoleacetate **2** was prepared in a manner similar to that previously described.[21]

5.1.1. (2*E*)-3-Dimethylamino-2-(1*H*-indol-3-yl)-propenoate. The first procedure for the preparation of this compound was already published in the literature[7] herein we report an alternative method:

Methyl 3-indoleacetate **2** (11.90 g, 62.91 mmol) was dissolved in DMF (30 mL) and DMFDMA (15.46 g, 125.82 mmol) was added into the solution. The mixture was refluxed for 7 h and then cooled to room temperature. The solvent and excess of DMFDMA were evaporated in vacuo to furnish dark brown oil. The oil was dissolved in MeOH (10 mL) and Et_2O (50 mL) was added to the solution. White precipitate that was formed was filtered off and washed with Et_2O (50 mL) to furnish 7.16 g (47%) of **3** as white crystalline powder. The remained filtrate was evaporated to give red-brown oil (7.55 g) which was treated with another portion of DMFDMA (9.80 g, 79.78 mmol). The mixture was refluxed for 7 h and then cooled to rt followed by the evaporation of the solvent and excess of DMFDMA. Following the same isolation procedure as described above another portion of **3** 5.86 g (38%) was isolated. Overall yield: 13.02 g (85%) as white needles; mp 156–157 °C (from ethanol); IR 3314, 2947, 1669, 1580, 1489 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 2.62 (6H, s, N(C*H*$_3$)$_2$), 3.45 (3H, s, C*H*$_3$), 6.95 (1H, dt, J=7.0, 1.1 Hz, *H*–C(5′)), 7.02–7.07 (2H, m, *H*–C(2′), *H*–C(6′)), 7.23 (1H, d, J=7.5 Hz, *H*–C(7′)), 7.33 (1H, d, J=8.3 Hz, *H*–C(4′)), 7.64 (1H, s, *H*–C(3)), 10.91 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 42.6, 51.2, 89.5, 110.4, 112.1, 119.4, 120.1, 121.6, 126.3, 130.6, 136.4, 151.3, 170.8; MS (EI) m/z: 244 (M+, 100), 213 (28), 201 (7), 185 (27), 170 (25), 155 (32), 144 (20), 130 (33), 115 (21), 88 (60); HRMS: found 244.1218, $C_{14}H_{16}N_2O_2$, requires 244.1212; Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.75; H, 6.74; N, 11.44.

5.2. General procedure for the preparation of methyl 2-(1*H*-indol-3-yl)-3-(3-substituted(thio)ureido)propenoates 4–9

A mixture of (*E*)-methyl 3-dimethylamino-2-(indole-3-yl) propenoate **3** and appropriate urea was dissolved in DMAA followed by the addition of an acid. The reaction mixture was stirred at 50 °C for 3 h for products **4–8** and 5 h for the product **9**. The solvent was evaporated in vacuo and the residue first treated with an ice-water and then quenched with saturated K_2CO_3 solution. The suspension was extracted with AcOEt. Organic extracts were combined, washed with brine and dried (Na_2SO_4). The solvent was evaporated in vacuo. The remaining crude product was purified by column chromatography over silica gel using $CHCl_3$/MeOH=10:1 or $CHCl_3$/MeOH=20:1 mixture as the eluent to afford (thio)ureidopropenoates **4–9**.

5.2.1. (*E*)-Methyl 2-(1*H*-indol-3-yl)-3-ureidopropenoate (4a), (*Z*)-methyl 2-(1*H*-indol-3-yl)-3-ureidopropenoate (4b) and methyl (2*E*)-3-[({[(1*E*)-3-methoxy-3-oxo-2-(1*H*-indol-3-yl)prop-1-enyl]amino}carbonyl)amino]-2-(1*H*-indol-3-yl)propenoate (4c). These compounds were

prepared from compound **3** (1.00 g, 4.09 mmol) and urea (0.74 g, 12.28 mmol) in the presence of $HCl_{37\%}$ (3.0 mL, 36.3 mmol) in DMAA (10 mL). Treated with ice-water (100 mL) and quenched with K_2CO_3 (25 mL) saturated solution. Extracted with (3×60 mL) AcOEt.

Data for major (*E*)*-isomer* **4a**. 619 mg (58%) as white crystalline powder; mp 136–139 °C (from PhMe/MeOH= 4:1); IR 3349, 3310, 1680, 1642, 1596, 1493, 1248 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.60 (3H, s, CH_3), 6.46 (2H, br s, NH_2), 6.98 (1H, ddd, J=7.9, 7.0, 1.1 Hz, *H*–C(5′)), 7.10 (1H, ddd, J=7.7, 7.0, 1.1 Hz, *H*–C(6′)), 7.19 (1H, dd, J= 7.9, 0.8 Hz, *H*–C(7′)), 7.26 (1H, d, J=2.6 Hz, *H*–C(2′)), 7.41 (1H, td, J=8.3, 0.8 Hz, *H*–C(4′)), 8.18 (2H, s, *H*–C(3), N*H*), 11.23 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 51.9, 101.3, 107.5, 112.4, 119.7, 120.0, 121.9, 126.2, 127.7, 137.0, 138.3, 155.1, 168.9; MS (EI) *m*/*z*: 259 (M+, 100), 242 (26), 227 (30), 216 (42), 184 (87), 155 (72), 141 (23), 129 (61); HRMS: found 259.0963, $C_{13}H_{13}N_3O_3$, requires 259.0957; Anal. Calcd for $C_{13}H_{13}N_3O_3$×2/3 CH_3OH: C, 58.49; H, 5.63; N, 14.97. Found: C, 58.17; H, 5.85; N, 14.69.

Data for minor (*Z*)*-isomer* **4b**. 140 mg (13%) as white crystals; mp 226–229 °C (from MeOH); IR 3434, 3339, 3158, 1695, 1646, 1550, 1493, 1206 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.67 (3H, s, CH_3), 6.94 (2H, br s, NH_2), 6.98 (1H, dt, J=7.2, 1.1 Hz, *H*–C(5′)), 7.08 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.26 (1H, d, J=2.6 Hz, *H*–C(2′)), 7.34–7.40 (2H, m, *H*–C(7′), *H*–C(4′)), 7.55 (1H, d, J=11.7 Hz, *H*–C(3)), 9.93 (1H, d, J=12.1 Hz, N*H*), 11.01 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 51.9, 98.8, 111.9, 112.4, 119.6, 119.8, 121.8, 125.1, 127.8, 136.8, 140.2, 155.1, 169.5; MS (EI) *m*/*z*: 259 (M+, 100), 242 (32), 227 (37), 216 (44), 184 (67), 155 (58), 141 (26), 129 (47); HRMS: found 259.0962, $C_{13}H_{13}N_3O_3$, requires 259.0957; Anal. Calcd for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.21. Found: C, 60.39; H, 5.12; N, 16.12.

Data for (*E*,*E*)*-isomer* (**4c**). 150 mg (16%) as white powder; mp 246–248 °C (from MeOH); IR 3351, 3303, 2953, 1686, 1643, 1511, 1435, 1194 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.62 (3H, s, CH_3), 6.94 (1H, dt, J=7.2, 1.1 Hz, *H*–C(5′)), 7.05 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.14 (1H, d, J=7.9 Hz, *H*–C(7′)), 7.22 (1H, d, J=2.6 Hz, *H*–C(2′)), 7.33 (1H, d, J=7.9 Hz, *H*–C(4′)), 8.14 (1H, d, J=11.7 Hz, *H*–C(3)), 9.04 (1H, d, J=11.7 Hz, N*H*), 11.20 (1H, d, J=2.3 Hz, N*H*); ^{13}C NMR (DMSO-d_6) δ: 52.2, 105.3, 106.9, 112.4, 119.8, 120.0, 122.0, 126.5, 127.5, 136.2, 136.9, 152.1, 168.5; MS (EI) *m*/*z*: 458 (M+, 78), 426 (10), 394 (6), 367 (6), 242 (25), 216 (100), 184 (83), 155 (63), 141 (16), 129 (26); HRMS: found 458.1596, $C_{25}H_{22}N_4O_5$, requires 458.1590; Anal. Calcd for $C_{25}H_{22}N_4O_5$: C, 65.49; H, 4.84; N, 12.22. Found: C, 65.32; H, 4.93; N, 12.08.

5.2.2. (*E*)-Methyl 2-(1*H*-indol-3-yl)-3-thioureidopropenoate (5a) and (*Z*)-methyl 2-(1*H*-indol-3-yl)-3-thioureidopropenoate (5b). These compounds were prepared from compound **3** (2.00 g, 8.19 mmol) and thiourea (1.870 g, 24.56 mmol) in the presence of $HCl_{37\%}$ (6.0 mL, 72.5 mmol) in DMAA (20 mL). Treated with ice-water (200 mL) and quenched with K_2CO_3 (50 mL) saturated solution. Extracted with (6×50 mL) AcOEt.

Data for major (*E*)*-isomer* **5a**. 1470 mg (65%) as light yellow crystalline powder; mp 145–150 °C (from PhMe/MeOH=4:1); IR 3445, 3404, 3314, 2950, 1699, 1634, 1601, 1550, 1390, 1208 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.63 (3H, s, CH_3), 7.00 (1H, ddd, J=8.0, 7.0, 1.1 Hz, *H*–C(5′)), 7.12 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.18 (1H, d, J= 7.9 Hz, *H*–C(7′)), 7.32 (1H, d, J=2.6 Hz, *H*–C(2′)), 7.42 (1H, td, J=7.9, 1.1 Hz, *H*–C(4′)), 7.88 (1H, br s, N*H*), 8.45 (1H, br s, N*H*), 8.70 (1H, d, J=11.3 Hz, *H*–C(3)), 9.12 (1H, d, J=11.3 Hz, N*H*), 11.31 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 52.2, 105.1, 107.1, 112.5, 2×C 119.9, 122.1, 126.6, 127.6, 137.1, 140.2, 168.6, 182.4; MS (EI) *m*/*z*: 275 (M+, 100), 258 (18), 243 (9), 233 (17), 216 (45), 201 (21), 184 (43), 173 (31), 155 (68), 141 (27), 129 (43); HRMS: found 275.0735, $C_{13}H_{13}N_3O_2S$, requires 275.0728; Anal. Calcd for $C_{13}H_{13}N_3O_2S$: C, 56.71; H, 4.76; N, 15.26. Found: C, 56.59; H, 4.88; N, 14.88.

Data for minor (*Z*)*-isomer* **5b**. 208 mg (9%) as yellow needles; mp 216–220 °C (from MeOH); IR 3469, 3360, 3285, 3163, 1673, 1638, 1622, 1548, 1385, 1196 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.72 (3H, s, CH_3), 7.01 (1H, dt, J=7.2, 1.1 Hz, *H*–C(5′)), 7.10 (1H, dt, J=6.8, 1.1 Hz, *H*–C(6′)), 7.34 (1H, d, J=2.6 Hz, *H*–C(2′)), 7.39 (1H, d, J=7.9 Hz, *H*–C(7′)), 7.50 (1H, d, J=7.9 Hz, *H*–C(4′)), 8.19 (1H, d, J=10.9 Hz, *H*–C(3)), 8.67, 8.72 (2H, 2 x br s, NH_2), 10.85 (1H, d, J=11.3 Hz, N*H*), 11.12 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 52.3, 102.2, 107.1, 111.2, 112.5, 119.8, 119.9, 122.0, 125.7, 127.4, 136.8, 141.4, 169.3, 182.3; MS (EI) *m*/*z*: 275 (M+, 100), 258 (12), 243 (14), 233 (14), 216 (43), 201 (22), 184 (42), 173 (28), 155 (50), 141 (23), 129 (35); HRMS: found 275.0735, $C_{13}H_{13}N_3O_2S$, requires 275.0728; Anal. Calcd for $C_{13}H_{13}N_3O_2S$: C, 56.71; H, 4.76; N, 15.26. Found: C, 56.78; H, 4.89; N, 15.08.

5.2.3. (*E*)-Methyl 3-(3-ethylureido)-2-(1*H*-indol-3-yl) propenoate (6a) and (*Z*)-methyl 3-(3-ethylureido)-2-(1*H*-indol-3-yl)propenoate (6b). These compounds were prepared from compound **3** (2.00 g, 8.19 mmol) and *N*-ethylurea (2.164 g, 24.56 mmol) in the presence of $HCl_{37\%}$ (6.0 mL, 72.5 mmol) in DMAA (20 mL). Treated with ice-water (200 mL) and quenched with K_2CO_3 (50 mL) saturated solution. Extracted with (6×50 mL) AcOEt.

Data for major (*E*)*-isomer* **6a**. 1600 mg (68%) as white crystalline powder; mp 212–215 °C (from PhMe/MeOH= 4:1); IR 3355, 2973, 1707, 1676, 1642, 1546, 1430, 1214 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 0.95 (3H, t, J= 7.1 Hz, CH_2CH_3), 3.02 (2H, dq, J=7.2, 5.3 Hz, CH_2CH_3), 3.58 (3H, s, CH_3), 6.85 (1H, br t, J=5.3 Hz, N*H*), 6.97 (1H, dt, J=7.1, 1.1 Hz, *H*–C(5′)), 7.09 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.16 (1H, d, J=7.9 Hz, *H*–C(7′)), 7.23 (1H, d, J=2.3 Hz, *H*–C(2′)), 7.40 (1H, d, J=8.3 Hz, *H*–C(4′)), 8.09 (1H, d, J=12.4 Hz, N*H*), 8.19 (1H, d, J=12.4 Hz, *H*–C(3)), 11.22 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 15.2, 34.5, 51.4, 104.0, 107.0, 111.9, 119.2, 119.5, 121.4, 125.7, 127.2, 136.5, 137.8, 153.7, 168.4; MS (EI) *m*/*z*: 287 (M+, 81), 255 (5), 242 (13), 216 (74), 184 (100), 156 (63), 141 (29), 129 (45); HRMS: found 287.1261, $C_{15}H_{17}N_3O_3$, requires 287.1269; Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63. Found: C, 62.80; H, 6.09; N, 14.62.

Data for minor (*Z*)-*isomer* **6b**. 465 mg (20%) as white crystalline powder; mp 165–167 °C (from PhMe/MeOH= 4:1); IR 3319, 2973, 1676, 1656, 1611, 1567, 1433, 1211 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.06 (3H, t, J= 7.2 Hz, CH_2CH_3), 3.14 (2H, dq, J=7.2, 5.3 Hz, CH_2CH_3), 3.67 (3H, s, CH_3), 6.98 (1H, dt, J=6.8, 1.1 Hz, *H*–C(5′)), 7.07 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.26 (1H, d, J= 2.3 Hz, *H*–C(2′)), 7.35–7.39 (2H, m, *H*–C(4′), *H*–C(7′)), 7.58 (1H, d, J=11.7 Hz, *H*–C(3)), 7.73 (1H, br t, J=5.3 Hz, N*H*), 9.94 (1H, d, J=12.0 Hz, N*H*), 11.02 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 15.9, 35.2, 51.9, 98.4, 111.9, 112.4, 119.6, 119.8, 121.8, 125.1, 127.8, 136.8, 140.1, 154.2, 169.5; MS (EI) *m*/*z*: 287 (M+, 99), 255 (10), 242 (16), 216 (74), 184 (100), 156 (60), 141 (30), 129 (44); HRMS: found 287.1263, $C_{15}H_{17}N_3O_3$, requires 287.1269; Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63. Found: C, 62.92; H, 6.19; N, 14.35.

5.2.4. (*E*)-Methyl 2-(1*H*-indol-3-yl)-3-(3-phenylureido) propenoate (7a) and (*Z*)-methyl 2-(1*H*-indol-3-yl)-3-(3-phenylureido)propenoate (7b). These compounds were prepared from compound **3** (2.00 g, 8.19 mmol) and *N*-phenylurea (3.344 g, 24.56 mmol) in the presence of $HCl_{37\%}$ (6.0 mL, 72.5 mmol) in DMAA (20 mL). Treated with ice-water (200 mL) and quenched with K_2CO_3 (50 mL) saturated solution. Extracted with (6×50 mL) AcOEt.

Data for major (*E*)-*isomer* **7a**. 1754 mg (64%) as white crystalline powder; mp 248–250 °C (from PhMe/MeOH= 4:1); IR 3411, 3257, 1687, 1655, 1598, 1566, 1432, 1194 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.64 (3H, s, CH_3), 6.96–7.03 (2H, m, *H*–C(5′), 1*H*–Ph), 7.13 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.22–7.29 (3H, m, 3*H*–Ph), 7.34–7.38 (3H, m, *H*–C(2′), *H*–C(7′), 1*H*–Ph), 7.45 (1H, td, J=8.0, 1.0 Hz, *H*–C(4′)), 8.24 (1H, d, J=12.1 Hz, *H*–C(3)), 8.39 (1H, d, J=12.1 Hz, N*H*); 9.26 (1H, s, N*H*), 11.33 (1H, d, J=1.5 Hz, N*H*); ^{13}C NMR (DMSO-d_6) δ: 51.6, 102.5, 106.7, 112.0, 118.6, 119.4, 119.5, 121.6, 123.0, 125.9, 127.1, 129.3, 136.5, 136.6, 139.0, 151.3, 168.2; MS (EI) *m*/*z*: 335 (M+, 92), 304 (5), 242 (16), 216 (65), 184 (100), 155 (64), 141 (22), 129 (40); HRMS: found 335.1278, $C_{19}H_{17}N_3O_3$, requires 335.1270; Anal. Calcd for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53. Found: C, 67.78; H, 5.21; N, 12.29.

Data for minor (*Z*)-*isomer* **7b**. 685 mg (25%) as white crystalline powder; mp 107–110 °C (from PhMe/MeOH= 4:1); IR 3419, 1678, 1620, 1545, 1458, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.70 (3H, s, CH_3), 6.96–7.04 (2H, m, *H*–C(5′), 1*H*–Ph), 7.08 (1H, dt, J=6.8, 1.1 Hz, *H*–C(6′)), 7.27–7.32 (3H, m, 2*H*–Ph, *H*–C(2′)), 7.37 (1H, d, J= 7.9 Hz, *H*–C(7′)), 7.42 (1H, d, J=7.5 Hz, *H*–C(4′)), 7.47–7.51 (2H, m, 2*H*–Ph), 7.63 (1H, d, J=11.7 Hz, *H*–C(3)), 10.08 (1H, s, N*H*), 10.28 (1H, d, J=11.3 Hz, N*H*), 11.06 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 51.6, 99.9, 111.1, 112.0, 118.9, 2×C 119.3, 121.4, 123.0, 124.8, 127.2, 129.3, 136.4, 138.4, 139.4, 151.5, 169.1; MS (EI) *m*/*z*: 335 (M+, 100), 303 (8), 242 (17), 216 (68), 184 (87), 155 (51), 141 (20), 129 (34); Anal. Calcd for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11; N, 12.53. Found: C, 67.79; H, 5.21; N, 12.35.

5.2.5. (*E*)-Methyl 2-(1*H*-indol-3-yl)-3-(3-phenylthioureido)-propenoate (8a) and (*Z*)-methyl 2-(1*H*-indol-3-yl)-3-(3-phenylthioureido)propenoate (8b). These compounds were prepared from compound **3** (2.00 g, 8.19 mmol) and *N*-phenylthiourea (3.737 g, 24.56 mmol) in the presence of $HCl_{37\%}$ (6.0 mL, 72.5 mmol) in DMAA (20 mL). Treated with ice-water (200 mL) and quenched with K_2CO_3 (50 mL) saturated solution. Extracted with (6×50 mL) AcOEt.

Data for major (*E*)-*isomer* **8a**. 1793 mg (62%) as yellow crystalline powder; mp 194–197 °C (from PhMe/MeOH= 4:1); IR 3340, 3186, 1695, 1639, 1543, 1494, 1302, 1187, 1098 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.66 (3H, s, CH_3), 7.02 (1H, dt, J=7.2, 1.1 Hz, *H*–C(5′)), 7.11–7.17 (2H, m, *H*–C(6′), 1*H*–Ph), 7.22 (1H, d, J=7.5 Hz, 1*H*–Ph), 7.29–7.34 (2H, m, *H*–C(7′), 1*H*–Ph), 7.40 (1H, d, J=2.3 Hz, *H*–C(2′)), 7.45 (1H, d, J=8.3 Hz, *H*–C(4′)), 7.51–7.54 (2H, m, 2*H*–Ph), 8.87 (1H, d, J=11.3 Hz, *H*–C(3)), 9.26 (1H, d, J= 10.6 Hz, N*H*), 10.56 (1H, s, N*H*), 11.36 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 52.3, 106.0, 107.1, 112.6, 119.9, 120.0, 122.2, 124.0, 126.1, 126.7, 127.7, 129.4, 137.1, 139.2, 139.3, 168.5, 178.9; MS (EI) *m*/*z*: 351 (M+, 100), 317 (10), 292 (58), 258 (34), 233 (25), 216 (45), 201 (25), 184 (69), 173 (25), 155 (49), 141 (36), 129 (34); HRMS: found 351.1035, $C_{19}H_{17}N_3O_2S$, requires 351.1041; Anal. Calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96. Found: C, 65.10; H, 4.99; N, 11.94.

Data for minor (*Z*)-*isomer* **8b**. 577 mg (20%) as yellow crystalline powder; mp 176–180 °C (from PhMe/MeOH= 4:1); IR 3376, 3146, 1710, 1635, 1536, 1476, 1301, 1231, 1134, 1095 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.71 (3H, s, CH_3), 7.03 (1H, dt, J=6.8, 1.1 Hz, *H*–C(5′)), 7.11 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.25 (1H, t, J=7.2 Hz, 1*H*–Ph), 7.38–7.45 (4H, m, *H*–C(2′), *H*–C(7′), 2*H*–Ph), 7.54 (1H, d, J=7.9 Hz, *H*–C(4′)), 7.59–7.62 (2H, m, 2*H*–Ph), 8.39 (1H, d, J=10.9 Hz, *H*–C(3)), 11.17 (1H, br s, N*H*), 11.19 (1H, d, J=11.1 Hz, N*H*), 11.24 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 52.0, 102.9, 110.5, 112.1, 119.2, 119.6, 121.6, 124.3, 125.4, 126.1, 126.8, 129.1, 129.2, 136.4, 140.0, 168.9, 178.6; MS (EI) *m*/*z*: 351 (M+, 100), 317 (7), 292 (54), 258 (14), 233 (23), 216 (44), 201 (23), 184 (76), 173 (23), 155 (51), 141 (33), 129 (33); HRMS: found 351.1050, $C_{19}H_{17}N_3O_2S$, requires 351.1042; Anal. Calcd for $C_{19}H_{17}N_3O_2S$: C, 64.94; H, 4.88; N, 11.96. Found: C, 65.15; H, 4.91; N, 11.84.

5.2.6. (*E*)-Methyl 2-(1*H*-indol-3-yl)-3-[3-(pyridin-2-yl) ureido]propenoate (9). This compound was prepared from compound **3** (416 mg, 1.70 mmol) and *N*-(2-pyridyl)-urea (700 mg, 5.10 mmol) in the presence of CF_3CO_2H (4.0 mL, 53.9 mmol) in DMAA (20 mL). Treated with ice-water (100 mL) and quenched with K_2CO_3 (50 mL) saturated solution. Extracted with (4×30 mL) AcOEt. Data for (*E*)-isomer **9**: 246 mg (43%) as white crystalline powder; mp 257–258 °C (from MeOH); IR 3301, 3230, 3086, 1700, 1635, 1580, 1546, 1515, 1435, 1305, 1265, 1211, 1099 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.69 (3H, s, CH_3), 6.86 (1H, ddd, J=7.3, 5.0, 1.0 Hz, *H*–C(5″)–Py), 6.96 (1H, dt, J=7.0, 1.0 Hz, *H*–C(5′)), 7.12 (1H, dt, J=7.0, 1.2 Hz, *H*–C(6′)), 7.23–7.28 (2H, m, *H*–C(7′), *H*–C(3″)–Py), 7.35 (1H, d, J=2.6 Hz, *H*–C(2′)), 7.47–7.50 (2H, m, *H*–C(4′), *H*–C(6″)–Py), 7.65 (1H, ddd, J=8.4, 7.2, 2.0 Hz,

H–C(4″)–Py), 8.27 (1H, d, $J=12.1$ Hz, *H*–C(3)), 9.77 (1H, s, N*H*), 10.78 (1H, br s, N*H*), 11.15 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 51.3, 104.2, 104.4, 106.5, 111.5, 112.0, 117.7, 118.9, 119.4, 121.1, 125.5, 126.3, 135.3, 136.1, 138.7, 151.5, 152.0, 167.6; MS (EI) *m/z*: 336 (M+, 54), 242 (100), 216 (23), 199 (4), 184 (16), 171 (8), 155 (36), 144 (8), 129 (18); HRMS: found 336.1227, $C_{18}H_{16}N_4O_3$, requires 336.1222; Anal. Calcd for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 16.66. Found: C, 63.97; H, 4.91; N, 16.35.

5.3. General procedure for the preparation of 5-(1*H*-indol-3-yl)-3-substituted-pyrimidine-2,4(1*H*,3*H*)-diones 10–14

Sodium (230 mg, 10.00 mmol) was dissolved in MeOH (4 mL) and DMAA (5 mL) was added to the obtained methoxide solution followed by the addition of **4**–**8**. The reaction mixture turned red-brown in few minutes after complete dissolution of **4**–**8** and was then stirred at rt for 3–9 h. The reaction mixture was neutralized with $HCl_{37\%}$ (1.8 mL) in H_2O (4 mL) and the obtained suspension was pured on ice-water. The suspension was stirred for 30 min at room temperature. The precipitate was filtered off washed with H_2O (20 mL) and dried to afford crude 5-(1*H*-indol-3-yl)-3-substituted-pyrimidine-2,4(1*H*,3*H*)-diones **10**–**14**. If necessary, products were further purified by dissolution in DMF/MeOH mixture addition of active carbon, refluxing for 30 min, filtration and evaporation of the solvent. Addition of MeOH precipitated pure diones **10**–**14**.

5.3.1. 5-(1*H*-Indol-3-yl)pyrimidine-2,4(1*H*,3*H*)-dione (10).

This compound was prepared from compounds **4a**,**b** (519 mg, 2.00 mmol) in 3.5 h. Data for **10**: 262 mg (58%) as white crystalline powder; mp +350 °C (from H_2O); IR 3274, 3214, 3052, 1698, 1650, 1435, 1418 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 7.03 (1H, dt, $J=6.8$, 1.1 Hz, *H*–C(5′)), 7.11 (1H, dt, $J=7.2$, 1.1 Hz, *H*–C(6′)), 7.41 (1H, d, $J=7.9$ Hz, *H*–C(7′)), 7.59–7.61 (2H, m, *H*–C(4′), *H*–C(6)), 7.69 (1H, d, $J=2.6$ Hz, *H*–C(2′)), 10.87 (1H, d, $J=4.9$ Hz, N*H*), 11.19 (2H, br s, 2×N*H*); ^{13}C NMR (DMSO-d_6) δ: 107.5, 108.8, 112.6, 120.0, 120.2, 122.1, 125.9, 126.2, 136.9, 137.3, 151.6, 164.4; MS (EI) *m/z*: 227 (M+, 100), 184 (28), 155 (34), 141 (14), 129 (22); HRMS: found 227.0605, $C_{12}H_9N_3O_2$, requires 227.0605; Anal. Calcd for $C_{12}H_9N_3O_2\times1/4$ CH_3OH: C, 62.55; H, 4.29; N, 17.86. Found: C, 62.38; H, 4.29; N, 17.77.

5.3.2. 2,3-Dihydro-5-(1*H*-indol-3-yl)-2-thioxopyrimidin-4(1*H*)-one (11).

This compound was prepared from compounds **5a**,**b** (550 mg, 2.00 mmol) in 5.5 h. Data for **11**: 404 mg (83%) as yellow crystalline powder; mp 307–312 °C (lit.,[42] 350 °C) (from H_2O); IR 3410, 3399, 2915, 1660, 1626, 1584, 1234, 1175 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 7.08 (1H, dt, $J=6.8$, 1.1 Hz, *H*–C(5′)), 7.14 (1H, dt, $J=6.8$, 1.1 Hz, *H*–C(6′)), 7.44 (1H, td, $J=7.5$, 1.1 Hz, *H*–C(7′)), 7.64 (1H, td, $J=8.5$, 1.3 Hz, *H*–C(4′)), 7.64 (1H, s, *H*–C(6)), 7.87 (1H, d, $J=2.6$ Hz, *H*–C(2′)), 11.35, 12.39, 12.56 (3H, 3×br s, 3×N*H*); ^{13}C NMR (DMSO-d_6) δ: 106.6, 112.8, 114.1, 120.2, 120.4, 122.4, 125.6, 126.9, 136.1, 137.0, 161.2, 173.7; MS (EI) *m/z*: 243 (M+, 100), 184 (17), 155 (36), 141 (24), 129 (18); HRMS: found 243.0472, $C_{12}H_9N_3OS$, requires 243.0466; Anal. Calcd for $C_{12}H_9N_3OS$: C, 59.24; H, 3.73; N, 17.27. Found: C, 59.00; H, 3.86; N, 16.90.

5.3.3. 3-Ethyl-5-(1*H*-indol-3-yl)pyrimidine-2,4(1*H*,3*H*)-dione (12).

This compound was prepared from compounds **6a**,**b** (575 mg, 2.00 mmol) in 7 h. Data for **12**: 425 mg (83%) as white crystalline powder; mp 307–310 °C (from H_2O); IR 3350, 3210, 2978, 1707, 1623, 1610, 1462 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 1.13 (3H, t, $J=7.2$ Hz, CH_2CH_3), 3.91 (2H, q, $J=7.2$ Hz, CH_2CH_3), 7.01 (1H, dt, $J=6.8$, 1.1 Hz, *H*–C(5′)), 7.10 (1H, dt, $J=7.0$, 1.1 Hz, *H*–C(6′)), 7.39 (1H, td, $J=8.0$, 1.0 Hz, *H*–C(7′)), 7.56–7.60 (2H, m, *H*–C(4′), *H*–C(6)), 7.68 (1H, d, $J=2.6$ Hz, *H*–C(2′)), 11.12 (1H, br d, $J=5.7$ Hz, N*H*), 11.19 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 13.2, 35.4, 107.3, 107.8, 112.0, 119.4, 119.7, 121.5, 125.4, 125.8, 135.4, 136.4, 150.8, 162.5; MS (EI) *m/z*: 255 (M+, 100), 227 (6), 184 (47), 155 (37), 141 (17), 129 (23); HRMS: found 255.1006, $C_{14}H_{13}N_3O_2$, requires 255.1008; Anal. Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.51; H, 5.28; N, 16.39.

5.3.4. 5-(1*H*-Indol-3-yl)-3-phenylpyrimidine-2,4(1*H*,3*H*)-dione (13).

This compound was prepared from compounds **7a**,**b** (671 mg, 2.00 mmol) in 3.5 h. Data for **13**: 501 mg (83%) as white crystalline powder; mp +350 °C (from H_2O); IR 3428, 3211, 2960, 1717, 1631, 1613, 1416 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 7.04 (1H, dt, $J=7.2$, 1.1 Hz, *H*–C(5′)), 7.12 (1H, dt, $J=7.2$, 1.1 Hz, *H*–C(6′)), 7.29–7.32 (2H, m, *H*–C(7′), 1*H*–Ph), 7.38–7.44 (2H, m, 2*H*–Ph), 7.45–7.52 (2H, m, 2*H*–Ph), 7.64 (1H, d, $J=7.9$ Hz, *H*–C(4′)), 7.67 (1H, d, $J=2.6$ Hz, *H*–C(2′)), 7.73 (1H, d, $J=5.7$ Hz, *H*–C(6)), 11.21 (1H, br s, N*H*), 11.31 (1H, br d, $J=5.7$ Hz, N*H*); ^{13}C NMR (DMSO-d_6) δ: 107.2, 108.3, 112.1, 119.5, 119.8, 121.6, 125.4, 125.7, 128.3, 129.2, 129.3, 136.1, 136.4, 136.4, 151.0, 163.0; MS (EI) *m/z*: 303 (M+, 100), 184 (57), 155 (36), 141 (15), 129 (16); HRMS: found 303.1010, $C_{18}H_{13}N_3O_2$, requires 303.1008; Anal. Calcd for $C_{18}H_{13}N_3O_2$: C, 71.28; H, 4.32; N, 13.85. Found: C, 70.86; H, 4.44; N, 13.56.

5.3.5. 2,3-Dihydro-5-(1*H*-indol-3-yl)-3-phenyl-2-thioxo-pyrimidin-4(1*H*)-one (14).

This compound was prepared from compounds **8a**,**b** (703 mg, 2.00 mmol) in 9 h. Data for **14**: 515 mg (81%) as yellow crystalline powder; mp 337–340 °C (from MeOH); IR 3406, 3138, 3011, 1678, 1630, 1523, 1261, 1124 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 7.07–7.18 (2H, m, *H*–C(5′), *H*–C(6′)), 7.25–7.28 (2H, m, *H*–C(7′), 1*H*–Ph), 7.38–7.52 (4H, m, 4*H*–Ph), 7.68 (1H, d, $J=7.5$ Hz, *H*–C(4′)), 7.77 (1H, s, *H*–C(6)), 7.83 (1H, d, $J=2.6$ Hz, *H*–C(2′)), 11.36 (1H, br s, N*H*), 12.77 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 106.0, 111.9, 112.8, 119.3, 119.5, 121.5, 124.8, 125.9, 128.0, 128.6, 129.0, 134.8, 136.1, 139.6, 160.3, 174.7; MS (EI) *m/z*: 319 (M+, 100), 184 (47), 155 (45), 141 (19), 129 (15); HRMS: found 319.0780, $C_{18}H_{13}N_3OS$, requires 319.0779; Anal. Calcd for $C_{18}H_{13}N_3OS$: C, 67.69; H, 4.10; N, 13.16. Found: C, 67.11; H, 4.22; N, 13.10.

5.3.6. 5-(1*H*-Indol-3-yl)-3-(pyridin-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione 15.

t-BuOK (620 mg, 5.37 mmol) was dissolved in DMAA (5 mL) followed by the addition of **9** (361 mg, 1.07 mmol). The reaction mixture turned red-brown immediately after after the addition of **9** and was then

stirred at rt for 3.5 h. The reaction mixture was neutralized with $HCl_{37\%}$ (1.0 mL) in ice water (10 mL). The solvent was evaporated in vacuo and the solid residue was treated with saturated K_2CO_3 solution (10 mL). The obtained light-brown solution was stirred for 15 min at room temperature. Water was evaporated in vacuo and the remained crude product was purified by column chromatography over silica gel using AcOEt/MeCN = 85:15 mixture as eluent to afford 5-(1*H*-indol-3-yl)-3-(pyridin-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione **15**.

5.3.7. 5-(1*H*-Indol-3-yl)-3-(pyridin-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (15). *Data for* **15**. 149 mg (46%) as white crystalline powder; mp 316–326 °C (from H_2O); IR 3320, 3109, 2913, 1714, 1650, 1592, 1423 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 7.05 (1H, dt, J=7.2, 1.1 Hz, *H*–C(5′)), 7.12 (1H, dt, J=7.2, 1.1 Hz, *H*–C(6′)), 7.41 (1H, d, J=7.5 Hz, *H*–C(7′)), 7.46 –7.51 (2H, m, *H*–C(5″)–Py, *H*–C(3″)–Py), 7.64 (1H, td, J=7.3, 1.3 Hz, *H*–C(4′)), 7.67 (1H, d, J= 2.6 Hz, *H*–C(2′)), 7.77 (1H, s, *H*–C(6)), 7.99 (1H, dt, J= 7.9, 1.9 Hz, *H*–C(4″)–Py), 8.61 (1H, dd, J=4.9, 1.9 Hz, *H*–C(6″)–Py), 11.23 (1H, br s, N*H*), 11.39 (1H, br s, N*H*); ^{13}C NMR (DMSO-d_6) δ: 107.5, 108.9, 112.6, 120.1, 120.3, 122.2, 124.9, 125.2, 125.9, 126.2, 136.9, 137.0, 139.6, 150.2, 150.5, 151.3, 163.4; MS (EI) *m/z*: 304 (M+, 100), 262 (56), 184 (35), 155 (44), 141 (22), 129 (33); HRMS: found 304.0971, $C_{17}H_{12}N_4O_2$, requires 304.0960; Anal. Calcd for $C_{17}H_{12}N_4O_2 \times 1/2\ H_2O$: C, 65.17; H, 4.18; N, 17.88. Found: C, 65.61; H, 4.06; N, 17.96.

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42. Compound **11** was already reported. However, it was prepared by another approach in a very low yield: condensation of methyl 3-hydroxy-2-(1*H*-indol-3-yl)propenoate with thiourea at 100 °C afforded 27% of **11** after 6 h. In addition, compound **11** was not characterised by NMR spectroscopy. Rozhkov, V. S.; Smushkevich, Y. I.; Suvorov, N. N. *Khim. Geterotsikl. Soedin.* **1980**, 55–58. *Chem. Abstr.* **1980**, *93*, 8124j.

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7-Deaza-2,8-diazaadenine containing oligonucleotides: synthesis, ring opening and base pairing of 7-halogenated nucleosides

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Dedicated to the late Professor John A. Montgomery

Abstract—Oligonucleotides containing 7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine (**3**) and 5-amino-3-bromo-4-carbamoyl-1-(2′-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole (**4**) were synthesized. Compound **3** was prepared from 7-bromo-8-aza-7-deaza-2′-deoxyadenosine (**5**) via the 1,N^6-etheno derivative **6** and was converted into the phosphoramidite **11**. The 7-bromo substituent of **3** increases oligonucleotide duplex stability compared to the non-halogenated nucleoside. Oligonucleotides incorporating **3** are transformed to those containing **4** during long time deprotection at elevated temperature (25% aq ammonia, 60 °C, 30 h). Compound **3** forms a strong base pair with dG. The base pair stability decreases in the order dG > dT > dA > dC. Similar recognition selectivity is observed for the pyrazole nucleoside **4**, however, due to decreased stacking and higher flexibility of the pyrazole moiety, duplexes are less stable than those containing **3**.

1. Introduction

2-Azapurines and their nucleosides attracted attention not only because of their broad biological activity,[1–9] but also of their unusual base pairing properties when they are constituents of oligonucleotides.[10,11] Up to now, only a few 2-azapurine nucleosides were incorporated into oligonucleotides because of their paucity and instability in alkaline solution.[10–12] Previous studies of our laboratory on oligonucleotides containing 2-aza-2′-deoxyadenosine (**1**)[10] and 7-deaza-2,8-diaza-2′-deoxyadenosine (**2**)[11] have shown that 2-azaadenines form strong base pairs with guanine and weaker ones with thymine with a relative stability order of dG > dT ≫ dA ~ dC (purine numbering is used throughout the general part). A likely explanation for this is the presence of an additional proton acceptor site at the 2-position of the purine moiety. The additional nitrogen generates also an increased π-electron deficiency within the aromatic system, in particular at nitrogen-1 being involved in Watson–Crick base pairing. On the other hand, it has been reported that 7-halogeno substituents introduced in 7-deazapurine or the 8-aza-7-deazapurine moieties induce oligonucleotide duplex stabilization.[13–21] The six-membered ring of 2-azapurines is prone to ring opening under alkaline conditions thereby forming substituted imidazole derivatives; the same is observed on the corresponding 7-deaza-2,8-diazapurine nucleosides making them applicable as precursors for substituted pyrazole moieties within an oligonucleotide chain. Recent experiments indicate that the ring opening reaction is accelerated when 7-halogeno substituents are present in the 7-deaza-2,8-diazapurine system. This manuscript reports on the synthesis of 7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine (**3**), its conversion into a building block for solid phase synthesis and the preparation of corresponding oligonucleotides. Oligonucleotides containing **3** are transformed to those containing 5-amino-3-bromo-4-carboxamidopyrazole (**4**) residues under alkaline conditions (Scheme 1). As it was observed that imidazole 4-carboxamide and pyrazole 4-carboxamide form either a dA- or a dI-like hydrogen bonding motif within oligonucleotide duplexes[22–24] the base pairing properties of duplexes containing compounds **3** and **4** are studied.

Keywords: Oligonucleotides; Base pairing; Duplex stability; Nucleosides; Azapurine; Pyrazole.
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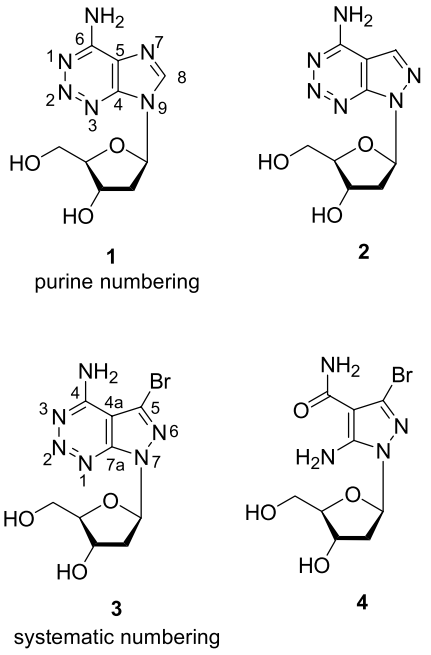


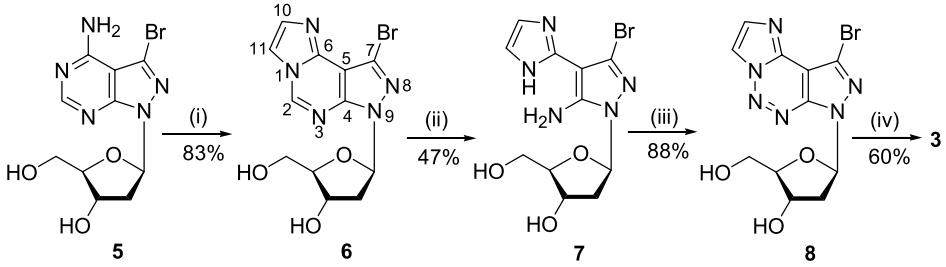
Scheme 1.

2. Results and discussion

2.1. Synthesis of the monomers

Among the several procedures developed for the synthesis of 2-azapurine nucleosides[2,8,25–37] the most attracting protocol is the one starting with the corresponding adenine nucleoside via 1,N^6-etheno adducts.[27–29] Herein, the synthesis of compound **3** is performed using 7-bromo-7-deaza-8-aza-2′-deoxyadenosine (**5**)[21] as starting material (Scheme 2). Reaction of **5** with chloroacetaldehyde at pH 4.5–5 yields 1,N^6-etheno-7-bromo-8-aza-7-deaza-2′-deoxyadenosine (**6**) (83%), which was treated with 1 M sodium hydroxide at room temperature to give the derivative **7** (47%). Subsequent treatment of **7** with sodium nitrite in 80% aqueous acetic acid afforded 1,N^6-etheno-7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine (**8**) in 88% yield. Afterwards compound **8** was treated with *N*-bromosuccinimide at pH 4–4.5 to result in **3** (60%, overall yield: 20%). All new compounds were characterized by ^{1}H and ^{13}C NMR spectra and by elemental analyses (see Table 1 and Section 4). The ^{13}C NMR resonances were assigned based on gated-decoupled spectra.

Next, the phosphoramidite **11** was prepared. Previous studies have shown that isobutyryl and acetyl groups are more suitable than the benzoyl residue for the protection of the amino group of 2-azapurine nucleosides.[11] However, when compound **3** was treated with trimethylsilyl chloride and isobutyric anhydride under the conditions of transient protection,[38] the resulting material was impure after extensive purification by column chromatography, as shown by the ^{1}H NMR spectra. This problem was overcome by using the dimethylaminomethylidene residue as protecting group. Treatment of **3** with dimethylformamide dimethylacetal provided 5-bromo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-(dimethylaminomethylidene)-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine (**9**) in 79% yield (Scheme 3). The 4,4′-dimethoxytritylation followed the standard protocol

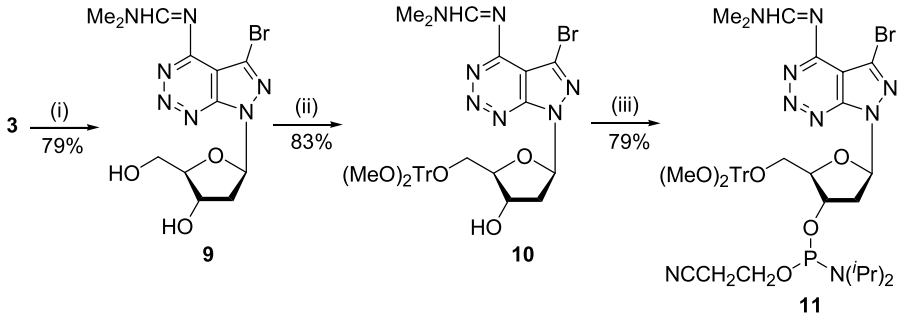


Scheme 2. Reagents and conditions: (i) $ClCH_2CHO$, 1 M aq NaOAc, pH 4.5–5, rt; (ii) 1 M NaOH, rt; (iii) $NaNO_2$, 80% aq HOAc, 0 °C–rt; (iv) NBS, 1 M aq NaOAc buffer, pH 4–4.5, rt.

Table 1. ^{13}C NMR chemical shifts of 2′-deoxyribonucleosides measured in (D_6) DMSO at 298 K

	C(2)[a] C(5)[d]	C(4)[a] C(7a)[b] C(5)[c] C(6a)[d]	C(5)[a] C(4a)[b] C(4)[c] C(9a)[d]	C(6)[a] C(4)[b] C(1″)[c] C(9b)[d]	C(7)[a] C(5)[b] C(3)[c] C(9)[d]	C(10)[a] C(3″)[c] C(2)[d]	C(11)[a] C(4″)[c] C(3)[d]	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)
2(11)		149.2	96.0	153.2	132.9			84.9	[e]	71.0	87.9	62.3
3		149.6	95.9	152.3	118.8			85.0	38.0	70.6	87.9	62.0
4		151.8	95.2	164.4	123.8			84.3	37.6	70.8	87.5	62.2
6	141.4	145.6	103.5	138.1	117.9	132.6[f]	113.2[f]	84.4	38.0	70.7	87.8	62.1
7		147.8[f]	94.0[f]	139.7[f]	115.5[f]	126.9[f]	121.9[f]	84.6	37.8	70.8	87.5	62.2
8		143.0	104.8	129.9	117.4	133.3[f]	115.8[f]	86.0	38.3	70.5	88.2	61.8
9		150.7	101.9	155.2[f]	120.5			84.1	38.0	70.6	87.9	62.0
10[g]		151.3	103.1	155.6	121.3			86.3	38.6	72.9	85.1	64.2

[a] Purine numbering.
[b] Systematic numbering for compounds **3**, **9** and **10**.
[c] Systematic numbering for compounds **4** and **7**.
[d] Systematic numbering for compounds **6** and **8**.
[e] Superimposed by the signal of DMSO-d_6.
[f] Tentative.
[g] Measured in $CDCl_3$.



Scheme 3. Reagents and conditions: (i) $Me_2NCH(OMe)_2$, MeOH, 40 °C; (ii) $(MeO)_2TrCl$, pyridine, rt; (iii) $^{i}Pr_2NP(Cl)OCH_2CH_2CN$, $^{i}Pr_2EtN$, CH_2Cl_2, rt.

Table 2. Molecular masses of oligonucleotides determinated by MALDI-TOF mass spectroscopy

Oligonucleotides	$[M+H]^+$ (calcd) [Da]	$[M+H]^+$ (found) [Da]
3′-d(ATC C**3**G TTA TGA) (**12**)	3725	3725
3′-d(ATC C**4**G TTA TGA) (**13**)	3715	3714
5′-d(TAG GTC **4**AT ACT) (**14**)	3715	3715
3′-d(ATC **4**AG TTA TGA) (**15**)	3739	3739
3′-d(AT**4** CAG TTA TGA) (**16**)	3739	3740
3′-d(ATC CA**G** TT **4** TGA) (**17**)	3715	3715

3=7-Bromo-7-deaza-2,8-diaza-2′-deoxyadenosine, **4**=5-amino-3-bromo-pyrazole-4-carboxamide 2′-deoxy-β-D-ribofuranoside.

yielding compound **10** in 83% yield.[39] Subsequent phosphitylation with chloro-(2-cyanoethoxy)-*N*,*N*-(diisopropylamino)phosphine resulted in the phosphoramidite **11** (79%).[40] All compounds were characterized by ^{1}H and ^{13}C NMR spectra and by elemental analysis (see Table 2 and Section 4).

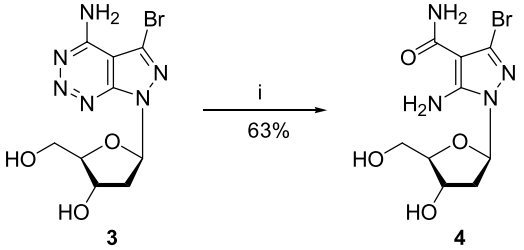
2.2. Synthesis and characterization of the oligonucleotides

The oligonucleotide synthesis was performed on an ABI 392-08 DNA synthesizer (Applied Biosystems, Weiterstadt, Germany) in a 1-μmol scale applying the phosphoramidite **11** in solid phase oligonucleotide chemistry.[41] Initially, the regularly protected canonical phosphoramidites were employed. After cleavage from the solid support, the oligonucleotides were deprotected according to the standard procedure (25% aq NH_3 solution, 60 °C, 16 h). However, it was difficult to obtain a pure oligonucleotides because of the instability of the 2-azapurines in alkaline medium at elevated temperature. This problem was overcome by using the ^{t}BPA-protected phosphoramidites and employing ultra mild deprotection conditions (25% aq NH_3, room temperature, 12 h). By this means the oligonucleotide **12** containing the nucleoside **3** residue was obtained.

To get the oligonucleotides containing pyrazole nucleoside **4** the oligonucleotides were deprotected by incubation with a 25% aq NH_3 solution at 60 °C for a period of 30 h. The deprotected oligonucleotides were purified by RP-18 HPLC. After removal of the 4,4′-dimethoxytrityl residue, the oligonucleotides were applied to RP-18 HPLC. The content of the main peak was separated from side products and was desalted to give the oligonucleotides **13**–**17** containing compound **4**.

When the pure oligonucleotide **12** containing nucleoside **3** was treated with aqueous ammonia at 60 °C for 30 h and was worked-up in the usual way two strong peaks are detected by HPLC. Enzymatic digestion of the peak contents with snake venom phosphodiesterase followed by alkaline phosphatase showed that only the faster migrating main peak contained the ring-opened nucleoside **4** while the modification of the second peak was not identified. To confirm the conversion on the oligonucleotide level the ring opening was performed on 4-amino-5-bromo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine (7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine) **3** which was converted to 5-amino-3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-carbarmoyl-1*H*-pyrazole (**4**). Compound **3** was heated in a sealed vessel in 25% aq NH_3 at 60 °C for 16 h. After work-up the ring-opened nucleoside was isolated in 63% yield. The structure was proven to be **4** by ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis (Scheme 4, Table 1 and Section 4).

In all cases the purity of the oligonucleotides was confirmed by the detection of a single peak (>99% area) in the HPLC profile. Furthermore, MALDI-TOF spectra (Table 2) and enzymatic analysis with snake venom phosphodiesterase followed by alkaline phosphate confirmed the composition of oligonucleotides (for the digest protocol see Section 4). The oligomers synthesized are shown in Table 3.



Scheme 4. Reagents and conditions: (i) NH_4OH, 60 °C, 16 h.

Table 3. T_m-values and thermodynamic data of oligonucleotide duplexes containing the base modified nucleosides **2**, **3** and **4**[a,b]

Duplex		T_m (°C)	ΔT_m[c]	ΔG^{310} (kcal/mol)[d]	Duplex		T_m (°C)	ΔT_m[c]	ΔG^{310} (kcal/mol)[d]
5′-d(TAGGTCAATACT)	**18**	47	0	−10.4	5′-d(TAGG**C**CAATACT)	**24**	36	0	
3′-d(ATCCAGTTATGA)	**19**				3′-d(ATCC**A**GTTATGA)	**23**			
5′-d(TAGG**T**CAATACT)	**18**	43	−4	−9.1	5′-d(TAGG**C**CAATACT)	**24**	36	0	−7.4
3′-d(ATCC**2**GTTATGA)	**21**				3′-d(ATCC**2**GTTATGA)	**21**			
5′-d(TAGG**T**CAATACT)	**18**	45	−2	−10.0	5′-d(TAGG**C**CAATACT)	**24**	35	−1	−7.4
3′-d(ATCC**3**GTTATGA)	**12**				3′-d(ATCC**3**GTTATGA)	**12**			
5′-d(TAGG**T**CAATACT)	**18**	42	−5	−9.2	5′-d(TAGG**C**CAATACT)	**24**	35	−1	−7.7
3′-d(ATCC**4**GTTATGA)	**13**				3′-d(ATCC**4**GTTATGA)	**13**			
5′-d(TAGG**G**CAATACT)	**20**	46	0	−10.5	5′-d(TAGGTC**4**ATACT)	**14**	36	−11	−7.7
3′-d(ATCC**A**GTTATGA)	**19**				3′-d(ATCCAGTTATGA)	**19**			
5′-d(TAGG**G**CAATACT)	**20**	48	+2	−10.6	5′-d(TAGGGCAATACT)	**20**	40	−6	−8.7
3′-d(ATCC**2**GTTATGA)	**21**				3′-d(ATCCAGTT**4**TGA)	**17**			
5′-d(TAGG**G**CAATACT)	**20**	50	+4	−11.6	5′-d(TAGGTC**4**ATACT)	**14**	35	−12	−7.7
3′-d(ATCC**3**GTTATGA)	**12**				3′-d(ATCC**4**GTTATGA)	**13**			
5′-d(TAGG**G**CAATACT)	**20**	45	−1	−10.1	5′-d(TAGG**G**CAATACT)	**20**	39	−7	−8.5
3′-d(ATCC**4**GTTATGA)	**13**				3′-d(ATC**4**AGTTATGA)	**15**			
5′-d(TAGG**A**CAATACT)	**22**	38	0		5′-d(TAGG**G**CAATACT)	**20**	36	−10	−7.7
3′-d(ATCC**A**GTTATGA)	**23**				3′-d(AT**4**CAGTTATGA)	**16**			
5′-d(TAGG**A**CAATACT)	**22**	37	−1	−7.8					
3′-d(ATCC**2**GTTATGA)	**21**								
5′-d(TAGG**A**CAATACT)	**22**	41	+3	−8.7					
3′-d(ATCC**3**GTTATGA)	**12**								
5′-d(TAGG**A**CAATACT)	**22**	34	−4	−7.3					
3′-d(ATCC**4**GTTATGA)	**13**								

[a] Determined in 10 mM sodium cacodylate-buffer, pH 7.0, containing 100 mM NaCl and 10 mM $MgCl_2$.
[b] **3**=7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine, **4**=5-amino-3-bromo-pyrazole-4-carboxamide-2′-deoxyfuranoside.
[c] Per modified base pair.
[d] 1 cal=4.184 J.

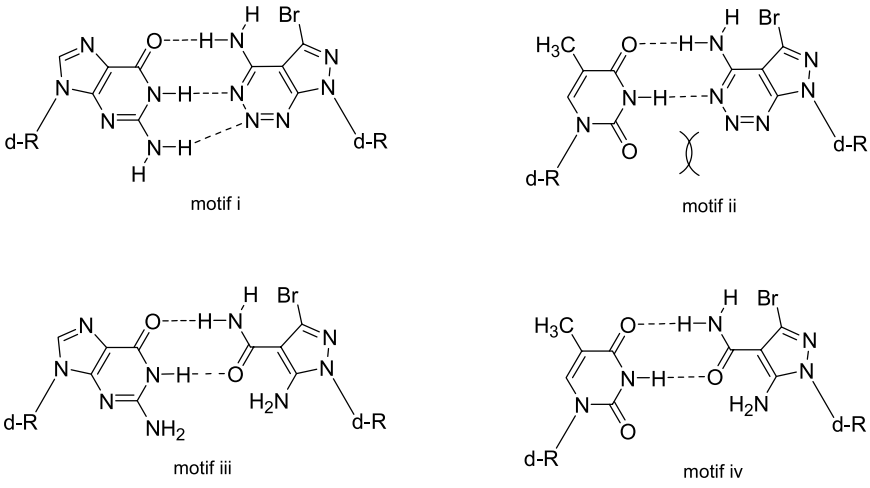
2.3. Duplex stability and base pairing selectivity

For the investigation of the duplex stability and the base pairing selectivity of oligonucleotides containing compounds **3** or **4**, the duplex 5′-d(TAG GTC AAT ACT) (**18**)·3′-d(ATC CAG TTA TGA)(**19**)[42] was modified in a central position (see Table 3). Within these duplexes the modified nucleosides were positioned opposite to the four canonical DNA-constituents. As it can be seen from the data of Table 3, compound **3** shows a different base recognition than the canonical 2′-deoxyadenosine. Compared to dA, compound **3** forms a weaker base pare with dT (duplex **18·12**), but stronger base pairs with dG (duplexes **20·12**). Compound **2** shows the same trend and compound **4** forms weaker base pairs in all cases. In cases when dA or dC was located opposite to **2**, **3** or **4**, the duplexes were significantly less stable (Table 2) than those with dG or dT opposite to **2**, **3**, or **4**. Thus, the base pairing preference of the compounds **3** and **4** is in the relative order dG > dT ≫ dA ~ dC. Compound **4** was also incorporated in various positions of oligonucleotide duplexes showing that the stability was depending on the position of incorporation (only shown for the 4-dT pair; Table 3).

Regarding base pairing we have already suggested motifs[11] including those for the base pairs of **2** with dT as well as that of **2** with dG. Similar motifs (i and ii) are now considered for compound **3** (Scheme 5). In both motifs, the bulky bromo substituents are well accommodated in the major groove of B-DNA. At the same time the bromo residues will increase base stacking as it has been observed in the case of pyrazolo[3,4-*d*]pyrimidine nucleosides. Consequently, the T_m values are increased in the case of oligonucleotide duplexes containing the bromo compound **3** over that of the non functionalized nucleoside **2**. For the assembly of base pairs of compound **4** with dT or dG, the most plausible motifs are iii and iv. As the nucleobase of compound **4** has a smaller surface area than that of compound **3**, stacking interactions are expected to decrease while conformational freedom increases. This results in lower T_m values in the case of **4** compared to **3** (see Table 3). It is worth to mention that the duplex containing two adenine-like moieties facing each other shows an exceptionally high T_m value (duplex **22·12**). Such a phenomenon has already been described for duplexes in which two identical purine or purine-like bases are located opposite to each other. This is correlated to the intra-strand stacking observed for the purine–purine nucleobases.[43]

3. Conclusion

Oligonucleotides containing 7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine (**3**) are obtained when nucleobase protecting groups are chosen which avoid harsh alkaline treatment at elevated temperature during deprotection. As base-labile protecting groups the dimethylaminomethylidene residue was selected for the nucleoside **3** and *p-tert*-butylphenoxyacetyl residues for the canonical phosphoramidites dA, dG and dC.[44] Oligonucleotides containing 7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine (**3**) are transformed into those incorporating 5-amino-3-bromo-4-carbarmoyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrazole (**4**) when they were treated with 25% aq ammonia at 60 °C for 30 h. Similar reaction conditions (25% aq ammonia at 60 °C for 16 h) did not convert the non-halogenated nucleoside **2** into



Scheme 5.

its ring opened derivative.[11] Oligonucleotide duplexes containing compounds **3** or **4** show base pairing selectivity when located opposite to the canonical DNA constituents. Nucleoside **3** forms a strong base pair with dG and a slightly weaker one with dT as it was reported for other 2-azapurine nucleosides such as 7-deaza-2,8-diaza-2′-deoxyadenosine.[11] Oligonucleotide duplexes incorporating the halogenated nucleoside **3** are thermally more stable than those containing the non-functionalized nucleoside **2**. Duplexes containing the pyrazole nucleoside **4** show a similar base pairing selectivity as those incorporating **3**. The pyrazole containing oligonucleotides are less stable than those containing the 'purine' base **3** which is related to a decreased surface area resulting in weaker stacking interactions and the higher conformational flexibility of the pyrazole moiety.

4. Experimental

4.1. General

Monomers. Flash chromatography (FC) was performed at 0.4 bar on silica gel 60 H (VWR, Darmstadt, Germany). Thin-layer chromatography (TLC) was carried out on TLC aluminium sheet covered with silica gel 60 F_{254} (0.2 mm, VWR, Germany). Reversed-phase HPLC was performed on a 4×250 mm RP-18 LiChrosorb column (10 μm, VWR, Germany) with a Merck-Hitachi HPLC pump (model 655 A-12) connected with a variable wavelength monitor (model D-2000). UV spectra were recorded on a U3200 spectrophotometer (Hitachi, Japan). λ_{max} in nm, ε in $dm^3\ mol^{-1}$. NMR spectra were measured on an Avance DPX-250 spectrometer or an AMX-500 spectrometer (Bruker, Rheinstetten, Germany) at 250.13 and 500.14 MHz for ^1H nuclei and 62.90 and 125.13 MHz for ^{13}C nuclei. Chemical shifts (δ) are in ppm relative to internal $SiMe_4$ (^1H, ^{13}C) or external 85% H_3PO_4 (^{31}P). Microanalyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). MALDI-TOF mass spectra were recorded on a Biflex-III spectrometer (Bruker, Leipzig, Germany) in the reflector mode. The average power of the nitrogen laser (337.1 nm) at 20 Hz was 3–4 mW (150–200 μJ/pulse) with a delay time of 600 ns. -Solvents: technical grade, distilled before use. Chemicals were purchased from ACROS, Fluka, or Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany).

Oligonucleotides. The oligonucleotide synthesis was performed applying phosphoramidite chemistry on a solid support in an automated DNA-synthesizer with Controlled Pore Glass (CPG) 500 serving as solid phase, to which the starting nucleoside was bound via its O-3′-position. The 4-*tert*-butylphenoxyacetyl-protected canonical phosphoramidites (Millipore) were used for the oligonucleotides synthesized. The syntheses were performed in a 1-μmol scale according to standard conditions[41] except that 145 s coupling time was used for the modified phosphoramidite (instead of 25 s) and the capping reagent CAP A contained 4-*tert*-butylphenoxyacetic anhydride instead of acetic anhydride. After cleavage from the support, the oligomers were incubated with a 25% aq NH_3 solution. The following conditions were employed: (i) room temperature, 12 h for oligonucleotides containing **3**; (ii) 60 °C, 30 h for oligonucleotides containing **4**. The deprotected oligomers were purified on a 4×250 mm RP-18 HPLC column (10 μm) with the following solvent gradient system: gradients of MeCN (A) and 0.1 M Et_3NHOAc (pH 7.0)/MeCN 95:5 (B); gradient: 3 min 15% A in B, 7 min 15–40% A in B, 10 min 40% A in B and 5 min 15% A in B, and with flow rate of 1.0 ml min^{-1}. The content of the main peak was collected and evaporated to dryness, and the residue was treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 3 min at room temperature to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified by reversed-phase HPLC (RP-18, 4×250 mm, 10 μm) with the following solvent gradient systems: gradients of MeCN (A) and 0.1 M Et_3NHOAc (pH 7.0)/MeCN 95:5 (B); gradient i: 25 min 0–20% A in B, 5 min 20% A in B, 5 min 20–0% A in B and 5 min B, with flow rate of 1 ml min^{-1} for oligonucleotide **5**; gradient ii: 30 min 0–15% A in B, 5 min 15–20% A in B, 5 min 20–0% A in B and 5 min B, with flow rate of 1 ml min^{-1} for oligonucleotides **6–10**. All the oligomers were desalted (RP-18, 4×100 mm, silica gel) and lyophilized on a Speed-Vac evaporator to yield colourless solids which were frozen at −24 °C.

The nucleoside composition of oligonucleotides was determined by enzymatic hydrolysis. The oligonucleotides were dissolved in 0.1 M Tris–HCl buffer (pH 8.3, 200 μl), and treated with snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) (3 μl) at 37 °C for 45 min and alkaline phosphatase (EC 3.1.3.1, *E. coli* from Roche Diagnostics GmbH, Germany) (3 μl) at 37 °C for another 30 min. The reaction mixture was analyzed by HPLC (RP-18, at 260 nm) solvent system 0.1 M (Et_3NH)OAc, pH 7.0/MeCN 95:5) showing the peaks of the modified and the unmodified nucleosides. The oligonucleotides were also characterized by MALDI-TOF-spectra. The masses were in agreement with the calculated values (Table 2).

The melting curves were measured with a Cary-1/3 UV/VIS spectrophotometer equipped with a Cary thermoelectrical controller. The thermodynamic data were calculated with the program Meltwin 3.0.[45]

4.1.1. 9-Bromo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-imidazo[1,2-*c*]-7*H*-pyrazolo[4,3-*e*]pyrimidine (6). To a stirred solution of compound **5** (0.63 g, 1.91 mmol) in aqueous sodium acetate (1 M, pH 4.5–5.0, 70 ml) chloroacetaldehyde (50% aqueous solution, 12 ml) was added at 40–50 °C. Stirring was continued at room temperature for 3 days. The reaction mixture was evaporated, and the residue was applied to FC (silica gel, 4×8 cm, CH_2Cl_2–MeOH 100:2 to 15:1) to give compound **6** (0.56 g, 83%) as colourless crystals (MeOH); mp 227 °C (dec.); R_f (CH_2Cl_2–MeOH 15:1) 0.25. UV (MeOH): λ_{max} 283 (3300), 260 (4800), 234 (29,600). ^{1}H NMR (D_6 (DMSO)), 2.35, 2.84 (2m, 2H, H_2–C(2′)); 3.39, 3.53 (2m, 2H, H_2–C(5′)); 3.86 (m, 1H, H–C(4′)); 4.47 (m, 1H, H-C(3′)); 4.73 (t, J=5.6 Hz, 1H, OH–C(5′)); 5.34 (d, J=4.5 Hz, 1H, OH–C(3′)); 6.68 (t, J=6.2 Hz, 1H, H–C(1′)); 7.57 (s, 1H, H–C(11)); 8.09 (s, 1H, H–C(10)); 9.35 (s, 1H, H–C(2)). Anal. Calcd for $C_{12}H_{12}BrN_5O_3$ (354.16): C 40.70, H 3.42, N 19.77; found: C 40.74, H 3.52, N 19.60.

4.1.2. 5-Amino-3-bromo-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-(imidazol-2-yl)-1*H*-pyrazole (7). A solution of compound **6** (0.53 g, 1.50 mmol) in aqueous NaOH (0.8 M, 38 ml) was stirred overnight at room temperature. The solution was acidified to pH 7.0 with hydrochloric acid (2 M), concentrated and purified by FC (silica gel, 4×6 cm, CH_2Cl_2–MeOH 20:1 to 15:1) to yield a colourless foam (0.24 g, 47%). R_f (CH_2Cl_2–MeOH 6:1) 0.3. UV (MeOH): λ_{max} 260 (9300). ^{1}H NMR (D_6 (DMSO)), 2.11, 2.66 (2m, 2H, H_2–C(2′)); 3.40, 3.47 (2m, 2H, H_2–C(5′)); 3.78 (m, 1H, H–C(4′)); 4.35 (m, 1H, H–C(3′)); 4.85 (m, 1H, OH–C(5′)); 5.22 (d, J=3.1 Hz, 1H, OH–C(3′)); 6.10 (t, J=6.3 Hz, 1H, H–C(1′)); 6.72 (s, 2H, NH_2); 7.00 (d, J=19.2 Hz, 2H, H–C(7), H–C(8)); 11.34 (s, 1H, NH). Anal. Calcd for $C_{11}H_{14}BrN_5O_3$ (344.16): C 38.39, H 4.10, N 20.35; found: C 38.56, H 4.23, N 20.46.

4.1.3. 9-Bromo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-imidazo[1,2-*c*]-7*H*-pyrazolo[4,3-*e*][1,2,3]triazine (8). A solution of compound **7** (0.22 g, 0.64 mmol) in 80% aq HOAc (20 ml) was treated with sodium nitrite (47 mg, 0.68 mmol) during one hour in an ice bath. The reaction solution was evaporated, dissolved in water and evaporated repeatedly to remove HOAc. The residue was then applied to FC (silica gel, column 2.5×6 cm, CH_2Cl_2–MeOH 20:1) to give **8** as yellowish crystals (0.20 g, 88%); mp 184 °C (dec.); R_f (CH_2Cl_2–MeOH 15:1) 0.18. UV (MeOH): λ_{max} 235 (27,400). ^{1}H NMR (D_6 (DMSO)), 2.48, 2.88 (2m, 2H, H_2–C(2′)); 3.39, 3.52 (2m, 2H, H_2–C(5′)); 3.87 (m, 1H, H–C(4′)); 4.51 (m, 1H, H–C(3′)); 4.70 (t, J=5.6 Hz, 1H, OH–C(5′)); 5.41 (d, J=4.7 Hz, 1H, OH–C(3′)); 6.72 (t, J=5.9 Hz, 1H, H–C(1′)); 7.83 (d, J=1.6 Hz, 1H, H–C(11)); 8.81 (d, J=1.6 Hz, 1H, H–C(10)). Anal. Calcd for $C_{11}H_{11}BrN_6O_3$ (355.15): C 37.20, H 3.12, N 23.66; found: C 36.98, H 3.30, N 23.51.

4.1.4. 4-Amino-5-bromo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine (7-bromo-7-deaza-2,8-diaza-2′-deoxyadenosine) (3). To a solution of compound **8** (135 mg, 0.38 mmol) in aq sodium acetate (1 M, pH 4.0–4.5, 30 ml) *N*-bromosuccinimide (0.65 g, 3.65 mmol) was added at 40–50 °C, and the reaction mixture was stirred at room temperature overnight. After evaporation the residue was applied to FC (silica gel, 2.5×8 cm, CH_2Cl_2–MeOH 15:1 to 9:1), and the content of the main zone was collected and crystallized from H_2O to yield yellow crystals (76 mg, 60%); mp 189 °C (dec.); R_f (CH_2Cl_2–MeOH 6:1) 0.44. UV (MeOH): λ_{max} 318 (6900), 255 (5700). ^{1}H NMR (D_6 (DMSO)), 2.36, 2.82 (2m, 2H, H_2–C(2′)), 3.38, 3.52 (m, 2H, H_2–C(5′)); 3.85 (m, 1H, H–C(4′)); 4.46 (m, 1H, H–C(3′)); 4.74 (t, J=5.7 Hz, 1H, OH–C(5′)); 5.35 (d, J=4.7 Hz, 1H, OH–C(3′)); 6.74 (t, J=6.2 Hz, 1H, H–C(1′)); 7.55, 8.60 (2br, 2H, NH_2). Anal. Calcd for $C_9H_{11}BrN_6O_3$ (331.13): C 32.65, H 3.35, N 25.38; found: C 32.67, H 3.32, N 25.21.

4.1.5. 5-Bromo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4-(dimethylaminomethylidene)-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine (9). To a solution of compound **3** (0.12 g, 0.36 mmol) in MeOH (30 ml) *N*,*N*-dimethylformamide dimethylacetal (0.18 ml, 1.34 mmol) was added while stirring at 40 °C for 2.5 h. The solution was evaporated, and the residue was purified on a silica gel column (2.5×8 cm, CH_2Cl_2–MeOH 20:1 to 15:1) to yield a colourless foam (0.11 g, 79%). R_f (CH_2Cl_2–MeOH 15:1) 0.2. UV (MeOH): λ_{max} 339 (20,900), 226 (10,500). ^{1}H NMR (D_6 (DMSO)), 2.37 (m, 1H, H_α–C(2′)); 2.85 (m, 1H, H_β–C(2′)); 3.26, 3.31 (2s, 6H, $2CH_3$); 3.54 (m, 1H, H–C(5′)); 3.85 (m, 1H, H–C(4′)); 4.45 (m, 1H, H–C(3′)); 4.74 (t, J=5.6 Hz, 1H, OH–C(5′)); 5.36 (d, J=4.6 Hz, 1H, OH–C(3′)); 6.76 (t, J=6.3 Hz, 1H, H–C(1′)); 9.16 (s, 1H, CH). Anal. Calcd for $C_{12}H_{16}BrN_7O_3$ (386.20): C 37.32, H 4.18, N 25.39; found: C 37.38, H 4.26, N 25.24.

4.1.6. 5-Bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-*erythro*-pentofuranosyl]-4-(dimethylaminomethylidene)-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine (10). Compound **9** (77 mg, 0.2 mmol) was coevaporated twice with anhydrous pyridine, dissolved in anhydrous pyridine (2 ml) and treated with 4,4′-dimethoxytriphenylmethyl chloride (84 mg, 0.25 mmol) at room temperature for 2 h while stirring. Thereupon, MeOH (1 ml) was added and the stirring was continued for 10 min. The solution was concentrated to half of the volume and CH_2Cl_2 (70 ml) was added. The organic phase was washed with aqueous

$NaHCO_3$ (twice, 5%, 2×15 ml), with water (twice, 2×15 ml) and once with a saturated NaCl (20 ml). The organic layer was separated and dried over Na_2SO_4, filtered, evaporated, and the residue was chromatographed on silica gel (column 2.5×6 cm, CH_2Cl_2–MeOH 200:1) to yield a yellowish foam (114 mg, 83%). R_f (CH_2Cl_2–MeOH 20:1) 0.35. UV (MeOH): λ_{max} 339 (17,000), 232 (25,700). ^1H NMR (D_6 (DMSO)) 2.50 (m, 1H, H_α–C(2′)); 3.04 (m, 1H, H_β–C(2′)); 3.29 (m, 8H, H_2–C(5′), $N(CH_3)_2$); 3.77 (s, 6H, $2OCH_3$); 4.06 (m, 1H, H–C(4′)); 4.84 (m, 1H, H–C(3′)); 6.95 (m, 1H, H–C(1′)); 7.16–7.41 (m, 9H, phenyl-H); 9.15 (s, 1H, CH). Anal. Calcd for $C_{33}H_{34}BrN_7O_5$ (688.57): C 57.56, H 4.98. Found: C 58.08, H 5.22.

4.1.7. 5-Bromo-7-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenyl)methyl-β-D-*erythro*-pentofuranosyl]-4-dimethylaminomethylidene-7*H*-pyrazolo[3,4-*d*][1,2,3]triazine 3′-[(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (11). Compound **10** (63 mg, 0.09 mmol) was coevaporated twice with anhydrous pyridine and dissolved in CH_2Cl_2 (4 ml). *N*,*N*-diisopropylethylamine (30 μl, 0.17 mmol) and chloro-(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine (30 μl, 0.13 mmol) were added under argon atmosphere at room temperature while stirring. The stirring was continued for 30 min and CH_2Cl_2 (20 ml) was added. The organic phase was washed twice with an aqueous solution of $NaHCO_3$ (5%, 2×10 ml) and once with a saturated solution of NaCl (10 ml). The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by FC (silica gel, 1.5×5 cm, cyclohexane–EtOAc 1:1) to furnish a colourless foam (64 mg, 79%). R_f (CH_2Cl_2–MeOH 20:1) 0.6. ^{31}P NMR ($CDCl_3$): 149.83, 149.68.

4.1.8. 5-Amino-3-bromo-4-carbarmoyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1*H*-pyrazole (4). Compound **3** (157 mg, 0.47 mmol) was stirred with 25% aq ammonia (20 ml) for 16 h at 60 °C in a sealed vessel. The reaction solution was evaporated to dryness, and the residue was applied to FC (silica gel, column 2.5×8 cm, CH_2Cl_2–MeOH 20:1 to 15:1) to yield a colourless foam (96 mg, 63%). R_f (CH_2Cl_2–MeOH 6:1) 0.41. UV (MeOH): λ_{max} 255 (7500). ^1H NMR (D_6 (DMSO)): 2.08, 2.61 (2m, 2H, H_2–C(2′)); 3.45 (m, 2H, H_2–C(5′)); 3.76 (m, 1H, H–C(4′)); 4.32 (m, 1H, H-C(3′)); 4.82 (t, J=5.4 Hz, 1H, OH–C(5′)); 5.22 (d, J=3.9 Hz, 1H, OH–C(3′)); 6.07 (t, J=6.3 Hz, 1H, H–C(1′)); 6.88 (br, 4H, $2NH_2$). Anal. Calcd for $C_9H_{13}BrN_4O_4$ (321.13): C 33.66, H 4.08, N 17.45; found: C 33.56, H 4.15, N 17.51.

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Bisphosphonate prodrugs: synthesis of new aromatic and aliphatic 1-hydroxy-1,1-bisphosphonate partial esters

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Abstract—Methods for the preparation of various 1-hydroxy-1,1-bisphosphonate partial esters were developed. They were obtained from (alkyl or phenyl) bis(trimethylsilyl) phosphite and aromatic or aliphatic acid chlorides, followed by methanolysis.

1. Introduction

1-Hydroxymethylene-1,1-bisphosphonic acids (HMBP) are an important class of drugs used clinically in the treatment of bone diseases involving excessive bone destruction or resorption such as Paget's disease, osteoporosis and bone metastases.[1,2] They are also routinely used as ^{99}Tc complexes in skeletal scintigraphy. They are structural analogues of natural pyrophosphates containing a P–C–P backbone and so are stable to enzymatic hydrolysis. More recently, bisphosphonates have been used for treatment of metastatic cancer. It has been shown that these compounds were able to inhibit bone metastases proliferation in prostate or breast cancer.[3–5] They also inhibit experimental angiogenesis in vitro and in vivo.[2,6–8] In addition, HMBP have also activity against several trypanosomatid and apicomplexan parasites.[9,10]

Unfortunately, the bio-availability of HBMP's is very poor because of their strong hydrophilicity and their negative charges due to their high ionization at physiological pH values. These properties characterize poor cell membrane permeability. Moreover, they also have powerful complexation properties towards calcium and other divalent metal cations decreasing their gastro-intestinal absorption. As such, only 3–7% of the drug is metabolized.[11] As the side chain of HMBP is responsible for most of the activity, the modification of some of the phosphonic acid functions should be a satisfying way to increase lipophilicity. Masking groups for the negative charge, introduced as phosphonoester, could be an interesting approach for a prodrug strategy. Few studies about the design of bisphosphonate prodrugs have been reported in the literature.[12–14] Only a few reports with phosphonoesters prodrugs[15,16] but such a modification is widely used in phosphate chemistry.[17,18]

Synthesis of 1-hydroxymethylene-1,1-bisphosphonate is usually achieved from condensation of a trialkylphosphite on an acid chloride leading to an α-ketophosphonate which then reacts with a dialkyl phosphite.[19–21] Different improvements of this method were proposed. Burgada et al.[22–24] described a one pot reaction between acid halides and a mixture of trialkyl and dialkyl phosphites and Ruel et al.[25] used anions of dialkyl phosphites to obtain directly the bisphosphonate tetraesters, at low temperature. The main drawback of these techniques are the thermal and basic instability of bisphosphonate tetraesters that promote their phosphonate–phosphate isomerisation.[26] Moreover the regioselective dealkylation to obtain partial esters is difficult and does not occur in good yield. Our group recently proposed a very mild and one-pot synthesis to obtain bisphosphonate methyl esters from bis(trimethylsilyl)-methyl phosphite and acyl chloride.[27]

Herein, we will present the extension of this synthesis to various 1-hydroxymethylene-1,1-bisphosphonate diesters using several alkyl or aryl substituents.

2. Results and discussion

Alkyl or arylbis(trimethylsilyl) phosphites **2** were obtained

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from corresponding dialkyl phosphite first by dealkylation with ammonia and then by silylation of the ammonium monoalkyl or aryl phosphite **1**. The silylphosphites were then reacted with acid chlorides to yield after hydrolysis, the corresponding HMBP symmetrical diesters **3** (Scheme 1).

Mono alkyl or aryl phosphites were synthesized as described by Hammond[28] from the dialkyl (or diphenyl) phosphites by reaction with a 30% ammonia solution. Reactions were exothermic and addition of the ammonia solution was carefully done at 0 °C. The course of the reactions were followed by $^{31}P\{^{1}H\}$ NMR and depending on the nature of the alkyl or aryl substituent the reaction time varied from one hour starting from dimethyl phosphite to one day for the less reactive ditetradecyl phosphite (Table 1). For all compounds **1a-e**, co-evaporation with dry pyridine and benzene was necessary to get rid of water at the end of the reaction. In the case of ammonium phenyl **1d**, or tetradecyl phosphites **1e**, products were further precipitated in dry ether and washed several times to remove phenol or tetradecanol formed. All the obtained compounds gave in $^{31}P\{^{1}H\}$ NMR a large doublet centred around 4–10 ppm with a characteristic coupling constant $^{1}J_{P-H} \approx 640$ Hz (Table 1).

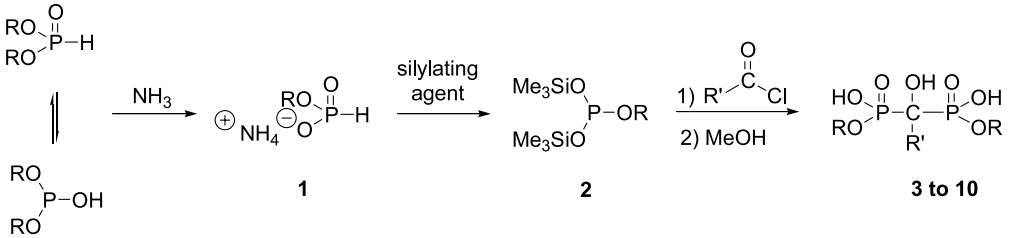
Numerous silylating reagents were described to silylate mono(alkyl or aryl) phosphites. Voronkov and Orlov[29,30] initially used trimethylsilyl chloride in pyridine and Sekine described use of HMDS, BSA, BSTFA.[31,32] In our case with alkyl phosphites, the use of hexamethyldisilazane gave good results. Reactions were conducted by heating the ammonium mono(alkyl or phenyl) phosphite in freshly distilled HMDS, under nitrogen and using dry vessels. Reaction evolution was followed by $^{31}P\{^{1}H\}$ NMR. We first observed the formation of the monosilylated phosphite giving a signal at approximately 13 ppm and then the formation of the alkyl or arylbis(trimethylsilyl) phosphite giving a characteristic signal between 115–125 ppm (Table 1). Once again we observed a difference in reactivity depending on the ammonium mono(alkyl or aryl) phosphite. Reaction time needed to achieve completion increased from methyl bis(trimethylsilyl) phosphite **2a** to tetradecyl (trimethylsilyl) phosphite **2e**. Moreover, in the case of the reaction of ammonium phenyl phosphite **1d** with HMDS, yield was lower than with ammonium alkyl phosphite. If heated to more than 90 °C a new signal was observed in $^{31}P\{^{1}H\}$ NMR at 114 ppm corresponding to the formation of tris(trimethylsilyl) phosphite. The same phenomenon was observed with tetradecyl phosphite but in a less extent. All (alkyl or phenyl) bis(trimethylsilyl) phosphite were distilled prior use except for the tetradecyl bis(trimethylsilyl) phosphite **2e** which was used, after HMDS evaporation, without further purification. To avoid the formation of tris(trimethylsilyl) phosphite when synthesizing phenyl bis(trimethylsilyl) phosphite, BSA could be used as silylating agent. In this case, the reaction was performed at −10 °C, in 1 h, using 5 equiv of BSA.

Table 1. Preparation of alkyl bis(trimethylsilyl) phosphites or aryl bis(trimethylsilyl) phosphites

Compound	R	Reaction conditions	$^{31}P\{^{1}H\}$ NMR (D_2O) ppm	Yield %
1a	CH_3	6 h, rt	9.2	95
1b	CH_3CH_2	6 h, rt	6.9	87
1c	$(CH_3)_2CH$	12 h, rt	4.9	50
1d	C_6H_5	2 h, rt	4.5	95
1e	$C_{14}H_{29}$	24 h, rt	6.2	85
2a	CH_3	6 h, reflux, HMDS	115.8	58
2b	CH_3CH_2	8 h, reflux, HMDS	116.1	55
2c	$(CH_3)_2CH$	24 h, reflux, HMDS	118.2	60
2d	C_6H_5	2 h, 90 °C, HMDS	121.5	45
2e	$C_{14}H_{29}$	24 h, reflux, HMDS	115.0	80

As previously described for reaction between tris(trimethylsilyl) phosphite with acid chlorides the procedure for (alkyl or phenyl) bis(trimethylsilyl) phosphites was still efficient in aromatic or aliphatic series. Various aliphatic acid chlorides, differently substituted aromatics acid chlorides and heterocyclic acid chlorides have been used, all giving good yields of HMBP (Table 2). The key features of this synthetic pathway were the use of silylated phosphites **2** which reacted readily with acid chlorides. A first equivalent reacted following an Arbuzov mechanism yielding a silylated α-ketophosphonate intermediate (Scheme 2). A second addition of the (alkyl or aryl) bis(trimethylsilyl) phosphite led to the fully silylated symmetrical bisphosphonate diester. This product was then hydrolyzed with methanol to the corresponding symmetrical bisphosphonate diester.

For each addition of the aryl or alkyl bis(trimethylsilyl) phosphite **2** the reaction always evolved towards the leaving of a silyl group rather than the alkyl or the aryl group. In fact, alkyl or aryl esters of phosphite were less reactive than silyl esters towards nucleophiles. Typically alkyl and aryl bis(trimethylsilyl) phosphites **2** and acid chloride were reacted for 2 h at room temperature under nitrogen without solvent or in a minimum of dry THF for solid acid chlorides. The reaction was strongly exothermic and the addition must be done in an ice bath. Reactions were followed by $^{31}P\{^{1}H\}$



Scheme 1.

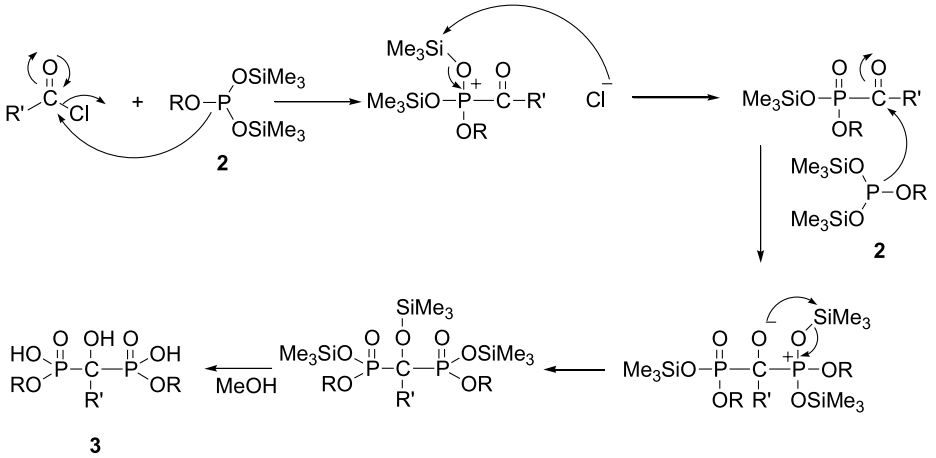
Table 2. Synthesis of HMBP partial esters **3** to **10** produced via Scheme 1

HMBP partial esters	R	R′	$^{31}P\{^{1}H\}$ NMR (D_2O) ppm	Yield %
3a	CH_3	CH_3	23.9	90
3b	CH_3CH_2	CH_3	19.9	95
3c	$(CH_3)_2CH$	CH_3	18.8	90
3d	C_6H_5	CH_3	17.4	65
3e	$C_{14}H_{29}$	CH_3	19.4	88
4a	CH_3	$(CH_3)_2CH$	24.2	85
4b	CH_3CH_2	$(CH_3)_2CH$	20.8	86
4c	$(CH_3)_2CH$	$(CH_3)_2CH$	20.4	92
4d	C_6H_5	$(CH_3)_2CH$	17.4	49
4e	$C_{14}H_{29}$	$(CH_3)_2CH$	20.9	81
5a	CH_3	$C_{15}H_{31}$	24.1	85
5b	CH_3CH_2	$C_{15}H_{31}$	20.2	95
5c	$(CH_3)_2CH$	$C_{15}H_{31}$	18.5	93
5d	C_6H_5	$C_{15}H_{31}$–	16.2	37
5e	$C_{14}H_{29}$	$C_{15}H_{31}$–	20.6	69
6a	CH_3	C_6H_5–CH_2	20.8	90
6b	CH_3CH_2	C_6H_5–CH_2	19.7	95
6c	$(CH_3)_2CH$	C_6H_5–CH_2	18.0	55
6d	C_6H_5	C_6H_5–CH_2	15.9	90
6e	$C_{14}H_{29}$	C_6H_5–CH_2	15.4	63
7a	CH_3	C_6H_5	18.2	90
7b	CH_3CH_2	C_6H_5	16.9	85
7c	$(CH_3)_2CH$	C_6H_5	16.4	95
7d	C_6H_5	C_6H_5	11.6	78
7e	$C_{14}H_{29}$	C_6H_5	15.3	77
8a	CH_3	C_6H_4–Br	17.3	90
8b	CH_3CH_2	C_6H_4–Br	16.4	72
8c	$(CH_3)_2CH$	C_6H_4–Br	15.6	80
8d	C_6H_5	C_6H_4–Br	12.8	70
8e	$C_{14}H_{29}$	C_6H_4–Br	14.6	80
9a	CH_3	C_6H_4–OCH_3	16.3	92
9b	CH_3CH_2	C_6H_4–OCH_3	17.3	60
9c	$(CH_3)_2CH$	C_6H_4–OCH_3	16.6	77
9d	C_6H_5	C_6H_4–OCH_3	13.5	81
9e	$C_{14}H_{29}$	C_6H_4–OCH_3	15.4	69
10a	CH_3	C_5H_4N	14.8	75
10b	CH_3CH_2	C_5H_4N	14.8	90
10c	$(CH_3)_2CH$	C_5H_4N	13.0	90
10d	C_6H_5	C_5H_4N	18.3	73
10e	$C_{14}H_{29}$	C_5H_4N	12.5	50

NMR. After evaporation of the volatile fractions and methanolysis, crude products were purified by precipitation. Products presented a unique signal in $^{31}P\{^{1}H\}$ NMR as expected for symmetric bisphosphonates. Observed chemical shifts were slightly lower than what was described for corresponding 1-hydroxymethylene-1,1-bisphosphonic acids. This decrease in the $^{31}P\{^{1}H\}$ NMR chemical shift value was more pronounced in the alkyl family from methyl to isopropyl and even more pronounced for phenyl esters. All compounds also presented in $^{13}C\{^{1}H\}$ NMR a triplet for the carbon bearing the two phosphonate groups with a coupling constant of approximately 145–155 Hz. Yields were usually good whether substrates were aliphatic, aromatic or heteroaromatic. They were slightly higher varying from 60 to 95% for alkyl esters compared to phenyl esters. This fact could be explained by the relative thermal instability of such diphenyl bisphosphonates. When reacting phenyl bis(trimethylsilyl) phosphite **2d** with acid chlorides, addition must be carried out carefully to avoid thermal dealkylation of the diphenyl bisphosphonate formed. For example, when reacting phenyl bis(trimethylsilyl) phosphite with palmitoyl chloride, if the addition was not done in an ice bath, new signals appeared in $^{31}P\{^{1}H\}$ NMR. After methanolysis, together with the signal at 15.7 ppm for the symmetrical bisphosphonate diphenyl ester **5d**, we observed two doublets centred on 16.2 and 20.1 ppm corresponding to the bisphosphonate monophenyl ester. This compound which was not symmetric possessed two different phosphorus atoms which coupled together with a 42 Hz coupling constant.

3. Conclusion

We have described a convenient route to a new class of bisphosphonate partial ester derivatives. This procedure offers many possibilities for combining together different aromatic and aliphatic phosphonic ester groups and aromatic and aliphatic substituents on 1-hydroxymethylene-1,1-bisphosphonic side chain group. It allows access to an extremely varied library of bisphosphonate partial esters with potent biological applications. Recently, we have shown that these compounds have anti-angiogenic and anti-tumor effects in breast carcinoma models.[33]



Scheme 2.

4. Experimental

4.1. General methods

Unless otherwise noted, all solvents and reagents were high-purity-grade materials and used without further purification. THF was distilled from benzophenone sodium. Diethylether, benzene and hexane were distilled from sodium. Pyridine was distilled from potassium hydroxide. Hexamethyldisilazane was distilled prior use. Solid acyl chlorides were used directly and liquid acid chlorides were distilled under reduced pressure. Ditetradecyl phosphite was obtained from transesterification of diphenyl phosphite as described by G Le Bolc'h.[34]

Boiling points are given in Torr (Bp_{value}). NMR spectra were recorded with a VARIAN Unity Inova 500 MHz (^{13}C: 125.9 MHz, ^{1}H: 500.6 MHz, ^{31}P: 200.7 MHz) or a VARIAN Gemini 200 MHz (^{13}C: 50.3 MHz, ^{1}H: 200 MHz, ^{31}P: 80.9 MHz) spectrometer in D_2O, $CDCl_3$, or DMSO-d^6. Chemical shifts (δ) are given in ppm. ^{31}P and ^{13}C NMR spectra were recorded with phosphoric acid and methanol as external references, respectively. ^{1}H NMR spectra were recorded using HOD or trimethylsilane as internal standard in D_2O or $CDCl_3$. Attribution of aromatic carbons and protons is given in the text by adding *o* for ortho, *m* for meta and *p* for para.

Mass spectra were recorded in positive reflectron mode with DHB as a matrix on a MALDI-TOF-MS (Bruker). Microanalyses were performed by the Service Central d'Analyse, CNRS, F-69390, Vernaison, France.

4.2. General procedure for synthesis of ammonium alkyl phosphites 1a to 1e

In a 250 mL round bottom three neck flask, equipped with a thermometer and a condenser, 20 mL of concentrated ammonia solution (33%) were added carefully over 30 min, to dialkylplhosphite (**1**, 75 mmol). An exothermic reaction took place for **1d** and **1e** and the solution was therefore kept at room temperature using an ice bath. When the addition was completed, the mixture was set aside at room temperature for 2 h for **1d**, 6 h for **1a** and **1b**, 12 h for **1c** and 24 h for **1e**. Except for **1d**, which gave an emulsion the other compounds gave clear solutions. Then solutions were concentrated in vacuo. The resulting solid was dried by repeated co-evaporation with dry benzene (3×20 mL), and then dry pyridine (3×20 mL) and finally, precipitated in diethylether for **1d** and **1e**.

4.2.1. Ammonium methyl *H*-phosphonate (1a). Yield: 95%. Mp 108 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, D_2O) δ 9.2. ^{1}H NMR (200 MHz, D_2O) δ 3.39 (d, 3H, $^{3}J_{P-H}=12.4$ Hz, OCH_3). ^{13}C NMR $\{^{1}H\}$ (125.9 MHz, D_2O) δ 53.9 (OCH_3).

4.2.2. Ammonium ethyl *H*-phosphonate (1b). Yield: 87%. Mp 93 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, D_2O) δ 6.9. ^{1}H NMR (200 MHz, D_2O) δ 1.09 (t, 3H, $^{3}J_{H-H}=7$ Hz, OCH_2CH_3) 3.75 (dt, 2H, $^{3}J_{H-H}=7$ Hz; $^{3}J_{P-H}=7$ Hz, OCH_2CH_3). ^{13}C NMR $\{^{1}H\}$ (125.9 MHz, D_2O) δ 18.4 (OCH_2CH_3), 63.1 (OCH_2CH_3).

4.2.3. Ammonium isopropyl *H*-phosphonate (1c). Yield: 50%. Mp 132 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, D_2O) δ 4.9. ^{1}H NMR (200 MHz, D_2O) δ 1.08 (d, 3H, $^{3}J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 1.18 (d, 3H, $^{3}J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 4.15–4.43 (m, 1H, $OCH(CH_3)_2$). ^{13}C NMR $\{^{1}H\}$ (125.9 MHz, D_2O) δ 26.6 ($OCH(CH_3)_2$), 72.3 ($OCH(CH_3)_2$).

4.2.4. Ammonium phenyl *H*-phosphonate (1d). Yield: 95%. Mp 133 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, D_2O) δ 4.5. ^{1}H NMR (200 MHz, D_2O) δ 6.94–7.07 (m, 3H, C_6H_5), 7.20–7.23 (m, 2H, C_6H_5). ^{13}C NMR $\{^{1}H\}$ (125.9 MHz, D_2O) δ 124.1 (*o*-C_6H_5), 127.9 (*p*-C_6H_5), 133.2 (*m*-C_6H_5), 153.9 (OC_6H_5).

4.2.5. Ammonium tetradecyl *H*-phosphonate (1e). Yield: 85%. Mp 52 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, D_2O) δ 6.2. ^{1}H NMR (200 MHz, D_2O) δ 0.85 (t, 3H, $^{3}J_{H-H}=6.5$ Hz, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.23–1.27 (m, 22H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.30–1.33 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$), 4.10–4.11 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$). ^{13}C NMR $\{^{1}H\}$ (125.9 MHz, D_2O) δ 17.1 ($OCH_2CH_2(CH_2)_{11}CH_3$), 26.0 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 29.2, 33.2, 33.7 ($OCH_2CH_2(CH_2)_{10}$–$CH_2CH_3$), 35.5 ($OCH_2CH_2(CH_2)_{11}CH_3$), 67.5 ($OCH_2CH_2(CH_2)_{10}$–$CH_2CH_3$).

4.3. General procedure for synthesis of alkyl or aryl bis(trimethylsilyl) phosphite 2

In a 100 mL round-bottom three-neck flask equipped with a condenser and a thermometer, ammonium alkyl (or phenyl) phosphite **1** (20 mmol) was mixed, under nitrogen, with freshly distilled hexamethyldisilazane (95 mmol, 20 mL). Except in the case of synthesis of **2d** which was heated at 90 °C for 2 h, all the other products were obtained by refluxing the ammonium salts in hexamethyldisilazane, respectively, 6 h for **2a**, 8 h for **2b** and 24 h for **2c** and **2e**. For each compound, reaction evolution was monitored by ^{31}P $\{^{1}H\}$ NMR. Hexamethyldisilazane was then evaporated in vacuo (0.1 Torr). Tetradecylbis(trimethylsilyl) phosphite **2e** was used without further purification. All the other silyl phosphites **2a** to **2d** were distilled under vacuum.

4.3.1. Methyl bis(trimethylsilyl) phosphite (2a). Yield: 58%. $Bp_{0.1}$ 42 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, $CDCl_3$) δ 115.8. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.28 (s, 18H, $Si(CH_3)_3$), 3.4 (d, 3H, $^{3}J_{P-H}=12.1$ Hz, OCH_3).

4.3.2. Ethyl bis(trimethylsilyl) phosphite (2b). Yield: 55%. $Bp_{0.1}$ 50 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, $CDCl_3$) δ 116.1. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.21 (s, 18H, $Si(CH_3)_3$), 1.23 (t, 3H, $^{3}J_{H-H}=7$ Hz, OCH_2CH_3), 3.75–3.89 (m, 2H, OCH_2CH_3).

4.3.3. Isopropyl bis(trimethylsilyl) phosphite (2c). Yield: 60%. $Bp_{0.1}$ 54 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, $CDCl_3$) δ 118.2. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.20 (s, 18H, $Si(CH_3)_3$), 1.20 (d, 3H, $^{3}J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 1.34 (d, 3H, $^{3}J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 4.40–450 (m, 1H, $OCH(CH_3)_2$).

4.3.4. Phenyl bis(trimethylsilyl) phosphite (2d). Yield: 45%. $Bp_{0.1}$ 83 °C. ^{31}P NMR $\{^{1}H\}$ (80.9 MHz, $CDCl_3$) δ

121.5. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.22 (s, 18H, Si(CH_3)$_3$), 7.00–7.08 (m, 2H, C_6H_5), 7.24–7.31 (m, 3H, C_6H_5).

4.3.5. Tetradecyl bis(trimethylsilyl) phosphite (2e). Yield: 80%. ^{31}P NMR {^{1}H} (80.9 MHz, $CDCl_3$) δ 115.0. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.33 (s, 18H, Si(CH_3)$_3$), 0.88 (t, 3H, $^{3}J_{H-H}$=7 Hz, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.16–1.41 (m, 22H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.60–1.80 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.96–4.07 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$).

4.4. General procedure for synthesis of bisphosphonate dialkyl or diaryl esters 3-10

In a 50 mL round-bottom three-neck flask equipped with a thermometer, acid chloride (2.5 mmol) was added dropwise, under argon, at −5 °C, to dialkyl or diaryl phosphite (5 mmol). When addition was completed, reaction mixture was allowed to stand at room temperature for 2 h. The evolution of the reaction was monitored by ^{31}P {^{1}H} NMR. Then, volatile fractions were evaporated under reduced pressure (0.1 Torr) before being hydrolyzed with methanol. After evaporation, crude products were precipitated in an appropriate mixture of solvent.

4.4.1. [1-Hydroxy-1-(hydroxy-methoxy-phosphoryl)-ethyl]-phosphonic acid monomethyl ester (3a). Precipitation in diethylether. Yield: 90%. Mp 76 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 23.9. ^{1}H NMR (500 MHz, D_2O) δ 1.04 (t, 3H, $^{3}J_{P-H}$=16 Hz, CH_3–C(OH)), 3.14–3.22 (m, 6H, OCH_3). ^{13}C NMR {^{1}H} (50.3 MHz, D_2O) δ 17.7 (CH_3–C(OH)), 51.6 (OCH_3), 69.1 (t, $^{1}J_{P-C}$=152.6 Hz, P–C(OH)–P). Anal. Calcd for $C_4H_{12}O_7P_2$: C, 20.52; H, 5.17; P, 26.46; Found: C, 20.57; H, 5.19; P, 26.51.

4.4.2. [1-Hydroxy-1-(hydroxy-ethoxy-phosphoryl)-ethyl]-phosphonic acid monoethyl ester (3b). Precipitation in diethylether. Yield: 95%. Mp>260 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 19.9. ^{1}H NMR (500 MHz, D_2O) δ 1.26 (t, 6H, $^{3}J_{H-H}$=7 Hz, OCH_2CH_3), 1.52 (t, 3H, $^{3}J_{P-H}$=15 Hz, C(OH)CH_3), 3.98–4.10 (m, 4H, OCH_2CH_3). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 19.7 (OCH_2CH_3), 23.7 (CH_3C(OH)), 65.2 (s, OCH_2CH_3), 75.5 (t, $^{1}J_{P-C}$=146.0 Hz, P–C(OH)–P). Anal. Calcd for $C_6H_{16}O_7P_2$: C, 27.49; H, 6.15; P, 23.63; Found: C, 27.43; H, 6.13; P, 23.58.

4.4.3. [1-Hydroxy-1-(hydroxy-isoproxy-phosphoryl)-ethyl]-phosphonic acid monoisopropyl ester (3c). Precipitation in diethylether/hexane: 80/20. Yield: 90%. Mp 128 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 18.8. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (d, 6H, $^{3}J_{H-H}$=6.0 Hz, $OCH(CH_3)_2$), 1.33 (d, 6H, $^{3}J_{H-H}$=6.0 Hz, $OCH(CH_3)_2$), 1.62 (t, 3H, $^{3}J_{P-H}$=16.0 Hz, CH_3C(OH)), 4.77–4.86 (m, 2H, $OCH(CH_3)_2$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 18.4 (CH_3C(OH)), 23.9 ($OCH(CH_3)_2$), 24.3 ($OCH(CH_3)_2$), 71.4 (t, $^{1}J_{P-C}$=154.4 Hz, P–C(OH)–P), 73.1 ($OCH(CH_3)_2$). Anal. Calcd for $C_8H_{20}O_7P_2$: C, 33.11; H, 6.95; P, 21.35; Found: C, 33.15; H, 6.96; P, 21.44.

4.4.4. Disodium salt of [1-hydroxy-1-(hydroxy-phenoxy-phosphoryl)-ethyl]-phosphonic acid monophenyl ester (3d). Precipitation in diethylether/hexane: 95/5. Yield: 65%. Mp>260 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 17.4. ^{1}H NMR (500 MHz, D_2O) δ 1.78 (t, 3H, $^{3}J_{P-H}$=16.0 Hz, CH_3C(OH)), 7.19 (t, 1H, $^{3}J_{H-H}$=7 Hz, p-C_6H_5), 7.26–7.29 (m, 2H, o-C_6H_5), 7.28–7.41 (m, 2H, m-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 23.7 (CH_3C(OH)), 75.5 (t, $^{1}J_{P-C}$=149.2 Hz, P–C(OH)–P), 124.2 (o-C_6H_5), 127.1 (p-C_6H_5), 132.7 (m-C_6H_5), 155.2 (OC_6H_5). Anal. Calcd for $C_{14}H_{14}Na_2O_7P_2$: C, 41.81; H, 3.51; P, 15.40; Found: C, 41.88; H, 3.53; P, 15.47.

4.4.5. [1-Hydroxy-1-(hydroxy-tetradecyloxy-phosphoryl)-ethyl]-phosphonic acid monotetra decyl ester (3e). Precipitation in diethylether. Yield: 88%. Mp<50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 19.4. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 6H, $^{3}J_{H-H}$=6.5 Hz, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.19–1.33 (m, 44H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.30–1.37 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.63 (t, 3H, $^{3}J_{P-H}$=16 Hz, CH_3–COH), 4.08–4.14 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 ($OCH_2CH_2(CH_2)_{11}CH_3$), 22.9 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 25.9 (s, CH_3–COH), 29.9–30.7 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 32.2 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 67.8 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 71.6 (t, $^{1}J_{P-C}$=152.7 Hz, P–C(OH)–P). MS ($C_{30}H_{64}O_7P_2$): m/z 621.3 [M+Na+H]$^{+}$, 599.3 [M+H]$^{+}$. Anal. Calcd for $C_{30}H_{64}O_7P_2$: C, 60.18; H, 10.77; P, 10.35; Found: C, 60.13; H, 10.75; P, 10.31.

4.4.6. [1-Hydroxy-1-(hydroxy-methoxy-phosphoryl)-2-methyl-propyl]-phosphonic acid mono methyl ester (4a). Precipitation in diethylether. Yield: 85%. Mp 125 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 24.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (d, 3H, $^{3}J_{H-H}$=7 Hz, $(CH_3)_2CH$), 1.10 (d, 3H, $^{3}J_{H-H}$=7 Hz, $(CH_3)_2CH$), 2.16–2.28 (m, 1H, $(CH_3)_2CH$), 3.64–3.66 (m, 6H, OCH_3). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 17.1 ($(CH_3)_2CH$), 39.4 ($(CH_3)_2CH$), 57.4 (OCH_3), 78.1 (t, $^{1}J_{P-C}$=152.2 Hz, P–C(OH)–P). Anal. Calcd for $C_6H_{16}O_7P_2$: C, 27.49; H, 6.15; P, 23.63; Found: C, 27.43; H, 6.13; P, 23.60.

4.4.7. [1-Hydroxy-1-(hydroxy-ethoxy-phosphoryl)-2-methyl-propyl]-phosphonic acid mono ethyl ester (4b). Precipitation in diethylether. Yield: 86%. Mp 216 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 20.8. ^{1}H NMR (500 MHz, D_2O) δ 1.07 (d, 6H, $^{3}J_{H-H}$=7 Hz, $(CH_3)_2CH$), 1.19 (t, 6H, $^{3}J_{H-H}$=7 Hz, OCH_2CH_3), 2.14–2.26 (m, 1H, $(CH_3)_2CH$), 4.02–4.08 (m, 4H, OCH_2CH_3). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 19.1 (OCH_2CH_3), 20.8 ($(CH_3)_2CH$), 37.0 ($(CH_3)_2CH$), 66.6 (OCH_2CH_3), 80.6 (t, $^{1}J_{P-C}$=146.5 Hz, P–C(OH)–P). Anal. Calcd for $C_8H_{20}O_7P_2$: C, 33.11; H, 6.95; P, 21.35; Found: C, 33.07; H, 6.86; P, 21.28.

4.4.8. [1-Hydroxy-1-(hydroxy-isopropoxy-phosphoryl)-2-methyl-propyl]-phosphonic acid mono isopropyl ester (4c). Precipitation in diethylether.Yield: 92%. Mp 200 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 20.4. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.19 (d, 6H, $^{3}J_{H-H}$=6.8 Hz, $(CH_3)_2CHCOH$), 1.32 (d, 6H, $^{3}J_{H-H}$=6 Hz, $OCH(CH_3)_2$), 1.34 (d, 6H, $^{3}J_{H-H}$=6 Hz, $OCH(CH_3)_2$), 2.30–2.42 (m, 1H, $(CH_3)_2CHCOH$), 4.76–4.86 (m, 2H, $OCH(CH_3)_2$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 18.3 ($(CH_3)_2CHCOH$), 24.0 ($OCH(CH_3)_2$), 24.4 ($OCH(CH_3)_2$), 32.9 ($(CH_3)_2CHCOH$), 72.5 ($OCH(CH_3)_2$), 77.4 (t, $^{1}J_{P-C}$=147.1 Hz, P–C(OH)–P). Anal. Calcd for $C_{10}H_{24}O_7P_2$: C, 37.74; H, 7.60; P, 19.47; Found: C, 37.82; H, 7.61; P, 19.51.

4.4.9. Disodium salt of [1-hydroxy-1-(hydroxy-phenoxy-phosphoryl)-2-methyl-propyl]-phosphonic acid monophenyl ester (4d). Precipitation in water as diacid form. Yield: 49%. Mp 122 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 17.4. ^{1}H NMR (500 MHz, D_2O) δ 1.34 (d, 6H, $^{3}J_{H-H}$= 6.8 Hz, (CH_3)$_2$CH), 2.50–2.59 (m, 1H, (CH_3)$_2$C*H*), 7.20–7.26 (m, 6H, C_6H_5), 7.39 (t, 4H, $^{3}J_{H-H}$=7.5 Hz, *m*–C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 21.2 ((CH_3)$_2$CH), 37.5 ((CH_3)$_2$*C*H), 82.1 (t, $^{1}J_{P-C}$=143.9 Hz, P–*C*(OH)–P), 124.2 (*o*-C_6H_5), 127.8 (*p*-C_6H_5), 132.9 (*o*-C_6H_5), 153.9 (OC_6H_5). Anal. Calcd for $C_{16}H_{18}Na_2O_7P_2$: C 44.67; H, 4.22; P, 14.40; Found: C, 44.72; H, 4.23; P, 14.48.

4.4.10. [1-Hydroxy-1-(hydroxy-tetradecyloxy-phosphoryl)-ethyl]-phosphonic acid mono tetradecyl ester (4e). Yellow oil. Yield: 81%. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 20.9. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 6H, $^{3}J_{H-H}$=6.5 Hz, OCH$_2$CH$_2$(CH$_2$)$_{11}$C*H*$_3$), 1.13–1.27 (m, 50H, OCH$_2$CH$_2$(C*H*$_2$)$_{11}$CH$_3$; (CH_3)$_2$CH), 1.32–1.33 (m, 4H, OCH$_2$C*H*$_2$(CH$_2$)$_{11}$CH$_3$), 2.46–2.53 (m, 1H, (CH_3)$_2$C*H*), 3.96–4.10 (m, 4H, OC*H*$_2$CH$_2$(CH$_2$)$_{11}$CH$_3$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 (OCH$_2$CH$_2$(CH$_2$)$_{11}$*C*H$_3$), 18.3 ((CH_3)$_2$CH), 22.9 (OCH$_2$CH$_2$(CH$_2$)$_{10}$*C*H$_2$CH$_3$), 25.7–30.7 (OCH$_2$CH$_2$(*C*H$_2$)$_{10}$CH$_2$CH$_3$), 32.1 (OCH$_2$*C*H$_2$(CH$_2$)$_{10}$CH$_2$CH$_3$), 32.8 ((CH_3)$_2$*C*H), 67.6 (O*C*H$_2$CH$_2$(CH$_2$)$_{10}$CH$_2$CH$_3$), 71.8 (t, $^{1}J_{P-C}$=152.7 Hz, P–*C*(OH)–P). MS ($C_{32}H_{66}O_7P_2Na_2$): *m/z* 670.8 [M+H]$^{+}$. Anal. Calcd for $C_{32}H_{68}O_7P_2$: C, 61.32; H, 10.93; P, 9.88; Found: C, 61.39; H, 10.95; P, 9.96.

4.4.11. [1-Hydroxy-1-(hydroxy-methoxy-phosphoryl)-hexadecyl]-phosphonic acid mono methyl ester (5a). Precipitation in diethylether. Yield: 85%. Mp 64 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 24.1. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, $^{3}J_{H-H}$=7 Hz, C*H*$_3$(CH$_2$)$_{14}$COH), 1.23–1.26 (m, 24H, CH$_3$(C*H*$_2$)$_{12}$CH$_2$CH$_2$COH), 1.52–1.64 (m, 2H, CH$_3$(CH$_2$)$_{12}$C*H*$_2$CH$_2$COH), 1.96–1.99 (m, 2H, CH$_3$(CH$_2$)$_{12}$CH$_2$C*H*$_2$COH), 3.82–3.84 (m, 6H, OC*H*$_3$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$(CH$_2$)$_{15}$COH), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_{13}$COH), 23.4–30.5 (CH$_3$CH$_2$(*C*H$_2$)$_{11}$CH$_2$CH$_2$COH), 32.1 (CH$_3$CH$_2$(CH$_2$)$_{11}$*C*H$_2$CH$_2$COH), 33.4 (CH$_3$CH$_2$(CH$_2$)$_{11}$CH$_2$*C*H$_2$COH), 54.1 (*C*H$_3$O), 75.0 (t, $^{1}J_{P-C}$=151.0 Hz, P–*C*(OH)–P). Anal. Calcd for $C_{17}H_{37}O_7P_2$: C 49.15; H, 8.98; P, 14.91; Found: C, 49.07; H, 8.96; P, 14.82.

4.4.12. [1-Hydroxy-1-(hydroxy-ethoxy-phosphoryl)-hexadecyl]-phosphonic acid monoethyl ester (5b). Precipitation in diethylether. Yield: 95%. Mp 66 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 20.2. ^{1}H NMR (500 MHz, D_2O) δ 0.74 (t, 3H, $^{3}J_{H-H}$=7 Hz, C*H*$_3$(CH$_2$)$_{14}$COH), 1.15 (m, 30H, CH$_3$(C*H*$_2$)$_{12}$CH$_2$CH$_2$COH; OCH$_2$C*H*$_3$), 1.55–1.63 (m, 2H, CH$_3$(CH$_2$)$_{12}$C*H*$_2$CH$_2$COH), 1.93–2.07 (m, 2H, CH$_3$(CH$_2$)$_{12}$CH$_2$C*H*$_2$COH), 4.18–4.28 (m, 4H, OC*H*$_2$CH$_3$). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 17.1 (*C*H$_3$(CH$_2$)$_{14}$COH), 19.5 (OCH$_2$*C*H$_3$), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_{13}$COH), 25.9–33.4 (CH$_3$CH$_2$(*C*H$_2$)$_{11}$CH$_2$CH$_2$COH), 35.3 (CH$_3$CH$_2$(CH$_2$)$_{11}$*C*H$_2$CH$_2$COH), 37.6 (CH$_3$CH$_2$(CH$_2$)$_{11}$CH$_2$*C*H$_2$COH), 65.9 (O*C*H$_2$CH$_3$), 75.1 (t, $^{1}J_{P-C}$=149.2 Hz, P–*C*(OH)–P). Anal. Calcd for $C_{20}H_{24}O_7P_2$: C, 52.39; H, 9.67; P, 13.51; Found: C, 52.30; H, 9.65; P, 13.43.

4.4.13. [1-Hydroxy-1-(hydroxy-isopropoxy-phosphoryl)-hexadecyl]-phosphonic acid mono isopropyl ester (5c). Precipitation in diethylether. Yield: 93%. Mp<50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 18.5. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3H, $^{3}J_{H-H}$=7 Hz, C*H*$_3$(CH$_2$)$_{14}$COH), 1.23–1.34 (m, 36H, CH$_3$(C*H*$_2$)$_{12}$CH$_2$CH$_2$COH; OCH(C*H*$_3$)$_2$), 1.53–1.62 (m, 2H, CH$_3$(CH$_2$)$_{12}$C*H*$_2$CH$_2$COH), 1.99–2.12 (m, 2H, CH$_3$(CH$_2$)$_{12}$CH$_2$C*H*$_2$COH), 4.79–4.83 (m, 2H, OC*H*(CH$_3$)$_2$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$(CH$_2$)$_{14}$COH), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_{13}$COH), 24.0 ((CH_3)$_2$CH), 24.3 ((CH_3)$_2$CH), 29.8 ((CH_3)$_2$*C*H), 32.1 (CH$_3$CH$_2$(CH$_2$)$_{11}$*C*H$_2$CH$_2$COH), 33.4 (CH$_3$CH$_2$(CH$_2$)$_{11}$CH$_2$*C*H$_2$COH), 72.7 (O*C*H(CH$_3$)$_2$), 74.3 (t, $^{1}J_{P-C}$=151.2 Hz, P–*C*(OH)–P). Anal. Calcd for $C_{21}H_{45}O_7P_2$: C, 53.49; H, 9.62; P, 13.14; Found: C, 53.54; H, 9.64; P, 13.20.

4.4.14. [1-Hydroxy-1-(hydroxy-phenoxy-phosphoryl)-hexadecyl]-phosphonic acid mono phenylester (5d). Yellow oil. Yield: 37%. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 16.2. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 3H, $^{3}J_{H-H}$=7 Hz, C*H*$_3$(CH$_2$)$_{14}$COH), 1.15–1.29 (m, 24H, CH$_3$(C*H*$_2$)$_{12}$CH$_2$CH$_2$COH), 1.54–1.65 (m, 2H, CH$_3$ (CH$_2$)$_{12}$C*H*$_2$CH$_2$COH), 2.30 (t, 2H, $^{3}J_{H-H}$=8 Hz, CH$_3$ (CH$_2$)$_{12}$CH$_2$C*H*$_2$COH), 6.99–7.24 (m, 10H, C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$(CH$_2$)$_{14}$COH), 22.9 (CH$_3$*C*H$_2$(CH$_2$)$_{13}$COH), 24.9–30.1 (CH$_3$CH$_2$(*C*H$_2$)$_{11}$ CH$_2$CH$_2$COH), 32.2 (CH$_3$CH$_2$(CH$_2$)$_{11}$*C*H$_2$CH$_2$COH), 34.2 (CH$_3$CH$_2$(CH$_2$)$_{11}$CH$_2$*C*H$_2$COH), 75.0 (t, $^{1}J_{P-C}$=152.9 Hz, P–*C*(OH)–P), 121.0 (*o*-C_6H_5), 124.9 (*p*-C_6H_5), 129.7 (*m*-C_6H_5), 150.7 (OC_6H_5). Anal. Calcd for $C_{28}H_{44}O_7P_2$: C 60.64; H, 8.00; P, 11.17; Found: C, 60.59; H, 7.99; P, 11.12.

4.4.15. [1-Hydroxy-1-(hydroxy-tetradecyloxy-phosphoryl)-hexadecyl]-phosphonic acid mono tetradecyleste r (5e). Precipitation in hexane. Yield: 69%. Mp<50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 20.6. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 9H, $^{3}J_{H-H}$=7 Hz, O(CH$_2$)$_{13}$C*H*$_3$; C*H*$_3$(CH$_2$)$_{14}$COH), 1.23–1.30 (m, 70H, OCH$_2$CH$_2$(C*H*$_2$)$_{11}$CH$_3$; CH$_3$(C*H*$_2$)$_{13}$CH$_2$COH), 1.56–1.61 (m, 4H, OCH$_2$C*H*$_2$(C*H*$_2$)$_{11}$CH$_3$), 1.86–2.00 (m, 2H, CH$_3$ (CH$_2$)$_{13}$C*H*$_2$COH), 4.01–4.09 (m, 4H, OC*H*$_2$CH$_2$(CH$_2$)$_{11}$CH$_3$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$ (CH$_2$)$_{14}$COH; OCH$_2$CH$_2$(CH$_2$)$_{11}$*C*H$_3$), 22.9 (OCH$_2$(CH$_2$)$_{11}$*C*H$_2$CH$_3$; CH$_3$*C*H$_2$(CH$_2$)$_{13}$COH), 26.0–30.9 (OCH$_2$CH$_2$ (*C*H$_2$)$_{10}$CH$_2$CH$_3$; CH$_3$CH$_2$(*C*H$_2$)$_{11}$CH$_2$CH$_2$COH), 32.2 (CH$_3$CH$_2$(CH$_2$)$_{11}$*C*H$_2$CH$_2$COH; OCH$_2$*C*H$_2$(CH$_2$)$_{10}$CH$_2$CH$_3$), 34.2 (CH$_3$CH$_2$(CH$_2$)$_{11}$CH$_2$*C*H$_2$COH), 67.1 (O*C*H$_2$CH$_2$(CH$_2$)$_{10}$CH$_2$CH$_3$), 74.7 (t, $^{1}J_{P-C}$=148.3 Hz, P–*C*(OH)–P). MS ($C_{44}H_{90}O_7P_2Na_2$, pH=7.5): *m/z* 817.6 [M+Na+H]$^{+}$, 795.6 [M+H]$^{+}$. Anal. Calcd for $C_{44}H_{92}O_7P_2$: C, 66.46; H, 11.66; P, 7.79; Found: C, 66.53; H, 11.68; P, 7.84.

4.4.16. [1-Hydroxy-1-(hydroxy-methoxy-phosphoryl)-2-phenyl-ethyl]-phosphonic acid mono-methyl ester (6a). Precipitation in diethylether. Yield: 90%. Mp 130 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 20.8. ^{1}H NMR (500 MHz, D_2O) δ 3.16 (t, 2H, $^{3}J_{P-H}$=13.5 Hz, C_6H_5C*H*$_2$-COH), 3.44–3.49 (m, 6H, OC*H*$_3$), 7.14–7.20 (m, 3H, C_6H_5), 7.26 (d, 2H, $^{3}J_{H-H}$=6.5 Hz, C_6H_5). ^{13}C NMR {^{1}H} (50.3 MHz, DMSO-d_6) δ 37.2 (C_6H_5–*C*H$_2$–COH), 51.1 (O*C*H$_3$), 73.4 (t, $^{1}J_{P-C}$=145.4 Hz, P–*C*(OH)–P), 125.1 (*p*-C_6H_5–CH$_2$), 126.1 (*m*-C_6H_5–CH$_2$), 129.5 (*o*-C_6H_5–CH$_2$), 134.1 (C_6H_5–CH$_2$). Anal. Calcd for $C_{10}H_{16}O_7P_2$: C, 38.72; H, 5.20; P, 19.97; Found: C, 38.65; H, 5.19; P, 19.88.

4.4.17. [1-Hydroxy-1-(hydroxy-ethoxy-phosphoryl)-2-phenyl-ethyl]-phosphonic acid monoethyl ester (6b). Precipitation in diethylether. Yield: 95%. Mp 134 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 19.7. ^{1}H NMR (500 MHz, D_2O) δ 1.04 (t, 6H, $^{3}J_{H-H}=7$ Hz, OCH_2CH_3), 3.17 (t, 2H, $^{3}J_{P-H}=13.3$ Hz, $C_6H_5CH_2COH$), 3.84–3.91 (m, 4H, OCH_2CH_3), 7.16–7.21 (m, 3H, C_6H_5), 7.26 (d, 2H, $^{3}J_{H-H}=6.5$ Hz, *o*-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 19.2 (OCH_2CH_3), 42.0 (C_6H_5–CH_2–COH), 66.4 (OCH_2CH_3), 74.4 (t, $^{1}J_{P-C}=143.8$ Hz, P–*C*(OH)–P), 130.2 (*p*-C_6H_5–CH_2), 131.2 (*m*-C_6H_5–CH_2), 134.5 (*o*-C_6H_5–CH_2), 138.8 (C_6H_5–CH_2). Anal. Calcd for $C_{12}H_{20}O_7P_2$: C, 42.61; H, 5.96; P, 18.32; Found: C, 42.70; H, 5.97; P, 18.39.

4.4.18. [1-Hydroxy-1-(hydroxy-isopropoxy-phosphoryl)-2-phenyl-ethyl]-phosphonic acid mono isopropyl ester (6c). Precipitation in diethylether. Yield: 55%. Mp 130 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 18.0. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (d, $^{3}J_{H-H}=6.5$ Hz, 6H, ($OCH(CH_3)_2$), 1.22 (d, 6H, $^{3}J_{H-H}=6.5$ Hz, ($OCH(CH_3)_2$), 3.46 (t, 2H, $^{3}J_{P-H}=13.5$ Hz, $C_6H_5CH_2COH$), 4.72–4.81 (m, 2H, $OCH(CH_3)_2$), 7.26–7.29 (m, 3H, C_6H_5), 7.36 (d, 2H, $^{3}J_{H-H}=6.5$ Hz, *o*-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 26.9 ($OCH(CH_3)_2$), 27.2 ($OCH(CH_3)_2$), 42.4 (C_6H_5–CH_2–COH), 72.7 ($OCH(CH_3)_2$), 78.9 (t, $^{1}J_{P-C}=$ 140.8 Hz, P–*C*(OH)–P), 129.6 (*p*-C_6H_5–CH_2), 130.9 (*m*-C_6H_5–CH_2), 134.9 (*o*-C_6H_5–CH_2), 140.7 (C_6H_5–CH_2). Anal. Calcd for $C_{14}H_{24}O_7P_2$: C, 45.91; H, 6.60; P, 16.91; Found: C, 45.82; H, 6.58; P, 16.83.

4.4.19. Disodium salt of [1-hydroxy-1-(hydroxy-phenoxy-phosphoryl)-2-phenyl-ethyl]-phosphonic acid monophenyl ester (6d). Yellow oil in the acidic form. Yield: 90%. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 15.9. ^{1}H NMR (500 MHz, D_2O) δ 3.56 (t, 2H, $^{3}J_{P-H}=13.0$ Hz, $C_6H_5CH_2COH$), 6.99–7.55 (m, 15H, C_6H_5). ^{13}C NMR {^{1}H} (50.3 MHz, D_2O) δ 38.2 (C_6H_5–CH_2–COH), 74.6 (t, $^{1}J_{P-C}=146.1$ Hz, P–*C*(OH)–P), 119.8 (*o*–C_6H_5), 125.2 (*p*–C_6H_5), 126.5 (*m*-C_6H_5), 128.2 (*p*-C_6H_5–CH_2), 130.4 (*m*-C_6H_5–CH_2), 135.7 (*o*-C_6H_5–CH_2), 137.6 (C_6H_5–CH_2), 150.7 (C_6H_5O). Anal. Calcd for $C_{20}H_{18}Na_2O_7P_2$: C, 50.27; H, 3.79; P, 12.95; Found: C, 50.35; H, 3.80; P, 12.99.

4.4.20. [1-Hydroxy-1-(hydroxy-tetradecyloxy-phosphoryl)-2-phenyl-ethyl]-phosphonic acid mono tetradecyl ester (6e). Precipitation in diethylether. Yield: 63%. Mp <50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 15.4. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 6H, $^{3}J_{H-H}=6.5$ Hz, $O(CH_2)_{13}CH_3$), 1.15–1.32 (m, 44H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.39–1.47 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.39 (t, 2H, $^{3}J_{P-H}=13.5$ Hz, $C_6H_5CH_2COH$), 3.84–4.01 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 7.20–7.25 (m, 3H, C_6H_5), 7.37 (d, 2H, $^{3}J_{H-H}=6.5$ Hz, *o*-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 ($OCH_2CH_2(CH_2)_{11}CH_3$), 22.9 ($OCH_2(CH_2)_{11}CH_2CH_3$), 29.9–30.4 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$) 30.4 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 41.0 ($C_6H_5$–$CH_2$–COH), 67.8 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 76.8 (t, $^{1}J_{P-C}=$ 145.6 Hz, P–*C*(OH)–P), 123.5 (*p*-C_6H_5–CH_2), 133.9 (*m*-C_6H_5–CH_2), 136.2 (*o*-C_6H_5–CH_2), 137.7 (C_6H_5–CH_2). MS ($C_{36}H_{66}O_7P_2Na_2$, pH=7.5): *m/z* 697.5 $[M+Na+H]^+$, 675.4 $[M+H]^+$. Anal. Calcd for $C_{36}H_{68}O_7P_2$: C, 64.07; H, 10.16; P, 9.18; Found: C, 64.08; H, 10.15; P, 9.10.

4.4.21. [1-Hydroxy-(1-hydroxy-methoxy-phosphoryl)-2-phenyl-methyl]-phosphonic acid monomethyl ester (7a). Precipitation in diethylether. Yield: 90%. Mp 195 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 18.2. ^{1}H NMR (500 MHz, D_2O) δ 3.49–3.52 (m, 6H, OCH_3), 7.24–7.29 (m, 1H, *p*-C_6H_5), 7.33 (t, 2H, $^{3}J_{H-H}=7$ Hz, *m*-C_6H_5), 7.63 (d, 2H, $^{3}J_{H-H}=7$ Hz, *o*-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 56.9 (OCH_3), 79.6 (t, $^{1}J_{P-C}=148.4$ Hz, P–*C*(OH)–P), 129.1 (*o*-C_6H_5), 131.1 (*p*-C_6H_5), 131.4 (*m*-C_6H_5), 138.5 ($C_6H_5C(OH)$). Anal. Calcd for $C_9H_{14}O_7P_2$: C, 36.50; H, 4.76; P, 20.92; Found: C, 36.43; H, 4.74; P, 20.90.

4.4.22. [1-Hydroxy-1-(hydroxy-ethoxy-phosphoryl)-2-phenyl-methyl]-phosphonic acid monoethyl ester (7b). Precipitation in diethylether. Yield: 85%. Mp 168 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 16.9. ^{1}H NMR (500 MHz, D_2O) δ 1.14 (t, 6H, $^{3}J_{H-H}=6.5$ Hz, OCH_2CH_3) 3.89–3.98 (m, 4H, OCH_2CH_3), 7.33–7.38 (m, 1H, *p*-C_6H_5), 7.41 (t, 2H, $^{3}J_{H-H}=7.0$ Hz, *m*-C_6H_5), 7.73 (d, 2H, $^{3}J_{H-H}=7.0$ Hz, *o*-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 19.1 (OCH_2CH_3), 67.2 (OCH_2CH_3), 79.5 (t, $^{1}J_{P-C}=148.9$ Hz, P–*C*(OH)–P), 129.2 (*o*-C_6H_5), 131.1 (*p*-C_6H_5), 131.4 (*m*-C_6H_5), 138.6 ($C_6H_5C(OH)$). Anal. Calcd for $C_{11}H_{18}O_7P_2$: C, 40.75; H, 5.60; P, 19.11; Found: C, 40.84; H, 5.62; P, 19.20.

4.4.23. [1-Hydroxy-(1-hydroxy-isopropoxy-phosphoryl)-2-phenyl-methyl]-phosphonic acid mono isopropyl ester (7c). Precipitation in diethylether. Yield: 95%. Mp 174 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 16.4. ^{1}H NMR (500 MHz, D_2O) δ 1.09 (d, 6H, $^{3}J_{H-H}=6.5$ Hz, ($OCH(CH_3)_2$), 1.20 (d, 6H, $^{3}J_{H-H}=6.5$ Hz, $OCH(CH_3)_2$), 4.45–4.53 (m, 2H, ($OCH(CH_3)_2$), 7.35–7.40 (m, 1H, *p*-C_6H_5), 7.43 (t, 2H, $^{3}J_{H-H}=7.0$ Hz, *m*-C_6H_5), 7.76 (d, 2H, $^{3}J_{H-H}=7.0$ Hz, *o*-C_6H_5). ^{13}C NMR{^{1}H} (125.9 MHz, D_2O) δ 26.2 ($OCH(CH_3)_2$), 26.6 ($OCH(CH_3)_2$), 76.3 ($OCH(CH_3)_2$), 79.3 (t, $^{1}J_{P-C}=143.1$ Hz, P–*C*(OH)–P), 129.4 (*o*-C_6H_5), 131.0 (*p*-C_6H_5), 131.3 (*m*-C_6H_5), 138.6 ($C_6H_5C(OH)$). Anal. Calcd for $C_{13}H_{22}O_7P_2$: C, 44.33; H, 6.30; P, 17.59; Found: C, 44.25; H, 6.29; P, 17.48.

4.4.24. Disodium salt of [1-hydroxy-(1-hydroxy-phenoxy-phosphoryl)-2-phenyl-methyl]-phosphonic acid monophenyl ester (7d). Precipitation in diethylether. Yield: 78%. Mp <50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 11.6. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.75–7.89 (m, 15H, C_6H_5). ^{13}C NMR {^{1}H} (50.3 MHz, D_2O) δ 76.9 (t, $^{1}J_{P-C}=143.1$ Hz, P–*C*(OH)–P), 119.9–128.6 (C_6H_5; $C_6H_5C(OH)$), 136.8 ($C_6H_5C(OH)$), 150.7 (C_6H_5O). Anal. Calcd for $C_{19}H_{16}Na_2O_7P_2$: C, 49.15; H, 3.47; P, 13.34; Found: C, 49.22; H, 3.48; P, 13.39.

4.4.25. [1-Hydroxy-(1-hydroxy-tetradecyloxy-phosphoryl)-2-phenyl-methyl]-phosphonic acid mono tetradecyl ester (7e). Precipitation in diethylether. Yield: 77%. Mp <50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 15.3. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 6H, $^{3}J_{H-H}=6.5$ Hz, $O(CH_2)_{13}CH_3$), 1.04–1.29 (m, 44H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.30–1.36 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.59–3.69 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.80–3.88 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$), 7.23–7.29 (m, 1H, *p*-C_6H_5), 7.33 (t, 2H, $^{3}J_{H-H}=8$ Hz, *m*-C_6H_5), 7.82 (d, 2H, $^{3}J_{H-H}=8$ Hz, *o*-C_6H_5). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.2

($OCH_2CH_2(CH_2)_{11}CH_3$), 22.8 ($OCH_2(CH_2)_{11}CH_2CH_3$), 29.2–30.3 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 30.3 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 32.1 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 76.5 (t, $^1J_{P-C}=153.7$ Hz, P–*C*(OH)–P), 126.2 (*o*-C_6H_5), 127.8 (*p*-C_6H_5), 128.0 (*m*-C_6H_5), 133.6 ($C_6H_5C(OH)$). MS ($C_{35}H_{64}O_7P_2Na_2$, pH =7.5): *m/z* 684.4 $[M+Na+H]^+$, 661.4 $[M+H]^+$. Anal. Calcd for $C_{35}H_{66}O_7P_2$: C, 63.61; H, 10.07; P, 9.37; Found: C, 63.70; H, 10.09; P, 9.45.

4.4.26. (4-Bromo-phenyl)-hydroxy-(hydroxy-methoxy-phosphoryl)-methyl]-phosphonic acid monomethyl ester (8a). Precipitation in diethylether. Yield: 90%. Mp 165 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 17.3. ^{1}H NMR (500 MHz, D_2O) δ 3.79 (d, 3H, $^3J_{P-H}=3.0$ Hz, OCH_3), 3.81 (d, 3H, $^3J_{P-H}=3.0$ Hz, OCH_3), 7.59 (d, 2H, $^3J_{H-H}=8.0$ Hz, *m*-C_6H_4), 7.66 (d, 2H, $^3J_{H-H}=8.0$ Hz, *o*-C_6H_4). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 56.8 (OCH_3), 74.6 (t, $^1J_{P-C}=$ 146.2 Hz, P–*C*(OH)–P), 124.4 (*p*-C_6H_4), 131.1 (*m*-C_6H_4), 134.3 (*o*-C_6H_4), 138.9 ($C_6H_4C(OH)$). Anal. Calcd for $C_9H_{13}BrO_7P_2$: C, 28.82; H, 3.49; P, 16.52; Found: C, 28.92; H, 3.50; P, 16.57.

4.4.27. (4-Bromo-phenyl)-hydroxy-(hydroxy-ethoxy-phosphoryl)-methyl]-phosphonic acid monoethyl ester (8b). Precipitation in diethylether.Yield: 72%. Mp 199 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 16.4. ^{1}H NMR (500 MHz, D_2O) δ 1.16 (t, 6H, $^3J_{H-H}=6.5$ Hz, OCH_2CH_3), 3.89–3.98 (m, 4H, OCH_2CH_3), 7.60 (d, 2H, $^3J_{H-H}=8.5$ Hz, *m*-C_6H_4), 7.68 (d, 2H, $^3J_{H-H}=8.5$ Hz, *o*-C_6H_4). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 19.2 (OCH_2CH_3), 66.9 (OCH_2CH_3), 79.1 (t, $^1J_{P-C}=138.2$ Hz, P–*C*(OH)–P), 119.5 (*p*-C_6H_4), 131.2 (*m*-C_6H_4), 134.2 (*o*-C_6H_4), 138.9 ($C_6H_4C(OH)$). Anal. Calcd for $C_{11}H_{17}BrO_7P_2$: C, 32.78; H, 4.25; P, 15.37; Found: C, 32.71; H, 4.24; P, 15.30.

4.4.28. (4-Bromo-phenyl)-hydroxy-(hydroxy-isopropoxy-phosphoryl)-methyl]-phosphonic acid monoisopropyl ester (8c). Precipitation in diethylether. Yield: 80%. Mp 198 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 15.6. ^{1}H NMR (500 MHz, D_2O) δ 1.09 (d, 3H, $^3J_{H-H}=6$ Hz, $OCH(CH_3)_2$), 1.20 (d, 6H, $^3J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 4.41–4.50 (m, 2H, $OCH(CH_3)_2$), 7.61 (d, 2H, $^3J_{H-H}=8.5$ Hz, *m*-C_6H_4), 7.69 (d, 2H, $^3J_{H-H}=8.5$ Hz, *o*-C_6H_4). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 26.5 ($OCH(CH_3)_2$), 27.0 ($OCH(CH_3)_2$), 74.0 ($OCH(CH_3)_2$), 76.8 (t, $^1J_{P-C}=$ 143.5 Hz, P–*C*(OH)–P), 123.1 (*p*-C_6H_4), 131.4 (*m*-C_6H_4), 133.6 (*o*-C_6H_4), 140.9 ($C_6H_4C(OH)$). Anal. Calcd for $C_{13}H_{21}BrO_7P_2$: C, 36.21; H, 4.91; P, 14.37; Found: C, 36.28; H, 4.93; P, 14.42.

4.4.29. (4-Bromo-phenyl)-hydroxy-(hydroxy-phenoxy-phosphoryl)-methyl]-phosphonic acid monophenyl ester (8d). Precipitation in diethylether.Yield: 70%. Mp 92 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 12.8. ^{1}H NMR (500 MHz, D_2O) δ 6.93–7.80 (m, 14H, C_6H_4; C_6H_5). ^{13}C NMR (50.3 MHz, DMSO-d_6) δ 77.1 (t, $^1J_{P-C}=145.4$ Hz, P–*C*(OH)–P), 116.1 (C_6H_5), 121.5 (C_6H_5), 124.6 (*p*-C_6H_4), 129.9 (C_6H_5), 130.7 (*m*-C_6H_4), 130.8 (*o*-C_6H_4), 137.5 ($C_6H_4C(OH)$), 152.3 (O–C_6H_5). Anal. Calcd for $C_{19}H_{17}BrO_7P_2$: C, 45.72; H, 3.43; P, 12.41; Found: C, 45.62; H, 3.42; P, 12.36.

4.4.30. (4-Bromo-phenyl)-hydroxy-(hydroxy-tetradecyloxy-phosphoryl)-methyl]-phosphonic acid monotetradecyl ester (8e). Precipitation in diethylether/hexane: 50/50. Yield: 80%. Mp 54 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 14.6. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 6H, $^3J_{H-H}=7.0$ Hz, $O(CH_2)_{13}CH_3$), 1.15–1.31 (m, 44H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.33–1.39 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.72–3.80 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.86–3.94 (m, 2H, $OCH_2CH_2(CH_2)_{11}CH_3$), 7.47 (d, 2H, $^3J_{H-H}=$ 8.0 Hz, *m*-C_6H_4), 7.70 (d, 2H, $^3J_{H-H}=8.0$ Hz, *o*-C_6H_4). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 ($O(CH_2)_{13}CH_3$), 22.9 ($O(CH_2)_{12}CH_2CH_3$), 25.3–30.4 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 32.1 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 69.0 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 76.2 (t, $^1J_{P-C}=148.6$ Hz, P–*C*(OH)–P), 122.4 (*p*-C_6H_4), 127.9 (*m*-C_6H_4), 131.4 (*o*-C_6H_4), 132.6 ($C_6H_4C(OH)$). MS ($C_{35}H_{63}O_7P_2Na_2$, pH=7.5): *m/z* 763.3 $[M+Na+2H]^+$739.3 $[M+H]^+$. Anal. Calcd for $C_{35}H_{65}BrO_7P_2$: C, 56.83; H, 8.86; P, 8.37; Found: C, 56.89; H, 8.88; P, 8.43.

4.4.31. [Hydroxy-(hydroxy-methoxy-phosphoryl)-(4-methoxy-phenyl)-methyl]-phosphonic acid monomethyl ester (9a). Precipitation in diethylether. Yield: 92%. Mp 225 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 16.3. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.38–3.60 (m, 6H, OCH_3), 3.80 (s, 3H, $C_6H_4OCH_3$), 6.86 (d, 2H, $^3J_{H-H}=8.0$ Hz, *m*-$C_6H_4OCH_3$), 7.5 (d, 2H, $^3J_{H-H}=8.0$ Hz, *o*-$C_6H_4OCH_3$). ^{13}C NMR {^{1}H} (50.3 MHz, DMSO-d_6) δ 53.5 (OCH_3), 55.0 ($C_6H_4OCH_3$), 75.8 (t, $^1J_{P-C}=145.8$ Hz, P–*C*(OH)–P), 112.5 (*m*-C_6H_4), 127.8 (*o*-C_6H_4), 128.9 ($C_6H_4C(OH)$), 158.1 (*p*-C_6H_4). Anal.Calcd for $C_{10}H_{16}O_8P_2$: C, 36.82; H, 4.94; P, 18.99; Found: C, 36.76; H, 4.93; P, 18.92.

4.4.32. [Hydroxy-(hydroxy-ethoxy-phosphoryl)-(4-methoxy-phenyl)-methyl]-phosphonic acid monoethyl ester (9b). Precipitation in diethylether. Yield: 60%. Mp 172 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 17.3. ^{1}H NMR (500 MHz, D_2O) δ 1.11 (t, 6H, $^3J_{H-H}=6.5$ Hz, OCH_2CH_3), 3.78 (s, 3H, $C_6H_4OCH_3$), 3.86–3.95 (m, 4H, OCH_2CH_3), 6.98 (d, 2H, $^3J_{H-H}=9.0$ Hz, *m*-$C_6H_4OCH_3$), 7.64 (d, 2H, $^3J_{H-H}=$ 9.0 Hz, *o*-$C_6H_4OCH_3$). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 19.1 (OCH_2CH_3), 58.6 ($C_6H_4OCH_3$), 67.3 (OCH_2CH_3), 79.0 (t, $^1J_{P-C}=145.9$ Hz, P–*C*(OH)–P), 116.8 (*m*-C_6H_4), 130.7 (*o*-C_6H_4), 130.8 ($C_6H_4C(OH)$), 161.7 (*p*-C_6H_4). Anal. Calcd for $C_{12}H_{20}O_8P_2$: C, 40.69; H, 5.69; P, 17.49; Found: C, 40.75; H, 5.71; P, 17.58.

4.4.33. [Hydroxy-(hydroxy-isopropoxy-phosphoryl)-(4-methoxy-phenyl)-methyl]-phosphonic acid monoisopropyl ester (9c). Precipitation in diethylether. Yield: 77%. Mp 187 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 16.6. ^{1}H NMR (500 MHz, D_2O) δ 1.09 (d, 6H, $^3J_{H-H}=6$ Hz, ($OCH(CH_3)_2$), 1.21 (d, 6H, $^3J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 3.85 (s, 3H, $C_6H_4OCH_3$), 4.45–4.49 (m, 2H, $OCH(CH_3)_2$), 7.04 (d, 2H, $^3J_{H-H}=9.0$ Hz, *m*-$C_6H_4OCH_3$), 7.69 (d, 2H, $^3J_{H-H}=9.0$ Hz, *o*-$C_6H_4OCH_3$). ^{13}C NMR {^{1}H} (125.9 MHz, DMSO-d_6) δ 26.2 ($OCH(CH_3)_2$), 26.6 ($OCH(CH_3)_2$), 58.6 ($C_6H_4OCH_3$), 76.2 ($OCH(CH_3)_2$), 76.6 (t, $^1J_{P-C}=144.6$ Hz, P–*C*(OH)–P), 116.7 (*m*-C_6H_4), 130.9 (*o*-C_6H_4), 131.0 ($C_6H_4C(OH)$), 161.7 (*p*-C_6H_4). Anal. Calcd for $C_{14}H_{24}O_8P_2$: C, 43.99; H, 6.33; P, 16.20; Found: C, 44.08; H, 6.34; P, 16.25.

4.4.34. Disodium salt of [hydroxy-(hydroxy-phenoxy-phosphoryl)-(4-methoxy-phenyl)-methyl]-phosphonic acid monophenyl ester (9d). Precipitation in diethylether/hexane: 80/20 in the acid form. Yield: 81%. Mp 198 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 13.5. ^{1}H NMR (500 MHz, D_2O) δ 3.87 (s, 3H, $C_6H_4OCH_3$), 6.92–7.82 (m, 14H, $C_6H_4OCH_3$; C_6H_5). ^{13}C NMR {^{1}H} (50.3 MHz, D_2O) δ 55.7 ($C_6H_4OCH_3$), 77.9 (t, $^{1}J_{P-C}=144.5$ Hz, P–*C*(OH)–P), 113.4, 119.0, 121.2, 123.8, 127.8, 129.4, 132.1 (C_6H_5; C_6H_4), 152.2 (OC_6H_5), 157.8 (*p*-C_6H_4). Anal. Calcd for $C_{20}H_{18}Na_2O_8P_2$: C, 48.60; H, 3.67; P, 12.53; Found: C 48,.51; H, 3.65; P, 12.49.

4.4.35. [Hydroxy-(hydroxy-tetradecyloxy-phosphoryl)-(4-methoxy-phenyl)-methyl]-phosphonic acid monotetradecyl ester (9e). Precipitation in diethylether/hexane: 80/20. Yield: 69%. Mp <50 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 15.4. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 6H, $^{3}J_{H-H}=6.5$ Hz, $O(CH_2)_{13}CH_3$), 1.02–1.29 (m, 44H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.32–1.35 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.78 (s, 3H, $C_6H_4OCH_3$), 3.82–3.86 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 6.87 (d, 2H, $^{3}J_{H-H}=9.0$ Hz, *m*-$C_6H_4OCH_3$), 7.73 (d, 2H, $^{3}J_{H-H}=9.0$ Hz, *p*-$C_6H_4OCH_3$). ^{13}C NMR {^{1}H} (125.9 MHz, $CDCl_3$) δ 14.3 ($O(CH_2)_{13}CH_3$), 22.9 ($O(CH_2)_{12}CH_2CH_3$), 29.5–30.3 ($OCH_2CH_2(CH_2)_{10}CH_2CH_3$), 32.8 ($OCH_2CH_2(CH_2)_{11}CH_3$), 55.4 ($C_6H_4OCH_3$), 69.0 ($OCH_2CH_2(CH_2)_{11}CH_3$), 76.2 (t, $^{1}J_{P-C}=145.9$ Hz, P–*C*(OH)–P), 113.6 (*m*-C_6H_4), 127.5 (*p*-C_6H_4), 132.8 ($C_6H_4C(OH)$), 159.5 (*p*-C_6H_4). MS ($C_{35}H_{63}O_7P_2Na_2$, pH=7.5) *m/z* 713.4 $[M+Na+H]^+$, 691.3 $[M+H]^+$. Anal. Calcd for $C_{36}H_{68}O_8P_2$: C, 64.07; H, 10.16; P, 9.18; Found: C, 65.10; H, 10.18; P, 9.24.

4.4.36. Sodium salt of [hydroxy-(hydroxy-methoxy-phosphoryl)-pyridin-3-yl-methyl]-phosphonic acid monomethyl ester (10a). Precipitation in diethylether in the acidic form. Yield: 75%. Mp 220 °C. ^{13}P NMR {^{1}H} (200.7 MHz, D_2O) δ 14.8. ^{1}H NMR (500 MHz, D_2O) δ 3.48 (d, 6H, $^{3}J_{P-H}=9.0$ Hz, OCH_3), 7.82–7.94 (m, 1H, *H5*–C_5H_4N), 8.47–8.55 (m, 1H, *H4*–C_5H_4N), 8.70–8.79 (m, 1H, *H6*–C_5H_4N), 8.86 (s, 1H, *H2*–C_5H_4N). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 54.9 (OCH_3), 78.9 (t, $^{1}J_{P-C}=142.1$ Hz, P–*C*(OH)–P), 129.6 (*C5*–C_5H_4N), 141.9 (*C3*–C_5H_4N), 142.5 (*C4*–C_5H_4N), 143.4 (*C6*–C_5H_4N), 147.4 (*C2*–C_5H_4N). Anal. Calcd for $C_8H_{12}NNaO_7P_2$: C, 30.11; H, 3.79; N, 4.39; P, 19.41; Found: C, 30.08; H, 3.78; N, 4.38; P, 19.35.

4.4.37. Sodium salt of [hydroxy-(hydroxy-ethoxy-phosphoryl)-pyridin-3-yl-methyl]-phosphonic acid monoethyl ester (10b). Precipitation in diethylether in the acid form. Yield: 90%. Mp >260 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 14.8. ^{1}H NMR (200 MHz, D_2O) δ 0.96 (t, 6H, $^{3}J_{H-H}=7.2$ Hz, OCH_2CH_3), 3.68–3.75 (m, 4H, OCH_2CH_3), 7.31–7.42 (m, 1H, *H5*–C_5H_4N), 8.06–8.20 (m, 1H, *H4*–C_5H_4N), 8.26–8.35 (m, 1H, *H6*–C_5H_4N), 8.72 (s, 1H, *H2*–C_5H_4N). ^{13}C NMR {^{1}H} (50.3MHz, D_2O) δ 14.3 (OCH_2CH_3), 60.8 (OCH_2CH_3), 74.8 (t, $^{1}J_{P-C}=140.1$ Hz, P–*C*(OH)–P), 121.8 (*C5*–C_5H_4N), 134.7 (*C3*–C_5H_4N), 143.3 (*C4*–C_5H_4N), 143.5 (*C6*–C_5H_4N), 147.2 (*C2*–C_5H_4N). Anal. Calcd for $C_{10}H_{16}NNaO_7P_2$: C, 34.60; H, 4.65; N, 4.05; P, 17.84; Found: C, 34.54; H, 4.64, N, 4.04; P, 17.80.

4.4.38. Sodium salt of [hydroxy-(hydroxy-isopropoxy-phosphoryl)-pyridin-3-yl-methyl]-phosphonic acid monoisopropyl ester (10c). Precipitation in diethylether in the acidic form. Yield: 90%. Mp 197 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 13.0. ^{1}H NMR (500 MHz, D_2O) δ 1.15 (d, 6H, $^{3}J_{H-H}=6$ Hz, $OCH(CH_3)_2$), 1.20 (d, 6H, $^{3}J_{H-H}=7$ Hz, $OCH(CH_3)_2$), 4.16–4.56 (m, 2H, $OCH(CH_3)_2$), 8.07 (dd, 1H, $^{3}J_{H-H}=5.0$, 8.0 Hz, *H5*–C_5H_4N), 8.72 (d, 1H, $^{3}J_{H-H}=5.0$ Hz, *H4*–C_5H_4N), 8.94 (d, 1H, $^{3}J_{H-H}=8.0$ Hz, *H6*–C_5H_4N), 9.05 (s, 1H, *H2*–C_5H_4N). ^{13} C NMR {^{1}H} (125.9 MHz, D_2O) δ 26.6 ($OCH(CH_3)_2$), 75.3 ($OCH(CH_3)_2$), 78.6 (t, $^{1}J_{P-C}=139.9$ Hz, P–*C*(OH)–P), 129.5 (*C5*–C_5H_4N), 141.9 (*C3*–C_5H_4N), 142.4 (*C4*–C_5H_4N), 143.5 (*C6*–C_5H_4N), 147.6 (*C2*–C_5H_4N). Anal. Calcd for $C_{12}H_{20}NNaO_7P_2$: C, 38.41; H, 5.37; N, 3.73; P, 16.51; Found: C, 38.55; H, 5.38; N, 3.74; P, 16.57.

4.4.39. Sodium salt of [hydroxy-(hydroxy-phenoxy-phosphoryl)-pyridin-3-yl-methyl]-phosphonic acid monophenyl ester (10d). Precipitation in methanol in the acid form. Yield: 73%. Mp 134 °C. ^{31}P NMR {^{1}H} (200.7 MHz, D_2O) δ 18.3. ^{1}H NMR (500 MHz, D_2O) δ 6.96–7.34 (m, 10H, C_6H_5), 7.74–7.95 (m, 1H, *H5*–C_5H_4N), 8.55–8.82 (m, 2H, *H4*–C_5H_4N; *H6*–C_5H_4N), 9.04 (s, 1H, *H2*–C_5H_4N). ^{13}C NMR {^{1}H} (125.9 MHz, D_2O) δ 77.6 (t, $^{1}J_{P-C}=140.4$ Hz, P–*C*(OH)–P), 117.7 (C_6H_5), 122.9 (C_6H_5), 129.8 (*C5*–C_5H_4N), 132.2 (C_6H_5), 141.6 (C_6H_5), 141.9 (*C3*–C_5H_4N), 144.6 (*C4*–C_5H_4N), 146.8 (*C6*–C_5H_4N), 149.2 (*C2*–C_5H_4N). Anal. Calcd for $C_{18}H_{16}NNaO_7P_2$: C, 48.77; H, 3.64; N, 3.16; P, 13.98; Found: C, 48.85; H, 3.65; N, 3.17; P, 14.04.

4.4.40. Sodium salt of [hydroxy-(hydroxy-tetradecyloxy-phosphoryl)-pyridin-3-yl-methyl]-phosphonic acid monotetradecyl ester (10e). Precipitation in methanol in the acidic form. Yield: 50%. Mp 146 °C. ^{31}P NMR {^{1}H} (200.7 MHz, $CDCl_3$) δ 12.5. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (t, 6H, $^{3}J_{H-H}=7.0$ Hz, $O(CH_2)_{13}CH_3$), 0.98–1.48 (m, 44H, $OCH_2CH_2(CH_2)_{11}CH_3$), 1.51–1.87 (m, 4H, $OCH_2CH_2(CH_2)_{11}CH_3$), 3.60–4.16 (m, 4H $OCH_2CH_2(CH_2)_{11}CH_3$), 7.50–7.85 (m, 1H, *H5*–C_5H_4N), 8.25–8.58 (m, 1H, *H4*–C_5H_4N), 8.65–8.94 (m, 1H, *H6*–C_5H_4N), 9.4 (s, 1H, *H2*–C_5H_4N). MS ($C_{34}H_{64}NNaO_7P_2$, pH=7.5) *m/z* 684.4 $[M+H]^+$. Anal. Calcd for $C_{34}H_{64}NNaO_7P_2$: C, 59.72; H, 9.43; N, 2.05; P, 9.06; Found: C, 59.81; H, 9.45; N, 2.08; P, 9.12.

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Lewis acid induced [4+3] cycloadditions of 2-silyloxyacroleins. Insights on the mechanism from a DFT analysis

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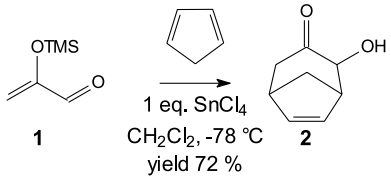
Abstract—The mechanism for the Lewis acid induced [4+3] cycloadditions of 2-(trimethylsilyloxy)acrolein with furan has been examined here through DFT calculations at B3LYP/6-31G* level. The mechanism is a three-step process initialized by the nucleophilic attack of furan to the β-conjugated position of acrolein yielding a zwitterionic intermediate. The key step on the formation of the seven-membered ring is the electrophilic attack of the furan residue to the carbonyl carbon in this intermediate. The *endo* selectivity experimentally observed is reproduced by the calculations.

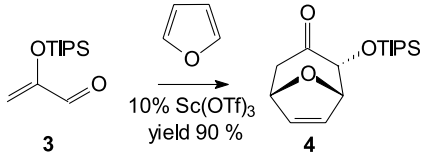
1. Introduction

The direct construction of seven-membered rings via [4+3] cycloadditions is the most attractive strategy for preparing this frequently observed natural product substructure.[1] α,β-Unsaturated carbonyl derivatives coordinated to Lewis acid (LA) have been widely used as the three-atom component of this particular cycloaddition. The use of 2-(silyloxy)acroleins in presence of a LA catalyst has received much interest in the last years. Sasaki et al.[2] demonstrated that treatment of aldehyde **1** with 1 equiv of $SnCl_4$ in the presence of a slight excess of cyclopentadiene (Cp) afforded, after acidic workup, the α-hydroxycycloheptenone **2** in 72% yield as a 2.7:1 mixture of *endo* and *exo* isomers, respectively (Scheme 1). The use of 2-(triisopropylsilyloxy)acrolein **3** has been recently reported by Harmata and Sharma.[3] This acrolein derivative



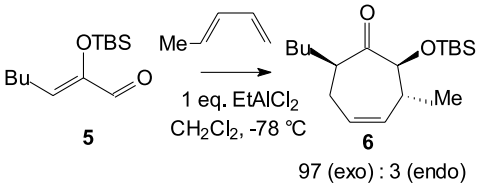
Scheme 1.

reacts with selected dienes, i.e. furan, in presence of catalytic amounts of scandium triflate to give products that are formally [4+3] cycloadducts (Scheme 2). An exception was in the case of butadiene, where only the [4+2] cycloadduct was observed.



Scheme 2.

More recently, Aungst and Funk[4] reported the LA catalyzed [4+3] cycloadditions of 2-(trialkylsilyloxy)-2-enals with a series of butadiene derivatives. In many instances, the [4+3] cycloadditions occurred with excellent regio- and/or stereoselectivity (Scheme 3). Several conclusions were obtained from these experiments: (i) in all cases, the silyl group is cleanly transferred to the aldehyde oxygen; (ii) the *endo* cycloadducts are uniformly preferred over the *exo* counterparts; and (iii) the stereoselectivity is better for



Scheme 3.

Keywords: [4+3] Cycloadditions; 2-Silyloxyacroleins; Lewis acid catalysts; Reaction mechanisms; DFT calculations.
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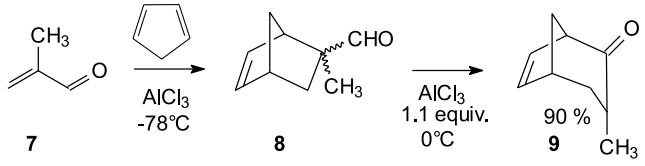
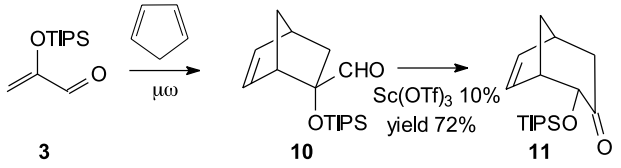
smaller silyl substituents, and the *endo*/*exo* ratio can also be significantly improved by the adequate choice of the LA catalyst.

The mechanism of these [4+3] cycloadditions involving LA coordinated 2-silyloxyacroleins is not absolutely clear. Harmata and Sharma[3] proposed that these cycloadditions with high levels of simple diastereoselectivity might be regarded as concerted processes. In addition, the occurrence of stereoisomers, as in the case of the reaction of **3** with Cp or the production of mixtures with 2,3-dimethylbutadiene, suggests the existence of an intermediate that can afford both [4+2] and [4+3] cycloadducts or [4+3] cycloadducts with eroded diastereoselectivity.[4]

Very recently, Davies and Dai[5] have reported the [4+3] cycloaddition between 2-alkylacroleins and Cp in the presence on 1.1 equiv of $AlCl_3$. For 2-methylacrolein, **7**, these authors found that at low temperature (−78 °C), the reaction yields the *endo* and *exo* [4+2] cycloadducts **8** (Scheme 4). When the reaction was warmed to 0 °C, the [4+3] cycloadduct **9** was the major product formed with a large diastereoselectivity (96% d.e.). These authors proposed a tandem Diels–Alder reaction/ring expansion for the formation of the [4+3] cycloadduct **9**. Davies and Dai proposed a similar mechanism for the Harmata's LA induced [4+3] cycloadditions of 2-silyloxyacroleins. But, all attempts to obtain the [4+2] cycloadduct from the scandium triflate-catalyzed Diels–Alder reaction of **3** with Cp yielded a mixture of the [4+3] cycloadducts **11**. The [4+2] cycloadduct **10** was obtained by a microwave (μω) induced cycloaddition reaction between **3** and Cp (Scheme 5). Furthermore, the isolated *exo* [4+2] cycloadduct **10** readily underwent a scandium triflate-catalyzed rearrangement under Harmata's conditions. These experiments drove Davies and Dai to propose that the Harmata's and related [4+3] cycloadditions are also examples of a tandem Diels–Alder reaction/ring expansion mechanism.[5]



Scheme 4.

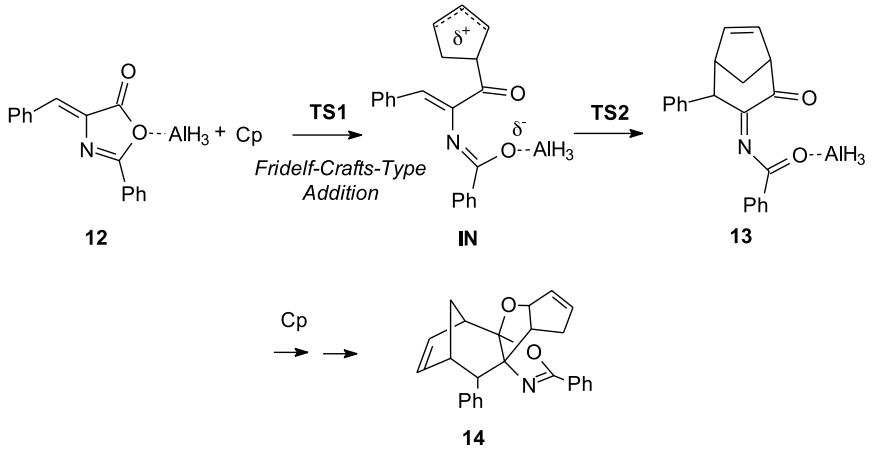


Scheme 5.

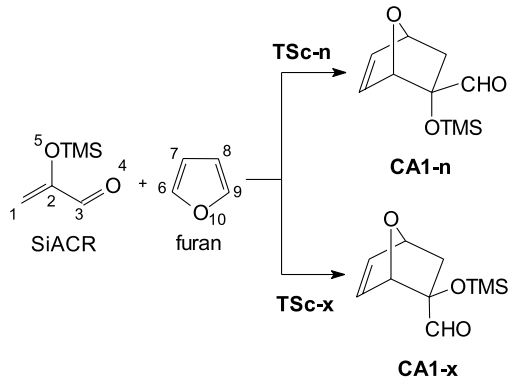
The structural information obtained by theoretical methods based on quantum mechanical calculations of possible intermediates and transition structures (TSs) provides powerful assistance for the study of organic reaction mechanisms.[6] These methods are accepted as a suitable tool for the interpretation of experimental results, since such data are rarely available from experiments.[6c]

Recently we have investigated[7] the mechanism of the domino reaction of the arylidenoxazolone **12** with an excess of Cp to give the adduct **14**.[8] An exhaustive exploration of the potential energy surface (PES) showed that formation of the formal [4+3] cycloadduct **13** requires a strong electrophilic activation of the carbonyl carbon atom of **12**.[7] This activation, modeled by coordination of the LA to the carboxyl oxygen atom of **12**, allows a Friedel–Crafts-type addition of **12** to Cp to yield the zwitterionic intermediate **IN** (see Scheme 6).[7] Further cyclization on the intermediate **IN** affords the [4+3] cycloadduct **13**, which is quickly nucleophilically captured by the excess of Cp to yield the final adduct **14**.

The LA induced [4+3] cycloadditions of 2-(silyloxy) acroleins has not been studied theoretically. Very recently, we have initiated a density functional theory (DFT) study for the mechanism of the LA induced [4+3] cycloadditions for 2-(trimethylsilyloxy)acrolein (SiACR-Al) with furan.[9] Now, a complete study for the role of the LA catalyst on the competitive [4+2] and [4+3] cycloadditions of 2-(silyloxy)acroleins has been carried out. In addition, the *endo* and *exo* channels have been explored in order to validate the method by the experimentally observed *endo* stereoselectivity. For this purpose, we have performed an exhaustive exploration of the PES for the reaction of



Scheme 6.



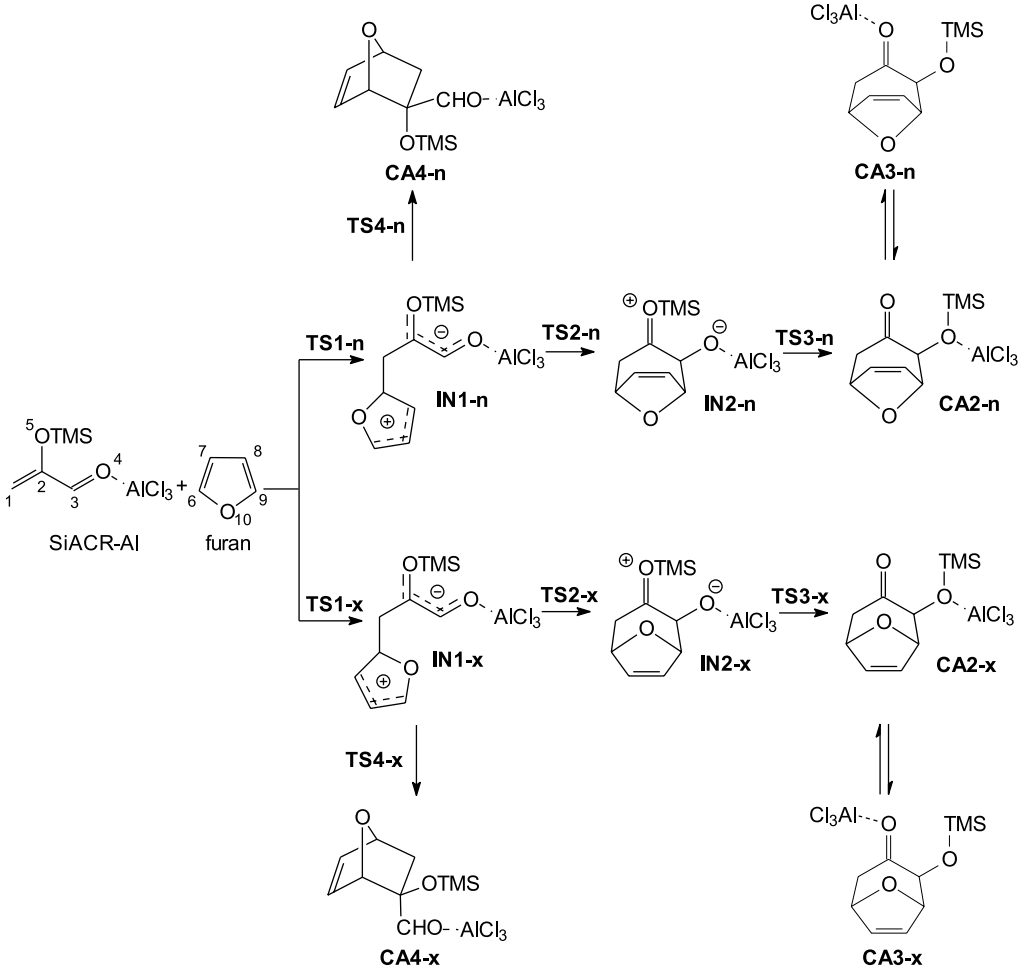
Scheme 7.

2-(trimethylsilyloxy)acrolein (SiACR) with furan in absence (Scheme 7) and in presence of $AlCl_3$ LA catalyst (Scheme 8) as model of the reaction studied by Aungst and Funk.[4]

2. Computational methods

In recent years, theoretical methods based on the DFT have emerged as an alternative to traditional ab initio methods in the study of structure and reactivity of chemical systems. Cycloaddition reactions have been object of several DFT studies showing that functionals that include gradient corrections and hybrid functionals for exchange and correlation, such as B3LYP,[10] together with the standard 6-31G* basis set,[11] lead to potential energy barriers in good agreement with the experimental results.[12] So, in the present study, geometrical optimizations of the stationary points were carried out using this methodology. The optimizations were performed using the Berny analytical gradient optimization method.[13] The stationary points were characterized by frequency calculations in order to verify that the TSs had one and only one imaginary frequency. The intrinsic reaction coordinate (IRC)[14] path was traced in order to check the energy profiles connecting each TS to the two associated minima of the proposed mechanism by using the second order González–Schlegel integration method.[15] The electronic structures of stationary points were analyzed by the natural bond orbital (NBO) method.[16] All calculations were carried out with the Gaussian 98 suite of programs.[17]

The values of the enthalpies, entropies and 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 and entropy have been scaled by 0.96.[18] The energies have been computed at 195.15 K, and the enthalpies and entropies



Scheme 8.

were calculated with the standard statistical thermodynamic formulae.[11]

The solvent effects of dichloromethane, modeled as a continuum model, have been considered by B3LYP/6-31G* single point calculations at the gas-phase optimized geometries using a self-consistent reaction field (SCRF)[19] based on the polarizable continuum model (PCM) of Tomasi's group.[20] The electronic energies in solution, E_{CH2Cl2}, have been obtained by adding the total electrostatic energies obtained from the PCM calculations to the electronic energies in vacuum. The PCM and solvent= dichloromethane options have been employed in the SCRF calculations.

The global electrophilicity index ω,[21] which measures the stabilization in energy when the system acquires an additional electronic charge ΔN from the environment, has been given the following simple expression,[21] $\omega=(\mu^2/2\eta)$, 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 $\eta\approx(\varepsilon_L-\varepsilon_H)$, respectively.[22]

3. Results and discussions

Firstly, in part (a) a DFT analysis based on the reactivity indexes of reagents involved in these cycloadditions will be performed. Then, the [4+2] cycloaddition between SiACR and furan will be considered in part (b) (see Scheme 7). Finally, in part (c) the presence of LA catalyst in these cycloadditions will be taken into account by coordination of LA to the carbonyl oxygen atom of SiACR (see Scheme 8).

3.1. Global electrophilicity analysis

Recent studies devoted to Diels–Alder[23] and 1,3-dipolar cycloaddition[24] reactions have shown that the global indexes defined in the context of DFT[25] are a powerful tool to understand the behavior of polar cycloadditions. The difference of global electrophilicity between the reagent pair,[23] $\Delta\omega$, can be used to predict the polar character of the process and thereby the feasibility of the cycloaddition. In Table 1 the static global properties: electronic chemical potential, μ, chemical hardness, η, and global electrophilicity, ω, of acrolein (ACR), SiACR, the corresponding LA coordinated acroleins, ACR-Al and SiACR-Al, Cp and furan are presented.

Table 1. Electronic chemical potential (μ, in au), chemical hardness (η, in au) and global electrophilicity (ω, in eV) of acrolein, ACR, 2-(trimethylsilyloxy)acrolein, SiACR, the corresponding LA coordinated acrolein, Cp and furan

	μ	η	ω
ACR-Al	−0.2190	0.1412	4.62
SiACR-Al	−0.2120	0.1493	4.09
ACR	−0.1611	0.1921	1.84
SiACR	−0.1535	0.1821	1.76
Cp	−0.1107	0.2016	0.83
Furan	−0.1024	0.2441	0.58

The electronic chemical potential, μ, of Cp and furan, −0.1107 and −0.1024 au, are lesser than those for SiACR and SiACR-Al, −0.1535 and −0.2120 au, respectively. Therefore, the charge transfer on these cycloaddition reactions will take place from the dienes, Cp or furan, to these 2-silyloxyacrolein derivatives, in complete agreement with the charge transfer analysis performed at the TSs (see later). SiACR and ACR present similar electrophilicity values: 1.76 and 1.84 eV, respectively. According to the absolute scale of electrophilicity based on the ω index, these compounds may be classified as strong electrophiles.[23] The presence of the electron-releasing trimethylsilyloxy group on the C2 position of ACR decreases slightly the electrophilicity of the 2-silyloxy derivative SiACR. Coordination of $AlCl_3$ to the carbonyl oxygen atom increases considerably the electrophilicity of ACR and SiACR: 4.62 eV (ACR-Al) and 4.09 eV (SiACR-Al). On the other hand, Cp and furan have very low electrophilicity values: 0.83 and 0.58 eV, respectively. Therefore, these dienes are classified as marginal electrophiles (good nucleophiles).[23] The large difference in electrophilicity for the SiACR-Al/furan pair, $\Delta\omega=3.51$ eV, indicates that this cycloaddition will have a large polar character.[23] This value is closer to that for the ACR-Al/Cp pair, 3.79 eV. The ACR/Cp and SiACR/furan pairs present lower $\Delta\omega$ values: 1.01 and 1.18 eV. These non-catalyzed cycloadditions will have a less polar character and a large activation barrier.

3.2. Study of the [4+2] cycloaddition between 2-(trimethylsilyloxy)acrolein, and furan

The cycloaddition between SiACR and furan can take place along two stereoisomeric reactive channels, the *endo* and *exo* (see Scheme 7). The two reactive channels associated with the *endo* and *exo* approach modes of the diene system of furan relative to the 2-silyloxy substituent of SiACR have been considered. An analysis of the gas-phase results

Table 2. Relative[a] enthalpies (ΔH, kcal/mol), entropies (ΔS, cal/mol K) and free energies (ΔG, kcal/mol) in vacuum at 195.15 K and 1 atm, corresponding to the stationary points of the cycloaddition reactions between SiACR and furan, and SiACR-Al and furan

	ΔH	ΔS	ΔG
TSc-n	21.0	−48.4	30.5
TSc-x	24.1	−48.2	33.6
CA1-n	5.7	−50.2	15.5
CA1-x	7.6	−50.5	17.5
TS1-n	4.3	−41.6	12.5
TS1-x	7.1	−40.7	15.1
IN1-n	2.1	−41.9	10.2
IN1-x	6.6	−41.5	14.7
TS2-n	3.5	−46.6	12.6
TS2-x	9.1	−47.3	18.3
IN2-n	0.7	−45.3	9.6
IN2-x	6.3	−47.2	15.5
TS3-n	10.0	−54.1	20.5
TS3-x	14.2	−52.5	24.5
CA2-n	0.9	−53.4	11.3
CA2-x	0.0	−50.5	9.9
CA3-n	−6.9	−45.1	1.9
CA3-x	−6.2	−44.3	2.5
TS4-n	8.6	−45.1	17.4
TS4-x	10.9	−43.5	19.4
CA4-n	6.5	−41.4	14.6
CA4-x	8.3	−41.3	16.3

[a] Relative to furan and SiACR or SiACR-Al.

indicates that these cycloadditions take place along concerted bond-formation processes to give the corresponding *endo* and *exo* [4+2] cycloadducts. Therefore, two TSs, **TSc-n** and **TSc-x**, and two cycloadducts, **CA1-n** and **CA1-x**, associated to the *endo* and *exo* reactive channels, named as **n** and **x**, have been located and characterized. The different stationary points of this cycloaddition have been depicted in Scheme 7 together with the atom numbering while the energetic results are listed in Table 2. The optimized geometries of the TSs are depicted in Figure 1.

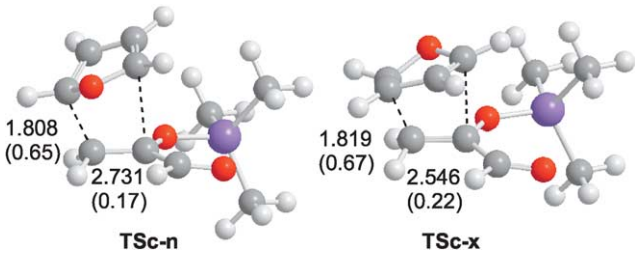


Figure 1. Optimized geometries of the transition structures for the *endo*, **TSc-n**, and *exo*, **TSc-x**, channels of the [4+2] cycloaddition between SiACR and furan. The distances directly involved in the forming-bond processes are given in angstroms. Bond order values are given in parentheses.

The activation enthalpies for the [4+2] cycloaddition reaction between SiACR and furan along the *endo* and *exo* reactive channels are: 21.0 (**TSc-n**) and 24.1 (**TSc-x**) kcal/mol. This cycloaddition presents a large *endo* selectivity. Inclusion of the activation entropy raises the activation free energies to 30.5 (**TSc-n**) and 33.6 (**TSc-x**) kcal/mol, as a consequence of the negative activation entropy associated with these [4+2] cycloadditions: ca. −48 cal/mol K. The large free activation energy for the non-catalyzed process prevents the [4+2] cycloaddition. These processes are endothermic by 5.7 (**CA1-n**) and 7.6 (**CA1-x**) kcal/mol.

The length of the C1–C6 and C2–C9 forming bonds at the TSs are 1.808 and 2.731 Å (**TSc-n**) and 1.819 and 2.546 Å (**TSc-x**), respectively. The extent of the asynchronicity of the cycloadditions can be measured by means of the difference between the lengths of the two σ bonds that are being formed, i.e $\Delta r = d(\text{C2–C9}) - d(\text{C1–C6})$. The asynchronicity at the TSs are: $\Delta r = 0.92$ (**TSc-n**) and 0.73 (**TSc-x**). Therefore, these TSs correspond to asynchronous bond-formation processes where the σ-bond at the β-position of acrolein is formed in a larger extent. The TS associated with the more favorable *endo* reaction channel is more asynchronous than that to the *exo* one. The BO values[26] of the C1–C6 and C2–C9 forming bonds at the TSs are 0.65 and 0.17 at **TSc-n**, and 0.67 and 0.22 at **TSc-x**, respectively.

The natural population analysis allows the evaluation of the charge transfer along these cycloaddition processes. The B3LYP/6-31G* natural atomic charges at the TSs have been partitioned between the SiACR and the furan frameworks. At the TSs, the negative charge that fluxes from the donor furan to the acceptor SiACR is 0.15 e at **TSc-n** and 0.16 e at **TSc-x**. These values are slightly larger than that computed at the *endo* TS associated with the butadiene/acrolein reaction, 0.11 e, which displays a lesser $\Delta\omega$, 0.77 eV.[23]

Table 3. Relative[a] (ΔE_{CH2Cl2}, in kcal/mol) energies in dichloromethane for the uncatalyzed and LA catalyzed reactions between SiACR and furan

	$\Delta E_{CH_2C_{12}}$		$\Delta E_{CH_2C_{12}}$
TSc-n	18.9	**TSc-x**	22.1
CA1-n	2.7	**CA1-x**	4.6
TS1-n	0.7	**TS1-x**	2.7
IN1-n	−4.2	**IN1-x**	0.4
TS2-n	−2.1	**TS2-x**	3.0
IN2-n	−6.5	**IN2-x**	−1.3
TS3-n	5.1	**TS3-x**	9.9
CA2-n	−1.5	**CA2-x**	−2.7
CA3-n	−15.4	**CA3-x**	−14.7
TS4-n	4.7	**TS4-x**	7.3
CA4-n	3.2	**CA4-x**	5.5

[a] Relative to furan and SiACR or SiACR-Al.

Finally, solvent effects in this cycloaddition were modeled using the PCM method. Table 3 reports the relative energies in dichloromethane. Solvent effects produce a slightly larger stabilization of TSs, 3.5 kcal/mol, than reagents, 2.2 kcal/mol. As consequence, solvent effects decrease the gas-phase activation barriers only in 1.3 kcal/mol. These poor solvent effects can be undertook as a consequence of the low polar character of these cycloadditions.

3.3. Study of the LA induced [4+3] cycloaddition between the $AlCl_3$ coordinated 2-(trimethylsilyloxy) acrolein and furan

The reaction between LA coordinated 2-silyloxyacrolein and furan can take place along two stereoisomeric reactive channels, the *endo* and *exo*.[4] The two reactive channels associated with the *endo* and *exo* approach modes of furan to SiACR-Al have been considered. The LA induced [4+3] cycloaddition between the LA coordinated 2-(trimethylsilyloxy)acrolein SiACR-Al and furan takes place along a three-step mechanism.[9] Therefore, six TSs, **TS1-n**, **TS2-n**, **TS3-n**, **TS1-x**, **TS2-x**, and **TS3-x**, four intermediates **IN1-n**, **IN2-n**, **IN1-x**, **IN2-x**, and two cycloadducts, **CA2-n** and **CA2-x**, associated to the *endo* and *exo* reactive channels, named as **n** and **x**, have been located and characterized. In addition, two TSs, **TS4-n** and **TS4-x**, connecting the intermediates **IN1-n** and **IN1-x** with the [4+2] cycloadducts **CA4-n** and **CA4-x** were also located and characterized. The different stationary points associated to the [4+3] and [4+2] cycloadditions have been depicted in Scheme 8 together with the atom numbering, while the energetic results are listed in Table 2. The optimized geometries of the TSs are depicted in Figure 2.

The first step of this LA promoted [4+3] cycloaddition is the nucleophilic attack of the C6 position of furan to the C1 conjugated position of the acrolein derivative SiACR-Al. The activation enthalpies associated to the nucleophilic attack along the *endo* and *exo* reactive channels are 4.3 (**TS1-n**) and 7.1 (**TS1-x**) kcal/mol. Coordination of the LA to the carbonyl oxygen atom decreases the activation enthalpy for the catalyzed process in 16.7 kcal/mol, as a consequence of the large increase of the electrophilicity of SiACR (see part a). Note that these reactions take place at very low temperature, −78 °C.[4] The nucleophilic attack along the *endo* channel is favored in 2.8 kcal/mol over the *exo* one. The zwitterionic intermediates **IN1-n** and **IN1-x**

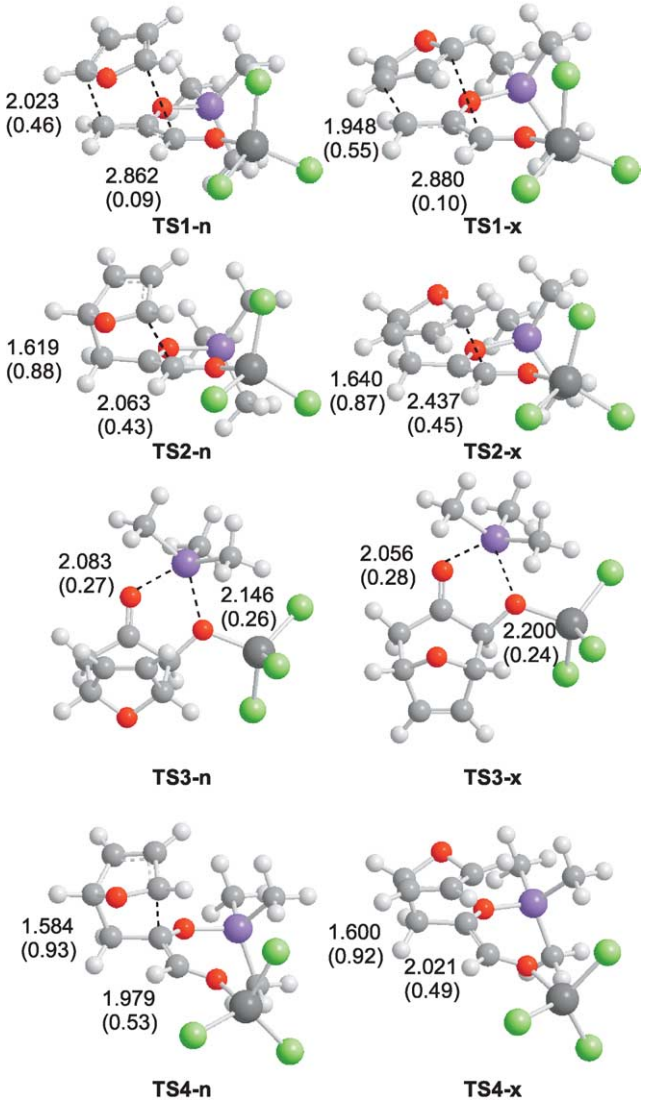


Figure 2. Optimized geometries of the transition structures for the *endo* and *exo* channels of the LA catalyzed [4+3] and [4+2] cycloadditions between SiACR-Al and furan. The distances directly involved in the forming-bond processes are given in angstroms. Bond order values are given in parentheses.

are located 2.1 and 6.6 kcal/mol above reagents. The second step, which is the electrophilic attack of the C9 carbon of the furan framework on the nucleophilically activated carbonyl C3 carbon of SiACR-Al with formation of the seven-membered ring presents also a very low activation enthalpy: 1.4 (**TS2-n**) and 2.5 (**TS2-n**) kcal/mol. The [4+3] cycloadduct intermediates **IN2-n** and **IN2-x** are 0.7 and 6.3 kcal/mol, respectively, above reagents. These cycloadducts experience a silyl migration from the O5 oxygen to carbonyl O4 oxygen atom. The activation enthalpies associated to these processes are 9.3 (**TS3-n**) and 7.9 (**TS3-x**) kcal/mol. Finally, the [4+3] cycloadducts formed after the silyl migrations, **CA2-n** and **CA2-x**, quickly equilibrate with the thermodynamically more stable cycloadducts **CA3-n** and **CA3-x** by a LA migration to the more basic O5 oxygen atom. Formation of these [4+3] cycloadducts are exothermic processes in −6.9 (**CA3-n**) and −6.2 (**CA3-x**) kcal/mol.

For this stepwise process, the silyl migration step corresponds to the rate-limiting of the overall process. The relative free energy associated to **TS3-n** remains 10.0 kcal/mol below to that associated to the non-catalyzed process, **TSc-n**. The *endo* **TS3-n** is 4.2 kcal/mol lesser in energy than the *exo* **TS3-x**. The *endo* stereoselectivity found along the nucleophilic attack of furan to SiACR-Al remains at the silyl migration step and, in consequence, the *endo* channel is favored over the *exo* one in clear agreement with the experimentally observed *endo* selectivity.[4]

An exhaustive exploration of the PES for the LA-catalyzed process allowed us to find also the TSs associated to the cyclization of the intermediates **IN1-n** and **IN1-x** at the C2 carbon atom of the acrolein residue yielding the [4+2] cycloadducts **CA4-n** and **CA4-x** (see Scheme 8). The activation enthalpies associated to the formation of the C2–C9 bond, 6.5 (**TS4-n**) and 4.3 (**TS4-x**) kcal/mol, are higher in energy than those associated to the C3–C9 formation bond, 1.4 (**TS2-n**) and 2.5 (**TS2-x**) kcal/mol. Formation of the [4+2] cycloadducts **CA4-n** and **CA4-x** are endothermic processes in 6.5 and 8.3 kcal/mol, respectively. Both kinetic and thermodynamic data clearly favor the formation of the [4+3] cycloadduct on the LA promoted process.

The length of the C1–C6 forming bond along the nucleophilic attack of furan to the β-conjugated position of SiACR-Al is 2.023 Å at **TS1-n** and 1.948 Å and **TS1-x**, while the distance between the C3 and C9 carbon atoms are 2.862 and 2.880 Å, respectively. The asynchronicity at the TSs are: $\Delta r = 0.84$ (**TS1-n**) and 0.93 (**TS1-x**). These TSs, which correspond with a two-center addition, are associated to a Michael-type addition of furan acting as nucleophile to the β conjugated position of the acrolein derivative SiACR-Al. At the intermediates **IN1-n** and **IN1-x**, the length of the C1–C6 bonds are 1.644 and 1.674 Å, respectively, while the distance between the C3 and C9 carbon atoms remains on 2.716 and 2.761 Å, respectively. The length of the C3–C9 forming-bond along the electrophilic attack of the furan residue to the carbonyl carbon of the acrolein moiety with formation of the seven-membered carbocycle is 2.063 Å at **TS2-n** and 2.437 Å **TS2-x**. At the TSs associated with the migration of the trimethyl silyl group, the lengths of the O5–Si breaking bond and O4–Si forming bond are 2.083 and 2.146 Å at **TS3-n** and 2.056 and 2.200 Å at **TS3-x**, respectively. Finally, the length of the C2–C9 forming bond along the electrophilic attack of the C9 carbon of the furan to the C2 carbon of the acrolein residue with formation of the six-membered carbocycles is 1.979 Å at **TS4-n** and 2.021 Å at **TS4-x**. The bond order values of the forming and breaking bonds along the *endo* and *exo* reactive channels are given in Figure 2.

At these LA induced [4+3] cycloadditions, the negative charge that fluxes from the donor furan to the acceptor SiACR-Al is 0.33 e at **TS1-n**, 0.43 e at **IN1-n**, 0.30 e at **TS2-n**, 0.37 e at **TS1-x**, 0.44 at **IN1-x**, and 0.29 e at **TS2-x**. These large values indicate the zwitterionic character of these species. The charge transfer increases along the nucleophilic attack of furan to the electrophilically activated β-position of SiACR-Al to formation of the intermediates

IN1-n and **IN-x**. Note that the charge transfer at **TS1-n** and **TS1-x** is twice than that for the uncatalyzed process. The larger polar character of the LA catalyzed cycloaddition relative to the uncatalyzed one is in agreement with the increase of the electrophilicity of the LA coordinated acrolein derivatives which raises the $\Delta\omega$ of the catalyzed reaction (see part a).

Finally, solvent effects produce a larger stabilization of TSs and intermediates, between 7.2 and 10.0 kcal/mol, than reagents, 5.4 kcal/mol, due to the zwitterionic character of the former. In consequence, in dichloromethane the relative energies to the TSs associated with the nucleophilic attack of furan to SiACR-Al are 0.7 and 2.7 kcal/mol (see Table 3). The *exo* **TS1-x** is 0.8 kcal/mol more stabilized than **TS1-n** as a consequence of the more character polar of the former. The energy barriers associated to the TSs of the silyl migration, **TS3-n** and **TS3-x**, evaluated from the corresponding intermediates, **IN2-n** and **IN2-x**, are 11.6 and 11.2 kcal/mol; the *endo* **TS3-n** remains 4.8 kcal/mol below than that associated with the *exo* **TS3-x**. With the inclusion of solvent effects, the barriers for the cyclization of the intermediates **IN1-n** and **IN1-x** with formation of the [4+2] cycloadducts, via **TS4-n** and **TS4-x**, remain 6.8 and 4.3 kcal/mol, respectively, above of those with formation of the [4+3] ones, via **TS2-n** and **TS2-x**, respectively (see Table 3). In consequence, inclusion of solvent effect on the electronic energies by the PCM model does not modify the analysis performed by the gas-phase calculations.

The present study of the LA induced [4+3] cycloaddition of 2-silyloxyacroleins with furan indicates that both [4+2] and [4+3] cycloadditions have polar stepwise mechanisms which share the TS associated with the nucleophilic attack of furan to SiACR-Al and the subsequent zwitterionic intermediate. Further cyclization at the C2 or C3 positions of the acrolein moiety affords the corresponding [4+2] and [4+3] cycloadducts. DFT calculations give the TS associated with the ring-closure with formation of the *endo* [4+2] cycloadduct 4.3 kcal/mol higher in energy than that associated to the formation of the *endo* [4+3] one. In addition, while formation of the [4+2] cycloadduct is endothermic in 6.5 kcal/mol, formation of the [4+3] cycloadduct is exothermic in −6.9 kcal/mol. As consequence, the [4+3] cycloadduct is ca. 14 kcal/mol lesser in energy than the [4+2] one because of the larger strain associated to a [2.2.1] bicyclic system than that associated with a [3.2.1] one. This gas-phase analysis is not modified with the inclusion of solvent effects, dichloromethane. These results are in agreement with the unfeasibility of obtaining the [4+2] cycloadduct in the scandium triflate-catalyzed cycloaddition reaction of the silylacrolein derivative **3** with Cp (see Scheme 4),[5] and with the easy LA promoted conversion of the [4+2] cycloadduct obtained via a microwave induced cycloadditions[5] into the thermodynamically more stable [4+3] cycloadduct. This conversion takes place by a LA induced retro-Diels–Alder reaction/[4+3] cycloaddition. We can conclude that these LA induced [4+3] cycloadditions do not take place through of a tandem Diels–Alder/ring expansion mechanism as proposed by Davies and Dai,[5] but through a direct polar stepwise mechanism.

4. Conclusions

The mechanisms for the [4+2] and [4+3] cycloadditions of furan with 2-(trimethylsilyloxy)acrolein in absence and in presence of a LA catalyst have been studied at B3LYP/6-31G* level. In absence of a LA the reaction is an asynchronous concerted bond-formation process yielding the *endo* [4+2] cycloadduct. The presence of a LA catalyst coordinated to 2-(trimethylsilyloxy)acrolein drastically changes the mechanism. The reaction is a three-step process yielding the *endo* [4+3] cycloadduct. The reaction is initialized by the nucleophilic attack of furan to the β-conjugated position of the LA coordinated 2-(trimethylsilyloxy)acrolein to give a zwitterionic intermediate. The key step on the formation of the seven-membered ring is the electrophilic attack of the furan residue to the nucleophilically activated carbonyl carbon at this intermediate. Both the presence of the electron-releasing silyloxy group at the α-position of acrolein and the presence of the LA coordinated to the carbonyl oxygen atom polarizes the electron density of the zwitterionic intermediate towards the carbonyl carbon favoring the subsequent cyclization at this carbon. The channel associated to the *endo* approach of furan to the LA coordinated 2-(trimethylsilyloxy)acrolein is favored over the *exo* one in clear agreement with the experimental results. Analysis of the PES for the LA induced process allows an explanation for the conversion of the [4+2] cycloadduct into the thermodynamically more stable [4+3] by a LA induced retro-Diels–Alder reaction/[4+3] cycloaddition. Inclusion of solvent effect on the energies does not modify substantially the analysis based on the gas-phase energy calculations.

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Stereoselective synthesis of microcarpalide

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Abstract—Microcarpalide is a strong microfilament disrupting agent. The convergent and stereoselective synthesis of microcarpalide was succeeded via Julia olefination and macrolactonization.

1. Introduction

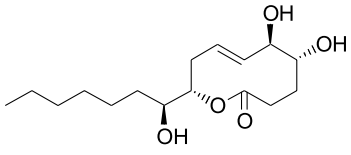
Microcarpalide, a 10-membered lactone, was isolated from the fermentation broth of an unidentified endophytic fungus by Hemscheidt and co-workers in 2001.[1] This compound acts as a strong microfilament disrupting agent and shows weak cytotoxicity to mammalian cells. Because of the large difference between the effective concentration for the antimicrofilament activity and the cytotoxicity, it is thought that this compound will be an effective tool for the studies of cell motility and metastasis. Then, we started the synthesis of microcarpalide. We have already published a rapid communication,[2] and here, we wish to report a full account of our work (Fig. 1).

2. Results and discussion

Our retrosynthesis is shown in Scheme 1. We selected lactonization as a ring-closing step. The precursor for the lactonization **A** would be prepared from aldehyde **B** and sulfone **C** via one-pot Julia coupling.[3] The aldehyde and the sulfone would be obtained by Sharpless asymmetric dihydroxylation[4] of olefins **D** and **E**, respectively. During our work in progress, other groups[5–10] also reported the total synthesis of microcarpalide, all of them using ring-closing metathesis as a key step.

The synthesis of the sulfone unit is shown in Scheme 2. The known olefinic alcohol **2**[11] was protected with PMB group and subjected to Sharpless asymmetric dihydroxylation[4,12] to give diol **4** (95% ee) as colorless crystals. Purification of the desired enantiomer could be realized by two times of recrystallization, affording **4** with >99% ee (determined by chiral HPLC). This diol was converted to *p*-methoxybenzylideneacetal **5** and the residual secondary alcohol was protected by MOM group. After removal of *p*-methoxybenzylidene group, the primary hydroxyl group of **7** was converted to corresponding 1-phenyl-1*H*-tetrazol-5-yl sulfone[13] **8** by Mitsunobu reaction and subsequent Mo(VI) catalyzed oxidation.[14] Preparation of the sulfone unit **9** was achieved by protection of the secondary alcohol.

On the other hand, synthesis of the aldehyde unit **16** is shown in Scheme 3. Starting from diol **10**,[15] olefinic ester **11** was prepared by Claisen rearrangement.[16,17] In our previous report,[2] ester **11** was temporarily hydrolyzed into carboxylic acid **12**, which was subjected to Sharpless asymmetric dihydroxylation[4] to afford the desired diol. This unstable diol was protected immediately together with re-esterification of the carboxyl group to give **13**. But the enantiomeric purity of this compound was determined to be only 60% ee. Therefore, we revised the synthetic route to intermediate **13** for the better enantiomeric purity. Direct dihydroxylation of **11** gave exclusively γ-lactone **14**, instead of the desired diol, as colorless crystals (95% ee). Purification of the desired enantiomer could be achieved by recrystallization, affording **14** with >99% ee (determined by chiral HPLC). Although,



Microcarpalide (**1**)

Figure 1.

Keywords: Microcarpalide; Microfilament disrupting agent; Macrolactonization; Julia olefination; Sharpless asymmetric dihydroxylation.
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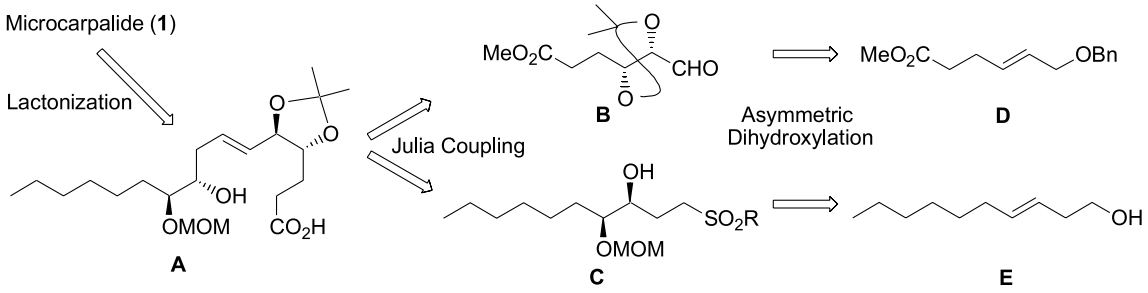
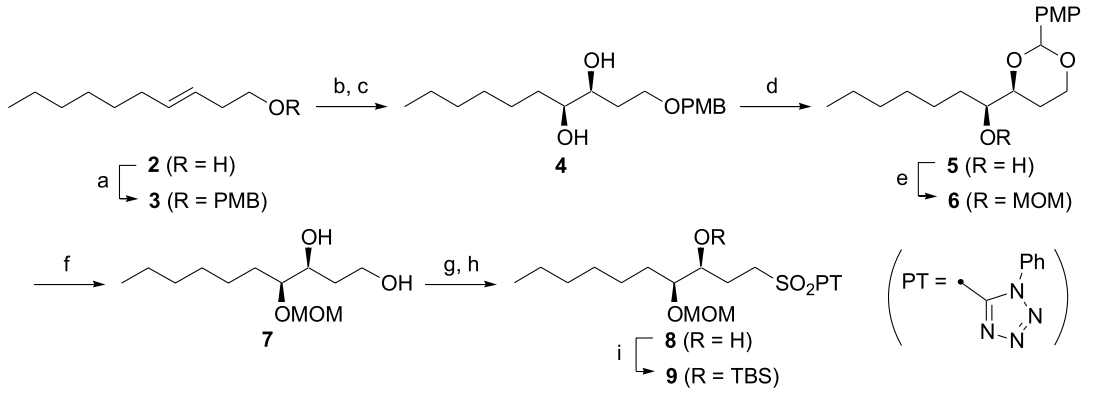
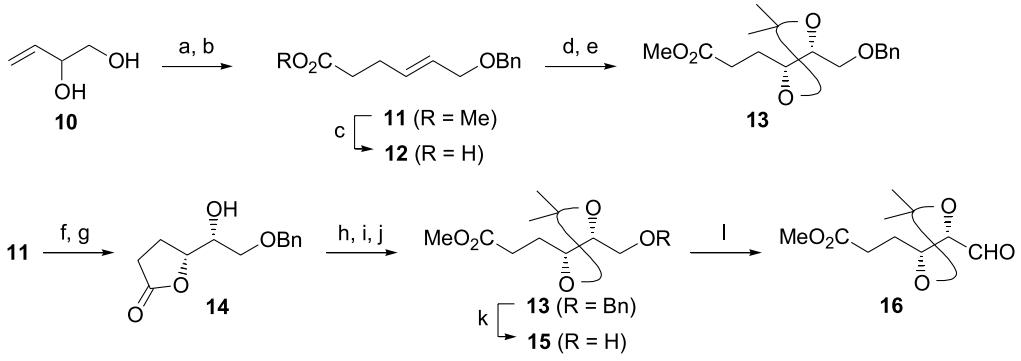


Scheme 1. Synthetic plan.



Scheme 2. Reagents and conditions: (a) PMBCl, NaH, TBAB, THF, reflux, quant.; (b) AD-mix-α, $MeSO_2NH_2$, *t*-BuOH, H_2O, 95% ee; (c) recrystn., >99% ee, 74% in two steps; (d) DDQ, CH_2Cl_2; (e) MOMCl, *i*-Pr_2EtN, CH_2Cl_2; (f) AcOH, H_2O, THF, 82% in three steps; (g) PTSH, PPh_3, DIAD, THF; (h) $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, H_2O_2, EtOH; (i) TBSOTf, 2,6-lutidine, CH_2Cl_2, 91% in three steps.



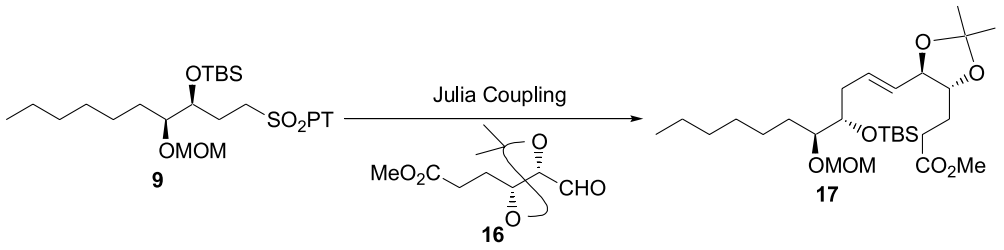
Scheme 3. Reagents and conditions: (a) BnBr, NaH, TBAI, THF; (b) $MeC(OMe)_3$, $EtCO_2H$, 140 °C, 48% in two steps; (c) LiOH, THF, H_2O, 95%; (d) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH, H_2O; (e) 2,2-dimethoxypropane, HCl, acetone, 74% in two steps; (f) AD-mix-β, $MeSO_2NH_2$, *t*-BuOH, H_2O, 95% ee, 83%; (g) recrystn., >99% ee, 86%; (h) LiOH, THF, H_2O; (i) 2,2-dimethoxypropane, acetone; (j) CH_2N_2, Et_2O, EtOAc, 88% in three steps; (k) H_2, 10% Pd/C, *i*-PrOH, quant.; (l) 4-MeO-TEMPO, KBr, NaOCl, $NaHCO_3$, CH_2Cl_2, H_2O, ca. 70%.

the yield in methanolysis of the lactone **14** was low (<40%), hydrolysis and subsequent protection afforded **13** in much better yield. After hydrogenolysis, **15** was successfully oxidized to the corresponding aldehyde **16** mediated by oxoammonium salt.[18]

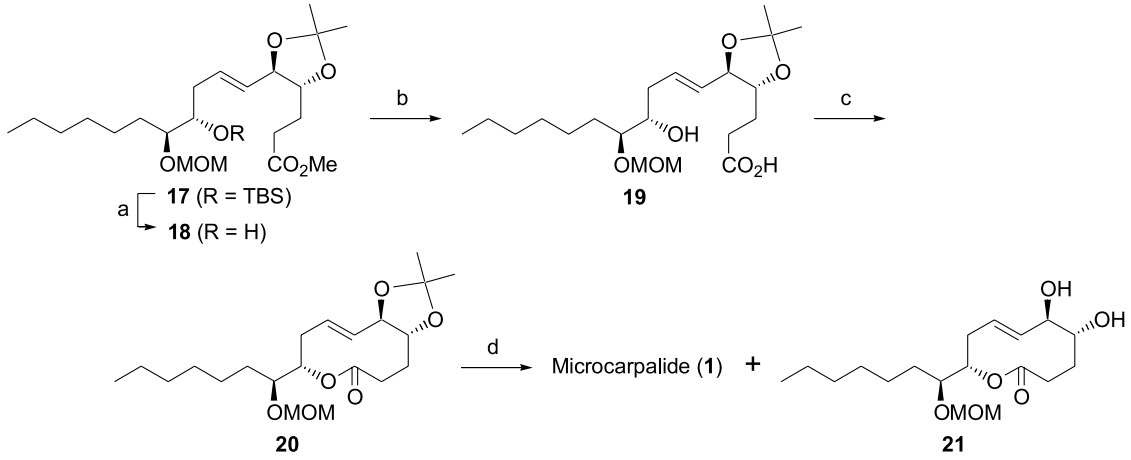
Now that both of the two units were obtained enantioselectively, we tried one-pot Julia coupling[3,13,19] in several conditions (Table 1). The reaction employing LiHMDS as a base gave the desired olefin **17** in poor yield (entries 1 and 2). On the other hand, good yield was realized when KHMDS was used as a base, but *E*/*Z* selectivity was not so high (entries 3 and 4). Because the low selectivity seemed to be caused by some chelation effects of oxygen functional groups in the sulfone **9** and the aldehyde **16** with potassium cation, we tried other conditions employing additives to prevent this chelation. When 18-c-6 was added (entry 6), *trans*-olefin **17** was successfully obtained in good yield and high selectivity. Separation of **17** from *cis*-isomer was easily succeeded by silica gel column chromatography.

Final steps including lactonization are shown in Scheme 4. The *trans*-olefin **17** was treated with TBAF and hydrolyzed to give hydroxy acid **19**, a lactonization precursor. It was subjected to Yamaguchi's method[20] to afford 10-membered lactone **20** in excellent yield. Formation of dimeric lactone was observed in higher concentration, but not in 1 mmol/L. Deprotection of **20** was performed in the similar method as Marco et al.[5] to give microcarpalide (**1**) successfully together with small amount of partially deprotected

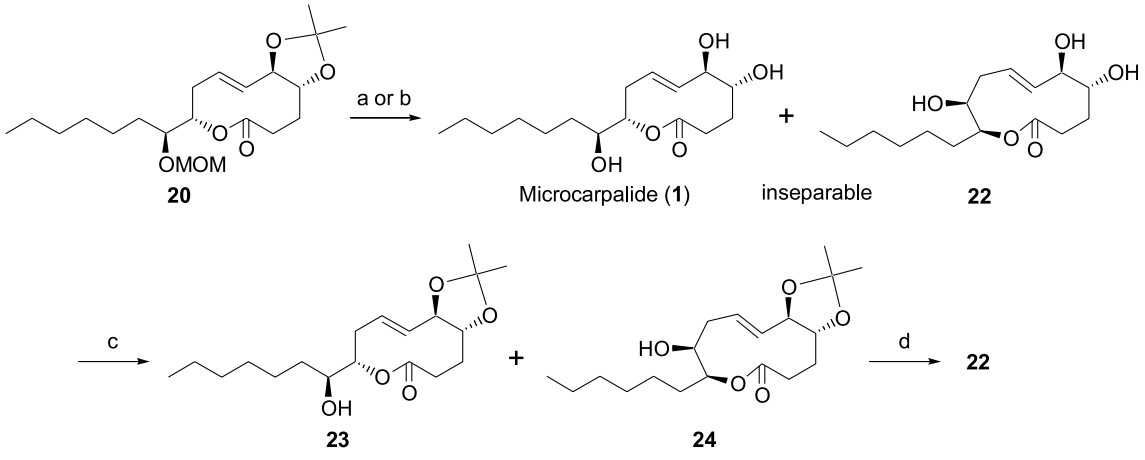
Table 1.



Entry	Base	Temperature (°C)	Additive	*E*:*Z*	Yield (%)
1	LiHMDS	−78	—	3:1	10
2	LiHMDS	−108	—	10:1	32
3	KHMDS	−78	—	2:1	64
4	KHMDS	−108	—	2:1	77
5	KHMDS	−108	HMPA	3:1	71
6	KHMDS	−108	18-c-6	10:1	72



Scheme 4. Reagents and conditions: (a) TBAF, THF, 99%; (b) LiOH, H_2O, THF; (c) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF, then DMAP, benzene, 94%; (d) $BF_3 \cdot OEt_2$, $(CH_2SH)_2$, CH_2Cl_2, −20 to −10 °C, 69% (**1**), 15% (**21**).



Scheme 5. Reagents and conditions: (a) DOWEX®-50W, MeOH, H_2O, 50 °C, 98%; (b) $BF_3 \cdot OEt_2$, $(CH_2SH)_2$, CH_2Cl_2, 0 °C, 70%; (c) 2,2-dimethoxypropane, PPTS, acetone; separation, 74% (**23**), 14% (**24**); (d) PPTS, MeOH, 85%.

compound **21**. The analytical and spectroscopic data of synthesized **1** were identical to the reported data.[1,5–10] As reported, the NMR spectra of **1** was observed as a mixture of two conformers in the ratio about 3.5:1.

Interestingly, acid-catalyzed isomerization of microcarpalide was clarified in the course of our examination of deprotection conditions as shown in Scheme 5. Treating **20** with DOWEX-50W (MeOH, H_2O, 50 °C, 4 days) or $BF_3 \cdot OEt_2$ and $(CH_2SH)_2$ (CH_2Cl_2, 0 °C, 2 h) gave inseparable mixture of microcarpalide (**1**) and small amount of unknown compound **22**. For structural determination of

22, this mixture was treated with 2,2-dimethoxypropane and PPTS to afford the corresponding acetonides, expected 10-membered lactone **23** and another lactone **24**. These compounds were easily separated and the structure of **24** was confirmed to be 11-membered lactone by ^{1}H-COSY spectrum. Deprotection of the isolated **24** gave **22** as a single product and its ^{1}H NMR spectrum was identical with that of the previous minor component in the inseparable mixture (**1** and **22**). Similarly, microcarpalide (**1**) was found to be partially isomerized affording the mixture of **1** and **22** by treatment with TsOH. ^{1}H NMR spectrum (in CD_3CN, 24 °C) showed this mixture comprised the major conformer of **1**, the minor conformer of **1** and 11-memberd lactone **22** (5:1.5:1).

In conclusion, we have accomplished a convergent and stereoselective synthesis of microcarpalide. All of the four chiral centers were introduced by Sharpless asymmetric dihydroxylation. One-pot Julia olefination gave the desired *trans*-olefin in good yield and high selectivity in the presence of crown ether and Yamaguchi's macrolactonization was also successful in excellent yield. The total yield was 23% in 13 steps. We also observed an interesting acid-catalyzed isomerization of microcarpalide.

3. Experimental

3.1. General

Optical rotations were recorded with a JASCO DIP-1000 polarimeter. IR spectra were measured with a JASCO FT/IR-230 spectrophotometer. ^{1}H and ^{13}C NMR were recorded on JEOL JNM AL300. Mass spectra were recorded on JEOL JMS-700T. Column chromatography was performed using Merck silica gel 60 (0.060–0.200 mm). TLC was carried out on Merck glass plates precoated with silica gel 60 F_{254} (0.25 mm). HPLC was performed using SHOWA DENKO shodex DS-4. Melting points are uncorrected values.

3.2. Synthetic studies

3.2.1. (*E*)-1-(4-Methoxybenzyloxy)-3-decene (3). To a solution of alcohol (**2**, 8.8 g, 56 mmol) in THF (160 mL) were added NaH (60%, 3.0 g, 78 mmol), PMBCl (9.0 mL, 66 mmol) and tetra-*n*-butylammonium bromide (100 mg, 0.31 mmol). The reaction mixture was heated under reflux for 2 days, poured into saturated NH_4Cl solution and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (10:1) gave **3** (15.5 g, quant.) as a colorless oil. $n_D^{25}=1.4957$. IR (film): $\nu=1613$, 1514, 1464, 1248, 1099, 1038 cm^{-1}. ^{1}H NMR ($CDCl_3$): $\delta=0.88$ (3H, t, $J=6.5$ Hz,), 1.2–1.4 (8H, m), 1.98 (2H, br q, $J=6.0$ Hz), 2.30 (2H, br q, $J=6.5$ Hz), 3.45 (2H, t, $J=7.0$ Hz), 3.81 (3H, s), 4.45 (2H, s), 5.41 (1H, dt, $J=15.0$, 6.0 Hz), 5.47 (1H, dt, $J=15.0$, 6.0 Hz), 6.88 (2H, d, $J=8.5$ Hz), 7.26 (2H, d, $J=8.5$ Hz). Anal. Calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 78.00; H, 10.12.

3.2.2. (3*S*,4*S*)-1-(4-Methoxybenzyloxy)decane-3,4-diol (4). A mixture of AD-mix-α (70 g) and methanesulfonamide (4.8 g, 50.5 mmol) in Bu^{t}OH (240 mL) and water (240 mL) was stirred at room temperature for 30 min. The mixture was cooled down to 0 °C and then, **3** (13.8 g, 50 mmol) was added to it. Stirred at 4 °C overnight, Na_2SO_3 (100 g) was added to the mixture. After stirring at room temperature for 30 min, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (3:1) gave crude **4** (95% ee) as colorless crystals. Crude product was recrystallized from hexane/EtOAc (20:1) to yield pure **4** (11.5 g, 74% recovery, >99% ee) as colorless crystals. HPLC [column: Daicel Chiralcel OD (0.46 cm×25 cm), eluent: hexane/ethanol (98:2), flow rate: 1.0 mL/min, detection: UV (254 nm)]: $t_R=21$ min [0.3%, (*R*,*R*)-isomer], 25 min [99.7%, (*S*,*S*)-isomer]. Mp=49.5–50.0 °C. $[\alpha]_D^{29}$ −2.2 (*c* 1.1, $CHCl_3$). IR (KBr): $\nu=3464$, 3374, 1513, 1249, 1086 cm^{-1}. ^{1}H NMR ($CDCl_3$): $\delta=0.88$ (3H, t, $J=6.6$ Hz,), 1.2–1.6 (10H, m), 1.7–1.9 (2H, m), 2.47 (1H, d, $J=5.5$ Hz), 3.17 (1H, d, $J=4.5$ Hz), 3.42 (1H, br m), 3.6–3.75 (3H, m), 3.81 (3H, s), 4.46 (2H, s), 6.89 (2H, d, $J=9.0$ Hz), 7.24 (2H, d, $J=9.0$ Hz). Anal. Calcd for $C_{18}H_{30}O_4$: C, 69.64; H, 9.74. Found: C, 69.69; H, 9.81.

3.2.3. (1*S*)-1-[(4*S*)-2-(4-Methoxyphenyl)-1,3-dioxan-4-yl] heptan-1-ol (5). DDQ (5.8 g, 19 mmol) was added to a solution of diol (**4**) in CH_2Cl_2 (120 mL) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was poured into 10% $Na_2S_2O_3$ solution and extracted with ether. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous sodium sulfate and concentrated in vacuo to provide *p*-methoxybenzylidene acetal **5** (5.8 g) as a yellow oil. This crude product was used in the next reaction without further purification. ^{1}H NMR (C_6D_6): $\delta=0.91$ (3H, t, $J=6.6$ Hz), 1.2–1.5 (10H, m), 1.6–1.8 (2H, m), 2.30 (1H, d, $J=4.0$ Hz), 3.26 (3H, s), 3.35–3.5 (3H, m), 3.96 (1H, ddd, $J=1.0$, 5.0, 11.0 Hz), 5.34 (1H, s), 6.82 (2H, d, $J=11.0$ Hz), 7.54 (2H, d, $J=11.0$ Hz).

3.2.4. (1*S*)-1-Methoxymethoxy-1-[(4*S*)-2-(4-methoxyphenyl)-1,3-dioxan-4-yl]heptane (6). To a solution of crude *p*-methoxybenzylidene acetal (5.8 g) in CH_2Cl_2 (180 mL) was added Pr^i_2EtN (9.0 mL, 52 mmol) and MOMCl (3.0 mL, 40 mmol). This solution was stirred at room temperature overnight, poured into water and extracted with ether. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous sodium sulfate and concentrated in vacuo to give MOM ether **6** (5.9 g) as a yellow oil. This crude product was used in the next reaction without further purification. ^{1}H NMR (C_6D_6): $\delta=0.90$ (3H, t, $J=6.6$ Hz,), 1.07 (1H, d, $J=13.0$ Hz), 1.2–1.85 (11H, m), 3.24 (6H, s), 3.56 (1H, dt, $J=2.5$, 12.0 Hz), 3.69 (1H, br m), 3.83 (1H, ddd, $J=2.5$, 6.0, 12.0 Hz), 4.02 (1H, br dd, $J=5.0$, 12.0 Hz), 4.66 (1H, d, $J=6.5$ Hz), 4.81 (1H, d, $J=6.5$ Hz), 5.42 (1H, s), 6.80 (2H, d, $J=8.5$ Hz), 7.61 (2H, d, $J=8.5$ Hz).

3.2.5. (3*S*,4*S*)-4-(Methoxymethoxy)decane-1,3-diol (7). A mixture of crude **6** (5.9 g), acetic acid (5.0 mL), water (5.0 mL) and THF (15 mL) was refluxed for 2 h. The reaction mixture was poured into saturated $NaHCO_3$

solution and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (1:1–1:3) gave **7** (3.6 g, 82% in three steps) as a colorless oil. $n_D^{23}=1.4527$. $[\alpha]_D^{22}$ +24 (*c* 1.0, $CHCl_3$). IR (film): $\nu=3400$, 1468, 1152, 1100, 1039 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=0.86$ (3H, t, $J=6.9$ Hz,), 1.25–1.6 (10H, m), 1.65–1.75 (2H, m), 2.81 (1H, t, $J=$ 5.5 Hz), 3.37 (1H, ddd, $J=4.5$, 6.5, 6.5 Hz), 3.43 (3H, s), 3.48 (1H, d, $J=3.0$ Hz), 3.7–1.8 (1H, m), 3.85 (2H, q, $J=$ 5.5 Hz), 4.69 (1H, d, $J=7.0$ Hz), 4.73 (1H, d, $J=7.0$ Hz). FAB-HRMS *m/z* calcd for $C_{12}H_{27}O_4$ $[M+H]^+$ 235.1909, found 235.1906.

3.2.6. (3*S*,4*S*)-4-(Methoxymethoxy)-1-(1-phenyl-1*H*-tetrazole-5-sulfonyl)decan-3-ol (8). The 40% solution of diisopropyl azodicarboxylate in toluene (5.8 mL, 11 mmol) was added to a solution of diol **7** (2.0 g, 8.5 mmol), PPh_3 (2.3 g, 8.8 mmol) and 1-phenyl-5-mercapto-1*H*-tetrazole (1.9 g, 11 mmol) in THF (60 mL) at −20 °C. Stirred at 0 °C for 20 min, the reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (2:1) gave crude sulfide as a colorless oil. To the solution of crude sulfide in ethanol (100 mL) was added the mixture of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (1.5 g, 1.2 mmol) and 30% H_2O_2 solution (6.0 mL, 53 mmol) at 0 °C. Stirred at room temperature for 6 h, the reaction mixture was poured into 10% $Na_2S_2O_3$ solution and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (4:1) gave crude **8** as a colorless oil. This crude product was used in the next reaction without further purification. ^1H NMR ($CDCl_3$): $\delta=0.88$ (3H, t, $J=$ 6.6 Hz), 1.2–1.6 (10H, m), 2.0–2.2 (2H, m), 3.17 (1H, d, $J=$ 5.0 Hz), 3.35 (1H, dt, $J=6.5$, 5.5 Hz), 3.41 (3H, s), 3.5–3.7 (1H, m), 3.8–4.1 (2H, m), 4.68 (1H, d, $J=6.5$ Hz), 4.70 (1H, d, $J=6.5$ Hz), 7.6–7.7 (5H, m).

3.2.7. (3*S*,4*S*)-3-(*tert*-Butyldimethylsilyloxy)-4-(methoxymethoxy)-1-(1-phenyl-1*H*-tetrazole-5-sulfonyl)decane (9). To a solution of crude **8** in CH_2Cl_2 (80 mL) was added TBSOTf (2.6 mL, 11 mmol) and 2,6-lutidine (1.7 mL, 15 mmol). The reaction mixture was stirred at room temperature for 30 min, poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (20:1) gave **9** (4.2 g, 91% in three steps) as a colorless oil. $n_D^{24}=1.4951$. $[\alpha]_D^{24}$ −18 (*c* 1.0, $CHCl_3$). IR (film): $\nu=1344$, 1151, 1097, 1039 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=0.10$ (3H, s), 0.11 (3H, s), 0.88 (3H, t, $J=$ 6.5 Hz), 0.90 (9H, s), 1.25–1.7 (10H, m), 2.01 (1H, m), 2.24 (1H, m), 3.38 (3H, s), 3.45 (1H, m), 3.82 (2H, m), 3.95 (1H, ddd, $J=4.2$, 4.2, 8.4 Hz), 4.61 (1H, d, $J=7.0$ Hz), 4.67 (1H, d, $J=7.0$ Hz), 7.6–7.7 (5H, m). FAB-HRMS *m/z* calcd for $C_{25}H_{45}N_4O_5SSi$ $[M+H]^+$ 541.2880, found 541.2877.

3.2.8. (*E*)-6-Benzyloxyhex-4-enoic acid methyl ester (11). According to the reported manner,[15,21] diol **10** (32 g, 0.36 mol) was treated with benzyl bromide to give monobenzyl ether as the mixture of primary and secondary alcohols. Propionic acid (1.6 mL) was added to the solution of this mixture in trimethyl orthoacetate (280 mL). The reaction mixture was refluxed for 2 h with absorbing generated methanol by molecular sieves 4 Å. After cooling, the reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (5:1) gave **11** (41 g, 48% in two steps) as a colorless oil. $n_D^{26}=1.5042$. IR (film): $\nu=$ 1739, 1437, 1362, 1166, 1116 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=$ 2.35–2.45 (4H, m), 3.68 (3H, s), 3.97 (2H, d, $J=5.0$ Hz), 4.50 (2H, s), 5.6–5.8 (2H, m), 7.25–7.35 (5H, m). Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.27; H, 7.77.

3.2.9. (5*R*)-5-[(1*R*)-2-Benzyloxy-1-hydroxyethyl]tetrahydrofuran-2-one (14). The mixture of AD-mix-β (160 g) and methanesulfonamide (11 g, 0.12 mol) in Bu^tOH (550 mL) and water (550 mL) was stirred at room temperature for 30 min. The mixture was cooled down to 0 °C and then **11** (27 g, 0.12 mol) was added to it. Stirred at 4 °C overnight, Na_2SO_3 (50 g) was added to the mixture. After stirring at room temperature for 30 min, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (2:1–1:1) gave crude **14** (22.5 g, 83%, 95% ee) as a colorless crystal. Crude product was recrystallized from hexane–EtOAc to yield pure **14** (19.3 g, 86% recovery, >99% ee) as colorless crystals. HPLC [column: Daicel Chiralcel OD-H (0.46 cm×25 cm), eluent: hexane/Pr^iOH (9:1), flow rate: 1.0 mL/min, detection: UV (254 nm)]: $t_R=19.4$ min [99.9%, (*R*,*R*)-isomer], 22.3 min [0.1%, (*S*,*S*)-isomer]. Mp=105.0–106.5 °C. $[\alpha]_D^{27}$ −47 (*c* 1.0, $CHCl_3$). IR (KBr): $\nu=3469$, 1759, 1197, 1083 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=2.2$–2.3 (2H, m), 2.4–2.7 (3H, m), 3.60 (2H, d, $J=6.0$ Hz), 3.83 (1H, m), 4.56 (2H, s), 4.55–4.6 (1H, m), 7.3–7.4 (5H, m). Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 66.11; H, 6.84.

3.2.10. 3-[(4*R*,5*R*)-5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl]propionic acid methyl ester (13). Lithium hydroxide monohydrate (4.0 g, 95 mmol) was added to the mixture of lactone **14** (10 g, 43 mmol), THF (100 mL) and water (100 mL). Stirred at room temperature for 1 h, the reaction mixture was poured into 0.3 N HCl and extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was dissolved in 2,2-dimethoxypropane (50 mL) and acetone (100 mL) and stirred at room temperature overnight. After evaporation, the residue was dissolved in ethyl acetate (100 mL) and treated with a solution of CH_2N_2 in ether. The reaction mixture was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (5:1) gave

13 (11 g, 88%) as a colorless oil. $n_D^{25}=1.4879$. $[\alpha]_D^{26}$ +15 (*c* 1.5, $CHCl_3$). IR (film): $\nu=1739$, 1449, 1373, 1081 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=1.39$ (3H, s), 1.40 (3H, s), 1.8–2.05 (2H, m), 2.4–2.6 (2H, m), 3.5–3.6 (2H, m), 3.67 (3H, s), 3.8–3.9 (2H, m), 4.59 (2H, s), 7.3–7.4 (5H, m). Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 65.90; H, 7.66.

3.2.11. 3-[(4*R*,5*R*)-5-Hydroxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl]propionic acid methyl ester (15). To a solution of **13** (5.0 g, 17 mmol) in Pr^iOH (135 mL) was added 10% Pd/C (400 mg) and the mixture was stirred overnight under H_2 atmosphere. The mixture was filtered through Celite® and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (2:1) gave **15** (3.7 g, quant.) as a colorless oil. $n_D^{27}=1.4472$. $[\alpha]_D^{24}$ +29 (*c* 1.0, $CHCl_3$). IR (film): $\nu=3476$, 1739, 1441, 1375, 1250, 1166, 1073 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=1.40$ (6H, s), 1.8–2.1 (3H, m), 2.4–2.6 (2H, m), 3.6–3.85 (3H, m), 3.69 (3H, s), 3.91 (1H, dt, $J=3.3$, 8.1 Hz). FAB-HRMS *m/z* calcd for $C_{10}H_{19}O_5$ $[M+H]^+$ 219.1233, found 219.1237.

3.2.12. 3-[(4*R*,5*S*)-5-Formyl-2,2-dimethyl-1,3-dioxolan-4-yl]propionic acid methyl ester (16). 4-Methoxy-2,2,6,6-tetramethylpiperidine 1-oxyl (750 mg, 4.0 mmol) was added to a mixture of alcohol **15** (2.8 g, 13 mmol), 0.8 M NaOCl solution (19 mL, 15 mmol), KBr (750 mg, 6.3 mmol), $NaHCO_3$ (1.6 g, 19 mmol), CH_2Cl_2 (160 mL) and water (80 mL) at 0 °C and the mixture was stirred at 0 °C for 15 min. The reaction mixture was poured into 10% $Na_2S_2O_3$ solution and extracted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (2:1–1:1) gave crude **16** (1.95 g, ca. 70%) as a colorless oil. This crude product was used in the next reaction without further purification. ^1H NMR ($CDCl_3$): $\delta=1.41$ (6H, s), 1.85–2.15 (2H, m), 2.4–2.6 (2H, m), 3.69 (3H, s), 3.98 (1H, dd, $J=2.0$, 7.5 Hz), 4.10 (1H, dt, $J=4.5$, 7.5 Hz), 9.74 (1H, d, $J=2.0$ Hz).

3.2.13. 3-[(4*R*,5*R*)-5-{(1*E*,4*S*,5*S*)-4-*tert*-Butyldimethylsilyloxy-5-(methoxymethoxy)undec-1-enyl}-2,2-dimethyl-1,3-dioxolan-4-yl]propionic acid methyl ester (17). To a solution of sulfone **9** (1.0 g, 1.9 mmol) and 18-crown-6 (740 mg, 2.8 mmol) in THF (40 mL) was added 0.5 M solution of KHMDS in toluene (4.4 mL, 2.2 mmol) at −100 °C. Stirred at −100 °C for 30 min, a solution of aldehyde **16** (600 mg, 2.78 mmol) in THF (10 mL) was dropped into this solution. The reaction mixture was allowed to warm to room temperature during 2 h and poured into saturated NH_4Cl solution. This mixture was extracted with ethyl acetate and the organic layer was washed with saturated $NaHCO_3$ solution and brine. After drying with anhydrous magnesium sulfate, solvent was removed in vacuo and the residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (10:1) gave **17** (645 mg, 66%) and *cis*-isomer (62 mg, 6%) as colorless oils. $n_D^{25}=1.4550$. $[\alpha]_D^{25}$ −20 (*c* 0.60, $CHCl_3$). IR (film): $\nu=1743$, 1252, 1162, 1044 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=0.05$ (6H, s), 0.89 (9H, s), 0.85–0.95 (3H, m), 1.25–1.5 (9H, m), 1.39 (6H, s), 1.5–1.7 (1H, m), 1.75–2.0 (2H, m), 2.15 (1H, ddd, $J=7.5$, 8.5, 15.0 Hz), 2.3–2.6 (3H, m), 3.37 (3H, s), 3.35–3.45 (1H, m), 3.6–3.7 (1H, m), 3.67 (3H, s), 3.75 (1H, dt, $J=8.5$, 4.3 Hz), 3.98 (1H, t, $J=7.5$ Hz), 4.62 (1H, d, $J=6.6$ Hz), 4.69 (1H, d, $J=6.6$ Hz), 5.46 (1H, dd, $J=7.5$, 15.0 Hz), 5.85 (1H, dt, $J=15.0$, 7.5 Hz). Anal. Calcd for $C_{28}H_{54}O_7Si$: C, 63.36; H, 10.25. Found: C, 63.36; H, 10.31.

3.2.14. 3-[(4*R*,5*R*)-5-{(1*E*,4*S*,5*S*)-4-Hydroxy-5-methoxymethoxyundec-1-enyl}-2,2-dimethyl-1,3-dioxolan-4-yl]propionic acid methyl ester (18). To a solution of **17** (530 mg, 1.0 mmol) in THF (10 mL) was added 1.0 M TBAF solution in THF (2.0 mL, 2 mmol) and the solution was stirred at room temperature for 2 h. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (4:1) gave **18** (412 mg, 99%) as a colorless oil. $n_D^{27}=1.4621$. $[\alpha]_D^{27}$ +13 (*c* 0.90, $CHCl_3$). IR (film): $\nu=3482$, 1740, 1441, 1373, 1221, 1163, 1037 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=0.88$ (3H, t, $J=6.6$ Hz), 1.25–1.65 (10H, m), 1.34 (6H, s), 1.75–2.0 (2H, m), 2.2–2.6 (4H, m), 2.75 (1H, d, $J=4.8$ Hz), 3.38 (1H, m), 3.41 (3H, s), 3.59 (1H, m), 3.67 (3H, s), 3.6–3.7 (1H, m), 4.01 (1H, t, $J=8.0$ Hz), 4.69 (2H, s), 5.52 (1H, dd, $J=8.0$, 15.3 Hz), 5.90 (1H, dt, $J=15.3$, 7.5 Hz). FAB-HRMS *m/z* calcd for $C_{22}H_{41}O_7$ $[M+H]^+$ 417.2852, found 417.2866.

3.2.15. 3-[(4*R*,5*R*)-5-{(1*E*,4*S*,5*S*)-4-Hydroxy-5-methoxymethoxyundec-1-enyl}-2,2-dimethyl-1,3-dioxolan-4-yl]propionic acid (19). A mixture of **18** (368 mg, 0.88 mmol), $LiOH\cdot H_2O$ (75 mg, 1.8 mmol), THF (7 mL) and water (5 mL) was stirred at room temperature for 2 h. The reaction mixture was poured into 10% tartaric acid solution and extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was suspended in $CHCl_3$, filtered through Celite® and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (1:1) gave crude **19** (400 mg) as a colorless oil. This crude product was used in the next reaction without further purification. IR (film): $\nu=3446$, 3300–2500 (br), 1714, 1377, 1219, 1157, 1101, 1038 cm^{-1}. ^1H NMR ($CDCl_3$): $\delta=0.89$ (3H, t, $J=6.6$ Hz), 1.25–1.65 (10H, m), 1.40 (6H, s), 1.8–2.05 (2H, m), 2.2–2.6 (4H, m), 3.35–3.45 (1H, m), 3.42 (3H, s), 3.6–3.75 (2H, m), 4.03 (1H, t, $J=8.0$ Hz), 4.70 (2H, s), 5.53 (1H, m), 5.90 (1H, dt, $J=15.0$, 7.2 Hz).

3.2.16. 4,5-*O*-Isopropylidene-10-*O*-methoxymethylmicrocarpalide (20). Triethylamine (280 μL, 2.0 mmol) and 2,4,6-trichlorobenzoyl chloride (200 μL, 1.3 mmol) were added to a solution of the crude hydroxy acid **19** (400 mg) in THF (7 mL). The reaction mixture was stirred at room temperature for 5 h and then filtered through Celite® under argon. The resultant solution was slowly added over 22 h using syringe pump to a refluxing solution of DMAP (2.2 g, 18 mmol) in dry benzene (1000 mL). After the addition was complete, the reaction mixture was stirred for additional 2 h and then concentrated in vacuo. The residue was dissolved in ethyl acetate and washed with 1 N HCl, saturated

$NaHCO_3$ solution and brine. The organic layer was dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with hexane/ethyl acetate (6:1) gave **20** (320 mg, 94%) as a colorless oil. $n_D^{26}=1.4742$. $[\alpha]_D^{24}$ −37 (*c* 0.60, $CHCl_3$). IR (film): $\nu=1735$, 1456, 1372, 1236, 1163, 1063 cm^{-1}. ^1H NMR (CD_3CN, observed as a mixture of two conformers in a ratio of 3:1) Major conformer: $\delta=0.89$ (3H, t, $J=6.6$ Hz), 1.25–1.65 (10H, m), 1.41 (6H, s), 1.9–1.25 (2H, m), 2.25–2.7 (4H, m), 3.41 (3H, s), 3.6–3.7 (2H, m), 3.93 (1H, t, $J=9.0$ Hz), 4.68 (1H, d, $J=7.2$ Hz), 4.71 (1H, d, $J=7.2$ Hz), 4.93 (1H, dt, $J=8.7$, 3.5 Hz), 5.33 (1H, dd, $J=9.0$, 15.0 Hz), 5.74 (1H, ddd, $J=4.5$, 11.0, 15.0 Hz). Minor conformer: $\delta=0.89$ (3H, t, $J=6.6$ Hz), 1.25–1.65 (11H, m), 1.41 (6H, s), 2.25–2.7 (5H, m), 3.41 (3H, s), 3.6–3.7 (1H, m), 3.76 (1H, dt, $J=2.5$, 9.5 Hz), 3.8–3.9 (1H, m), 4.68 (1H, d, $J=7.2$ Hz), 4.71 (1H, d, $J=7.2$ Hz), 5.09 (1H, br m), 5.7–5.8 (1H, m), 5.90 (1H, dt, $J=16.0$, 8.0 Hz). FAB-HRMS *m/z* calcd for $C_{21}H_{37}O_6$ $[M+H]^+$ 385.2590, found 385.2596.

3.2.17. Microcarpalide (1). To a solution of **20** (89 mg, 0.23 mmol) and 1,2-ethanedithiol (90 μL, 1.1 mmol) in CH_2Cl_2 (10 mL) was added $BF_3\cdot OEt_2$ (55 μL, 0.43 mmol) at −20 °C and the mixture was stirred at −20–−10 °C for 40 min. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel. Elution with $CHCl_3$/MeOH (20:1) gave **1** (46 mg, 66%) and MOM ether **21** (12 mg, 15%) both as colorless oils. $n_D^{19}=1.4965$. $[\alpha]_D^{26}$ −29 (*c* 0.67, MeOH). IR (film): $\nu=3438$, 2929, 2857, 1712, 1225, 1155, 1065 cm^{-1}. ^1H NMR (CD_3CN, observed as a mixture of two conformers in a ratio of 3.5:1) Major conformer: δ (ppm) 0.88 (3H, t, $J=6.9$ Hz), 1.2–1.4 (8H, m), 1.3–1.5 (2H, m), 1.7–1.8 (1H, m), 2.0–2.2 (2H, m), 2.1–2.3 (2H, m), 2.4–2.6 (1H, m), 2.8–2.9 (2H, br m), 3.12 (1H, br d), 3.54 (1H, br m), 3.77 (1H, br), 4.10 (1H, br), 4.81 (1H, ddd, $J=3.3$, 4.8, 11.1 Hz), 5.49 (1H, dddd, $J=2.1$, 5.1, 9.9, 15.6 Hz), 5.69 (1H, dd, $J=2.4$, 15.6 Hz). Minor conformer: δ (ppm) 0.88 (3H, t, $J=6.9$ Hz), 1.2–1.4 (8H, m), 1.3–1.5 (2H, m), 1.7–1.8 (1H, m), 2.0 (1H, m), 2.0–2.2 (1H, m), 2.2–2.4 (1H, m), 2.4–2.6 (2H, m), 2.8–2.9 (2H, br m), 3.2–3.3 (2H, br m), 3.5–3.6 (2H, m), 4.60 (1H, ddd, $J=2.7$, 4.5, 8.1 Hz), 5.05 (1H, dd, $J=9.3$, 15.6 Hz), 5.6–5.7 (1H, m). ^{13}C NMR (CD_3CN, observed as a mixture of two conformers): δ (ppm) 14.4, 23.3, 26.1, 26.3, 26.3, 26.4, 29.0, 29.9, 32.1, 32.2, 32.5, 33.8, 34.1, 35.9, 36.7, 72.4, 72.8, 73.4, 73.8, 76.4, 76.9, 79.5, 79.7, 126.6, 130.0, 133.7, 134.5, 173.5, 176.4. FAB-HRMS *m/z* calcd for $C_{16}H_{29}O_5$ $[M+H]^+$ 301.2015, found 301.2003.

3.2.18. 4,5-*O*-Isopropylidene-microcarpalide (23) and (4*R*,5*R*,6*E*,9*S*,10*S*)-4,5-Isopropylidenedioxy-9-hydroxy-6-hexadecen-10-olide (24). To a solution of **20** (112 mg, 0.29 mmol) in MeOH (6 mL) and water (1.2 mL) was added DOWEX®-50W X8 (1.0 g) and the mixture was stirred at 50 °C for 5 days. The reaction mixture was diluted with ethyl acetate and filtered through Celite®. After concentration, the residue was chromatographed over silica gel. Elution with $CHCl_3$/MeOH (20:1) gave a mixture of **1** and **22** (86 mg, 98%) as a colorless oil. This mixture (45 mg, 0.15 mmol) was dissolved in 2,2-dimethoxypropane (1 mL) and acetone (1 mL) and treated with PPTS (10 mg, 0.04 mmol). Stirred at room temperature overnight, the reaction mixture was diluted with ethyl acetate and washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by preparative TLC [hexane/ethyl acetate (3:1)] to give **23** (38 mg, 74%) and **24** (7 mg, 14%) both as colorless oils.

Compound **23**. $[\alpha]_D^{27}$ −36 (*c* 0.40, $CHCl_3$). ^1H NMR ($CDCl_3$, observed as a mixture of two conformers in a ratio of 3.5:1) Major conformer: δ (ppm) 0.88 (3H, t, $J=6.5$ Hz), 1.2–1.5 (10H, m), 1.41 (6H, s), 1.97 (1H, dddd, $J=3.5$, 8.0, 12.0, 15.0 Hz), 2.09 (1H, br ddd, $J=4.5$, 5.5, 15.0 Hz), 2.32 (1H, ddd, $J=4.5$, 12.0, 13.5 Hz), 2.42 (1H, br ddd, $J=2.5$, 4.5, 12.0 Hz), 2.54 (1H, ddd, $J=3.5$, 5.5, 13.5 Hz), 2.67 (1H, ddd, $J=9.0$, 11.0, 12.0 Hz), 3.6–3.7 (2H, m), 3.92 (1H, t, $J=9.0$ Hz), 4.71 (1H, ddd, $J=2.5$, 4.5, 9.0 Hz), 5.33 (1H, dd, $J=9.5$, 15.5 Hz), 5.78 (1H, ddd, $J=4.5$, 11.0, 15.5 Hz). Minor conformer: δ (ppm) 0.88 (3H, t, $J=6.5$ Hz), 1.2–1.7 (11H, m), 1.41 (6H, s), 2.0–2.7 (5H, m), 3.6–3.65 (1H, m), 3.77 (1H, br dt, $J=4.0$, 10.0 Hz), 3.8–3.9 (1H, m), 4.94 (1H, br m), 5.65–5.75 (1H, m), 5.85–5.95 (1H, m). FAB-HRMS *m/z* calcd for $C_{19}H_{33}O_5$ $[M+H]^+$ 341.2328, found 341.2301.

Compound **24**. ^1H NMR ($CDCl_3$): $\delta=0.87$ (3H, t, $J=6.5$ Hz), 1.2–1.8 (10H, m), 1.39 (3H, s), 1.40 (3H, s), 2.05–2.55 (6H, m), 3.63 (1H, m), 3.87 (1H, dt, $J=9.0$, 5.0 Hz), 3.97 (1H, t, $J=8.5$ Hz), 4.92 (1H, dd, $J=5.0$, 9.0 Hz), 5.53 (1H, dd, $J=8.5$, 16.0 Hz), 5.84 (1H, dt, $J=16.0$, 8.0 Hz). FAB-HRMS *m/z* calcd for $C_{19}H_{33}O_5$ $[M+H]^+$ 341.2328, found 341.2301.

3.2.19. (4*R*,5*R*,6*E*,9*S*,10*S*)-4,5,9-Trihydroxy-6-hexadecen-10-olide (22). To a solution of **24** (6.0 mg, 0.018 mmol) in MeOH (1.5 mL) was added PPTS (1 mg, 0.004 mmol) and the mixture was stirred at room temperature for 3 days. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried with anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by preparative TLC [$CHCl_3$/MeOH (10:1)] to give **22** (4.5 mg, 85%) as a colorless oil. $[\alpha]_D^{26}$ −17 (*c* 0.32, MeOH). ^1H NMR (CD_3CN): δ (ppm) 0.87 (3H, t, $J=6.5$ Hz), 1.2–1.35 (8H, m), 1.60 (2H, m), 1.8–1.9 (2H, m), 2.1–2.2 (2H, m), 2.3–2.4 (2H, m), 2.97 (1H, d, $J=7.0$ Hz), 3.11 (1H, d, $J=3.5$ Hz), 3.20 (1H, d, $J=3.5$ Hz), 3.40 (1H, m), 3.63 (1H, dt, $J=3.5$, 7.0 Hz), 3.88 (1H, tt, $J=2.5$, 7.0 Hz), 4.81 (1H, dt, $J=2.5$, 7.0 Hz), 5.47 (1H, ddt, $J=7.0$, 15.0, 1.0 Hz), 5.76 (1H, dt, $J=15.0$, 7.0 Hz). FAB-HRMS *m/z* calcd for $C_{16}H_{29}O_5$ $[M+H]^+$ 301.2015, found 301.2036.

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Reactions of (*Z*)-3-aryl-3-chloropropenals with nucleophiles: stereoselective formation of (*E*)-vinylogous esters, (*E*)-vinylogous amides, and vinamidinium salts

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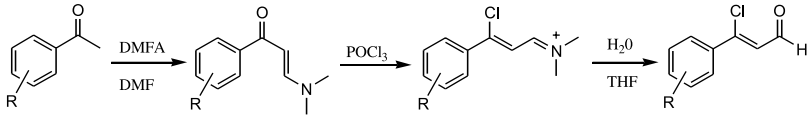
Abstract—The highly stereoselective conversions of (*Z*)-3-aryl-3-chloropropenals to (*E*)-3-alkoxy-3-arylpropenals, to (*E*)-3-aryl-3-morphorlinopropenals, and to vinamidinium salts are reported. The stereochemical assignments were based on 2D-NMR experiments.

1. Introduction

For the last 100 years 1,3-dicarbonyl compounds[1–4] and their functional group equivalents have served admirably as precursors to a plethora of heterocyclic compounds. In some of these cases the regiochemistry of the ring forming reactions has been a focus of interest. The use of 3-aryl-3-chloropropenimium salts, vinamidinium salts, and 3-chloroenals has received considerable attention.[5–7] In our labs we have developed routes to convert 3-aryl-3-chloropropenals to 5-chloropentadienoates and pent-2-en-4-ynoates and to such heterocyclics as pyrroles and pyrazoles.[8–10] Our interest in these transformations results from the anti-tumor activity exhibited by a number of heterocyclic containing natural products which suggests that general routes allowing regioselective syntheses of pyrroles and pyrazoles offer potential for use in drug discovery and synthesis.[11–14]

The (*Z*)-chloroenals employed in this study were readily prepared from substituted acetophenones which were converted to vinylogous amides with dimethyl formamide dimethyl acetal (Scheme 1). Subsequent treatment with $POCl_3$ and in situ hydrolysis of the intermediate chloropropeniminium salts resulted in good yields of the (*Z*)-chloroenals (**1**).[15]



a R = H
b R = p-tolyl
c R = 4-methoxyphenyl
d R = 4-chlorophenyl
e R = 4-fluorophenyl
f R = 3,4-methylenedioxyphenyl

1 a-f

Scheme 1.

Keywords: 3-Chloropropenals; Vinylogous esters; Vinylogous amides; Vinamidinium salts.
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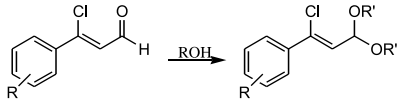
In the course of our studies with the chloroenals we encountered some difficulty in reproducibility when using alcohols as solvents. To better understand what was happening we looked more closely at the reactions of alcohols with the chloroenals.

In doing so we found that the 3-chloroenals can be regioselectively derivatized with alcohols, the regioselectivity being remarkably controlled by reaction conditions as indicated by the following examples. These derivatives offer the potential of serving as useful synthetic intermediates.

2. Results and discussion

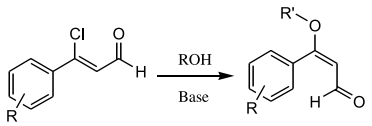
For example, stirring 3-chloro-3-(*p*-tolyl)-propenal **1b** with methanol or ethanol at room temperature results in the rapid formation of the corresponding acetals **2b** and **3b** (Scheme 2). The stereochemistry of the acetals are clear from NOESY experiments which show a strong interaction between the 2′-hydrogen on the aromatic ring and the vinyl hydrogen but no interaction with the hydrogen on the acetal carbon. Gas chromatographic analysis of the reaction mixture indicates that conversion is 90% within 5 min. Stirring overnight takes the reaction to greater than 95% conversion. Essentially no other products are observed. Although acetals are generally formed under acid catalyzed conditions, the presence of acid was not necessary in this case. Evaporation of the alcohol resulted in good yields of the chloroacetals **2**. Furthermore, the reaction seemed to be general thus allowing for the formation of **2a–e** and **3a** and **3b**. The acetals were not particularly stable and often tended not to survive for high resolution mass spectral analysis, but when first formed appeared by proton and carbon NMR and GC–MS analysis to be better than 95% pure. Successful HRMS analyses were obtained for several in the series.

If cesium carbonate is added to the alcohol prior to the addition of the enal **1b**, stirring at room temperature results in a clean addition elimination reaction forming the 3-*p*-tolyl-3-alkoxypropenals **4** and **5** (Scheme 3). Again this proved to be a general reaction proceeding equally well with all members of the series (**a–d**,**f**).



1 a-e **2 a-e** R' = methyl
3 a-b R' = ethyl

Scheme 2.



1 **4** R' = methyl
5 R' = ethyl

Scheme 3.

This reaction of **1b** with methanol was also followed by gas chromatography. Surprisingly the formation of the chloroacetal **2b** was not observed. Instead the conversion to **4b** appeared to be effectively complete within 5 min. The stereoselectivity observed was also remarkable. The 3-aryl-3-chloroenals as prepared from the corresponding vinylogous amides are in all cases more than 95% the (*Z*)-isomer. The 3-alkoxy-3-arylpropenals isolated are in all cases more than 95% the (*E*)-isomer. The stereochemical assignments were made on the basis of NOESY experiments. Strong interactions were noted for Ha and Hb in the chloroenals **1** whereas strong connections were observed for Hb and Hc in the vinylogous esters **4** and **5** (see Fig. 1). These assignments are compatible with the observed relative positions of the aldehydic protons in the NMR spectra assuming that when the aldehyde is *cis* to the aryl group it is shifted up-field.[16]

Interestingly, the acetals **2** are only slowly converted to the 3-alkoxyenals **4** under the reaction conditions suggesting that **1** is directly converted to **4**, and **2** is not directly involved in the conversion. The presence of base appears to inhibit the formation of the acetal and provides for clean conjugate addition/elimination.

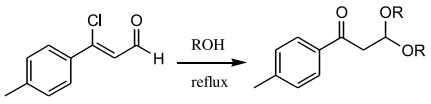
Hudlicky, Olivo, and Natchus have shown that β-methoxyenones are useful synthons for a wide range of synthetic targets.[17] These 3-alkoxyenals analogs (**4** and **5**) offer the same potential. As an example, the reaction of 3-alkoxypropenals with glycine esters to form 2-substituted pyrroles was demonstrated by Walizei and Breitmaier.[18]

Other related synthons, the β-keto dimethylacetals, are of some interest as useful synthetic intermediates.[19] Refluxing the chloroenal **1b** in methanol or ethanol results in the formation of 1-*p*-tolyl-3,3-dialkoxy-1-propanones **6b** and **7b** accompanied by some 4′-methyl acetophenone (Scheme 4). The ketone is presumably the result of the addition of water to the alkene followed by the elimination of HCl.

There was some lack of reproducibility in the time required for this conversion to take place. The reaction of **1b** was thus followed by GC–MS. As indicated above, as soon as the chloroenal was dissolved in methanol it was converted to the chloroacetal **2**. Refluxing for up to 2 h did nothing but push the conversion to equilibrium. After 5 h of reflux there was an approximately 50% conversion of the chloroacetal cleanly to the dialkoxypropanone **6b** accompanied by a trace of 4′-methylacetophenone. After 6 h of reflux the conversion to **6b** was essentially complete although the appearance of the acetophenone was more pronounced. The observation that refluxing for several hours produced no discernable reaction product but that after that point in time conversion to the propanone **6b** was relatively rapid

1 **4,5**

Figure 1.



1b **6b** R = methyl
7b R = ethyl

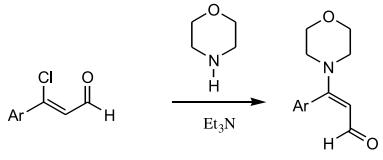
Scheme 4.

suggests that auto-catalysis by the HCl generated in the reaction is occurring. Careful monitoring of the reaction is required to get clean ketone without significant contamination by *p*-acetophenone since the solvolysis of the ketoacetals to form 4′-methylacetophenone becomes problematic if the reaction is refluxed too long.

The above observations demonstrate how subtle differences in reaction conditions in some instances can have a dramatic effect on the outcome of a seemingly simple reaction. These new derivatives, formed cleanly and easily in high yield, offer the potential to fine tune the regioselectivity of reactions of the 1,3-dicarbonyl functional group equivalents.

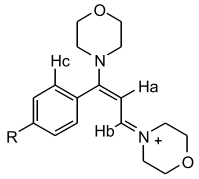
The related reactions of the chloroenals (**1**) with morpholine were also explored as behavior similar to that observed with alcohols under basic conditions was expected. Molho and Giraud have reported the similar reaction of dimethylamine with 3-chloro-3-phenylpropenal (**1a**) resulting in the formation of 3-dimethylamino-3-phenylpropenal.[20] Roy, Kar, and Ray have also reported the reaction of **1a** with anilines to form vinamidinium salts which were subsequently thermally cyclized to form 2-phenyl-6-substituted quinolines.[21] Indeed, stirring the 3-aryl-3-chloroenals with 1 equiv of morpholine and 3 equiv of triethylamine in acetonitrile provided a route to the vinylogous amides (**8**) (Scheme 5). NOESY experiments demonstrated a close through–space relationship of the aldehydic and aromatic hydrogens thus confirming the (*E*) stereochemistry of the vinylogous amides.

Stirring the chloroenals in acetonitrile with 2 equiv of morpholine followed by treatment with sodium hexafluorophosphate resulted in high yields of the corresponding 1,3-di-4-morpholino-1-(*p*-tolyl)-2-propenylium hexafluorophosphates (**9**) (vinamidinium salts) in each case, thus providing a new and convenient synthesis of this system. The vinamidinium salts (**9**) were shown to hydrolyze to the vinylogous amides (**6**) when stirred in water/THF. Vinamidinium salts have proven to be useful synthons in a variety of applications.[21–28] Our observations are not surprising since *N*-(3-aryl-3-methylthioallylidene)ammonium iodides have been shown to convert to vinamidinium salts.[29] We find the stereochemical consequence of the reaction to be quite interesting, however. Restricted rotation



1 a,b **8 a,b**

Scheme 5.



9

Figure 2.

is assumed about all of the bonds in the pi system since all eight of the methylene groups on the morpholino rings are resolved in the proton and the carbon spectra. Barriers to rotation have been estimated to be in the neighborhood of 17–18 kcal/mol in one related vinamidinium salt.[30] In every case in this study NOESY experiments showed strong interactions between Hb and Hc (see Fig. 2) which is consistent with the geometry shown.

These conclusions are in accord with the reported X-ray crystallographic analysis of an analogous vinamidinium salt, 2-phenyl-1,3-bis(dimethylamino)trimethinium hexafluorophosphate, which exists in an all *trans* 'W' configuration.[31]

Hartree–Fock calculations[32] using a 6-31G* basis set were performed on the (*Z*)- and (*E*)-isomers of the phenyl substituted chloroenal, vinylogous amide, and vinamidinium salt. The best equilibrium conformer was used as the starting point for the geometry optimization. We found it interesting that in each case the isomer that was isolated corresponded to the isomer with the lower calculated total energy suggesting that the stereochemistry is the result of thermodynamic control (see Table 1). The calculations also suggest that the aromatic ring is twisted out of overlap with the propenal π-system. The shielding region of the aromatic ring explains the relative chemical shifts of the aldehydic protons in the (*Z*)- and (*E*)-isomers.

3. Conclusions

We have shown that the reactions of (*Z*)-3-aryl-3-chloropropenals with alcohols (methanol and ethanol) to form one of three derivatives depending on the reaction conditions: propenal acetals (**2** and **3**), vinylogous esters (**4** and **5**), or ketoacetals (**6** and **7**). These reactions appear to be general proceeding in good yields with a variety of electron withdrawing and electron donating groups on the aryl substituent. Additionally, the reactions with morpholine provide useful routes to vinylogous amides **8** and vinamidinium salts (**9**). Furthermore, the stereochemistry is highly selective, converting the (*Z*)-isomers of **1** into the (*E*)-isomers of **4**, **5**, **8**, and **9**. This stereochemistry appears to be thermodynamically controlled.

Table 1. Relative energies (*Z* vs *E*)

Compound	$E_Z - E_E$ (kcal/mol)
3-Chloroenal **1a**	−1.237
3-Methoxyenal **4a**	1.242
Vinylogous amide **8a**	1.921
Vinamidinium salt **9a**	5.233

4. Experimental

4.1. General

The 300 and 75.5 MHz NMR data was collected with a GE Omega 300 MHz instrument. The 500 and 125.8 MHz NMR data were collected on a Bruker 500. The IR data was collected using a Nicolet Avatar fitted with an HATR accessory. The low resolution mass spectral data was obtained using a Shimadzu Model QP 5050 GC–MS equipped with a direct insert sampling device. HRMS data was provided by the Nebraska Center for Mass Spectrometry at the University of Nebraska-Lincoln. The purity of the samples were estimated at better than 95% based on either GC or NMR analysis or both. Solvents and reagents were used as received from the suppliers (Aldrich Chemicals and Fisher Scientific).

4.1.1. (*Z*)-1-Chloro-3,3-dimethoxy-1-phenyl-1-propene (2a). Methanol (40 mL) was added to 3-chloro-3-phenyl-2-propenal (**1a**) (0.210 g, 1.26 mmol) and stirred at room temperature for 17 h. Ethyl acetate (25 mL) was added and the product was washed with 10% $NaHCO_3$ (25 mL). A milk white emulsion formed and was broken with water. The organic layer was dried with $MgSO_4$ and the solvent was removed in vacuo leaving 0.217 g (81.3%) of **2a** as an orange oil. A Kugelrohr distillation provided an analytical sample: bp 190 °C (3 mmHg); IR (HATR) 3063, 2986, 2935, 2827, 1639, 1491, 1445, 1358, 1127, 1048, 754, and 689 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.46 (s, 6H), 5.38 (d, $J=6.4$ Hz, 1H), 6.24 (d, $J=6.4$ Hz, 1H), 7.39 (m,3H), 7.64 (m, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 53.3, 101.1, 123.9, 126.7, 128.4, 129.4, 136.6, 137.0; MS *m/z* 212 (M+, 2), 211 (1.5), 197 (5), 183 (34), 181 (100), 177 (64), 149 (46), 145 (25), 115 (26), 103 (25), 102 (24), and 79 (33); HRMS (EI, M+) calcd for $C_{11}H_{13}O_2Cl$ 212.0604, found 212.0596.

4.1.2. (*Z*)-1-Chloro-1-3,3-dimethoxy-1-(*p*-tolyl)-1-propene (2b). This compound was prepared from **1b** in 97% yield using the procedure described for the synthesis of **2a**: IR (HATR) 2932, 2823, 1668, 1500, 1358, 1045, 1124, 1053, 969, 905, and 813 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.34 (s, 3H), 3.45 (s, 6H), 5.40 (d, $J=6.5$ Hz, 1H), 6.23 (d, $J=6.5$ Hz, 1H), 7.18 (d, $J=8.1$ Hz, 2H), 7.54 (d, $J=8.1$ Hz, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 21.1, 53.2, 101.2, 123.1, 126.6, 129.1, 134.2, 136.6, 139.5; mass spec *m/z* 226 (M+, 6), 211 (10), 197 (32), 195 (100), 163 (47), 115 (69), 91 (24), and 79 (49); HRMS (EI, M+) calcd for $C_{12}H_{15}O_2Cl$ 226.0761, found 226.0759.

4.1.3. (*Z*)-1-Chloro-3,3-dimethoxy-1-(4-methoxyphenyl)-1-propene (2c). This compound was prepared from **1c** in 93% yield using the procedure described for the synthesis of **2a**: IR (HATR) 2996, 2955, 2930, 2899, 2827, 1609, 1516, 1255, 1050 and 815 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.44 (s, 6H), 3.83 (s,3H), 5.36 (d, $J=6.5$ Hz, 1H), 6.13 (d, $J=6.5$ Hz, 1H), 6.54 (d, $J=8.9$ Hz, 2H), 7.57 (d, $J=8.9$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 53.3, 55.4, 101.3, 113.7, 122.0, 128.0, 129.5, 136.4, 160.5; mass spec *m/z* 244 (4), 242 (M+,11), 229 (3), 227 (10), 213 (33), 211 (100), 181 (15), 179 (42), 133 (15), 89 (17), and 79 (30). This compound hydrolyzed to **1c** during shipping for HRMS analysis: HRMS (EI, M+) calcd for $C_{10}H_7O_3Cl$ 198.0262, found 198.0261.

4.1.4. (*Z*)-1-Chloro-1-(*p*-chlorophenyl)-3,3-dimethoxy-1-propene (2d). This compound was prepared from **1d** in 94% yield using the procedure described for the synthesis of **2a**: IR (HATR) 2991, 2935, 2822, 1639, 1593, 1486, and 1052; ^1H NMR (300 MHz, $CDCl_3$) δ 3.42 (s, 6H), 5.33 (d, $J=6.4$ Hz, IH), 6.21 (d, $J=6.4$ Hz, 1H), 7.33 (m, 2H), 7.54 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 53.3, 101.0, 124.4, 227.9, 128.6, 135.3, 135.4, 135.4; mass spec *m/z* 248 (2), 246 (M+, 3), 233 (4), 231 (6), 217 (63), 215 (100), 213 (16), 211 (46), 185 (16), 183 (24), 167 (5), 165 (15), 102 (20), 101 (26), 81 (21), and 79 (66). This compound hydrolyzed to back to **1d** during shipment for HRMS analysis: HRMS (EI, M+) calcd for $C_{11}H_{12}O_2Cl_2$ 246.0214, found 246.0204.

4.1.5. (*Z*)-1-Chloro-1-(*p*-fluorophenyl)-3,3-dimethoxy-1-propene (2e). This compound was prepared from **1e** in 74% yield using the procedure described for the synthesis of **2a**. A Kugelrohr distillation provided an analytical sample: bp 143 °C (1.5 mmHg); IR (HATR) 3053, 2996, 2935, 2827 1598, 1496, 1230, 1127, 1048, 972, 824, and 672 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.41 (s,6H), 5.32 (d, $J=6.4$ Hz, 1H), 6.12 (d, $J=6.4$ Hz, 1H), 7.03 (m, 3H), 7.58 (m, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 53.3. 101.1, 115.4 (d, $J=22.1$ Hz), 123.9, 128.5 (d, $J=8.4$ Hz), 133.2, 135.4, 163.2 (d, $J=250.7$ Hz); mass spec *m/z* 232 (0.8), 230 (M+, 2), 215 (6),201 (34), 199 (100), 195 (45),167 (21), 149 (6), 133 (15), 120 (20), 101 (15), 81 (26), and 79 (72); HRMS (EI, M+) calcd for $C_{11}H_{12}O_2ClF$ 230.0510, found 230.0502.

4.1.6. (*Z*)-1-Chloro-3,3-diethoxy-1-phenyl-1-propene (3a). 3-Chloro-3-phenyl-propenal (**1a**) was dissolved in 25 mL of absolute ethanol. The solution was stirred at room temperature for 22 h. Ethyl acetate (45 mL) was added and the solution was washed with 25 mL of 5% sodium bicarbonate. Water was added to clarify the aqueous layer. The organic layer was dried ($MgSO_4$) and the ethyl acetate was removed using a rotary evaporator leaving 0.16 g (56%) of **3a**. IR (HATR) 2971, 2874, 1674, 1449, 1122,1049, 994, 757, and 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.29 (t, $J=7.0$ Hz, 6H), 3.66 (d ($J=9.3$ Hz) of q ($J=7.0$ Hz), 2H, 3.77 (d ($J=9.3$ Hz) of q ($J=7.0$ Hz), 2H, 5.51 (d, $J=6.6$ Hz, 1H), 6.27 (d, $J=6.6$ Hz, 1H), 7.39 (m, 3H), 7.65 (m, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 15.3, 61.7, 99.4, 124.9, 126.7, 128.4, 129.3, 135.8, 137.1; mass spec *m/z* 205 (50), 195 (50), 167 (46), 117 (21), 103 (100), 89 (25), and 77 (29). This sample hydrolyzed back to **1a** when it was shipped to Indiana for high resolution mass spectral analysis: HRMS (EI M+) calcd for C_9H_7ClO 166.0185, found 166.0189.

4.1.7. (*Z*)-1-Chloro-3,3-diethoxy-1-(*p*-tolyl)-1-propene (3b). 3-Chloro-3-*p*-tolylpropenal (**1b**) was dissolved in 25 mL of absolute ethanol. The solution was stirred for 28 h at room temperature. Ethyl acetate (20 mL) was added and the solution was washed with 30 mL of 5% sodium bicarbonate. Water was added to clarify the aqueous layer. The organic layer was dried ($MgSO_4$) and the ethyl acetate was removed in vacuo leaving 158 mg (56%) of a crude brown oil. Kugelrohr distillation at 179 °C (0.2 mmHg)

provided an analytical sample: IR (HATR) 2971, 2866, 1635, 1111, 1052, 990, and 808 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, $J=7.1$ Hz, 6H), 2.36 (s, 3H), 3.70 (m, 4H), 5.50 (d, $J=6.7$ Hz, 1H), 6.23 (d, $J=6.7$ Hz, 1H), 7.18 (d, $J=8.2$ Hz, 2H), 7.53 (d, $J=8.2$ Hz, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) d 15.3, 21.1, 61.6, 99.4, 123.8, 126.5, 129.0, 134.3, 135.9, 139.3; mass spec *m/z* 254 (M+,1), 219 (65), 211 (36), 209 (100), 183 (28), 181 (35), 165 (17), 163 (22), 145 (16), 117 (55), 115 (38), and 91 (12); HRMS (EI, M+) calcd for $C_{14}H_{19}ClO_2$ 254.1074, found 254.1078.

4.1.8. (*Z*)-3-Methoxy-3-phenyl-2-propenal (4a). 3-Chloro-3-phenyl-2-propenal (**1a**) (0.200 g, 1.2 mmol) was added to a solution of cesium carbonate (1.26 g, 3.9 mmol) in 40 mL of methanol and the mixture was stirred at room temperature for 40 h. The methanol was removed in vacuo and the residue was taken up in ethyl acetate and washed with water. After drying ($MgSO_4$) the solvent was evaporated to yield 0.18 g (92%) of **4a**. Kugelrohr distillation afforded an analytical sample: bp 170–175 °C (0.7 mmHg); IR (HATR) 3058, 3017, 2940, 2843, 2776, 2735, 1655, 1588, 758, and 702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.79 (s, 3H), 5.61 (d, $J=7.8$ Hz, 1H), 7.40 (m, 5H), 9.42 (d, $J=7.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 56.6, 105.6, 128.4, 129.6, 130.8, 177.6, 192.5; GC–MS *m/z* 162 (M+, 29), 161 (100), 147 (3),131 (12), 115 (18), 102 (26), 91 (24), 77 (44), 69 (56) and 51 (18); HRMS (EI, M+) calcd for $C_{10}H_{10}0_2$ 162.0681, found 162.0674.

4.1.9. (*E*)-3-Methoxy-3-(4′-methylphenyl)-2-propenal (4b). This compound was prepared from **1b** in 95% yield using the procedure described for the synthesis of **4a**: mp 64–65 °C; IR (HATR) 2955, 2919, 2853, 2786, 2735, 1644, and 1588 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.19 (s, 3H), 3.89 (s, 3H), 5.66 (d, $J=7.7$ Hz, 1H), 7.27 (d, $J=8.0$ Hz, 2H), 7.40 (d, $J=8.0$ Hz, 2H), 9.49 (d, $J=7.7$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 21.3, 56.5, 105.4, 129.1, 129.7, 130.6, 141.4, 177.8, 192.9; GC–MS *m/z* 176 (M+, 36), 175 (100), 161 (95), 145 (9), 131 (13), 115 (48), 105 (9), 103 (11), 91 (43), 77 (14), 69 (56), 65 (19), and 51 (12); HRMS (EI, M+) calcd for $C_{11}H_{12}O_2$ 176.0837, found 176.0833.

4.1.10. (*E*)-3-Methoxy-3-(4-methoxyphenyl)-2-propenal (4c). This compound was prepared from **1c** in 78% yield using the procedure described for the synthesis of **4a**: Kugelrohr distillation afforded an analytical sample: bp 185–195 °C (0.4 mmHg); IR (HATR) 3007, 2935, 2904, 2838, 2771, 2735, 1650, and 1588 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.86 (s, 3H), 3.87 (s, 3H), 5.63 (d, $J=7.7$ Hz, 1H), 6.96 (d, $J=9.0$ Hz, 2H), 7.45 (d, $J=9.0$ Hz, 2H), 9.49 (d, $J=7.7$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 55.4, 56.6, 105.0, 113.7, 125.6, 131.3, 161.9, 177.5, 192.6; GC–MS *m/z* 192 (M+, 52), 191 (100), 177 (19), 161 (53), 149 (8), 135 (36), 121 (12), 105 (10), 91 (25), 89 (27), 77 (36), 69 (69), 63 (28), and 51 (21); HRMS (EI, M+) calcd for $C_{11}H_{12}0_3$ gives 192.0786, found 192.0780.

4.1.11. (*E*)-3-(4-Chlorophenyl)-3-methoxy-2-propenal (4d). This compound was prepared from **1d** in 85% yield using the procedure described for the synthesis of **4a**: Kugelrohr distillation afforded an analytical sample: bp 155–178 °C (1.3 mmHg); IR (HATR) 2945, 2843, 2776, 2735, 1660, and 1588 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.89 (s, 3H), 5.68 (d, $J=7.8$ Hz, 1H), 7.44 (s, 4H), 9.46 (d, $J=7.8$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 56.7, 105.9, 128.8, 131.1, 131.6, 137.1, 176.3, 192.0 ppm; GC–MS *m/z* 198 (12), 197 (37), 196 (M+, 37), 195 (100), 181 (4), 167 (5), 165 (9), 161 (28), 139 (23), 136 (28), 131 (19), 115 (23), 111 (17), 101 (23), 89 (20), 75 (34), 69 (95), 59 (29), and 51 (18); HRMS (EI, M+) calcd for $C_{10}H_9O_2Cl$ 196.0291, found 196.0292.

4.1.12. (*E*)-3-Methoxy-3-(3′,4′-methylenedioxyphenyl)-2-propenal (4f). This compound was prepared from **1e** in 78% yield using the procedure described for the synthesis of **4a**: Kugelrohr distillation yielded an analytical sample as a yellow solid: mp 72–74 °C; IR (HATR) 3075, 2991, 2919, 2848, 2771, 1655, and 1588 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 3H), 5.62 (d, $J=7.7$ Hz, 1H), 6.05 (s, 2H), 6.86 (d, $J=8.0$ Hz, 1H), 6.98 (d, $J=1.6$ Hz, 1H), 7.01 (dd, $J=1.6$, 8.0 Hz, 1H), 9.49 (d, $J=7.7$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDC1_3$) δ 56.6, 101.7, 105.4, 108.0, 109.5, 125.0, 126.0, 147.8, 150.0, 177.0, 192.5; GC–MS *m/z* 206 (M+, 75), 205 (73), 191 (8), 175 (25), 149 (27), 121 (16), 89 (19), 77 (21), and 69 (100); HRMS (EI, M+) calcd for $C_{11}H_{10}0_3$ 206.0580, found 206.0587.

4.1.13. (*E*)-3-Ethoxy-3-phenyl-2-propenal (5a). 3-Chloro-3-phenyl-2-propenal (0.220 g, 1.2 mmol) was added to a solution of cesium carbonate (1.26 g, 3.9 mmol) in 40 mL of ethanol and the mixture was stirred at room temperature for 4.5 h. The ethanol was removed in vacuo and the residue was taken up in ethyl acetate and washed with water. After drying ($MgSO_4$) the solvent was evaporated to yield 0.19 g (90%) of **5a**. Kugelrohr distillation afforded an analytical sample: bp 157–167 °C (0.8 mmHg); IR (HATR) 3058, 2981, 2935, 2894, 2827, 2761, 2730, 1655, 1588, 758, and 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (t, $J=7.1$ Hz, 3H), 4.09 (q, $J=7.1$ Hz, 2H), 5.65 (d, $J=7.8$ Hz, 1H), 7.47 (m, 5H), 9.45 (d, $J=7.8$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 65.4, 106.1, 128.4, 129.8, 130.7, 133.5, 177.0, 192.9; GC–MS *m/z* 176 (M+, 21), 175 (38), 159 (3), 147 (40), 131 (29), 120 (4), 105 (34), 91 (24), 77 (70), 69 (100), 51 (46); HRMS (EI, M+) calcd for $C_{11}H_{12}O_2$ 175.0837, found 176.0761.

4.1.14. (*E*)-3-Ethoxy-3-(*p*-tolyl)-2-propenal (5b). This compound was prepared from **1b** in 66% yield using the procedure described for the synthesis of **5a**: flash chromatography (Biotage Horizon HFC instrument with a #1542-2 silica cartridge and ethyl acetate/hexane as the eluant) was used to produce an analytical sample, a yellow solid: mp 60–61 °C; IR (HATR) 3274, 2986, 2913, 2835, 2769, 2727, 2357, 1654, 1588, 1344, 1212,1123, and 812 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.46 (t, $J=7.0$ Hz, 3H), 2.41 (s, 3H), 4.08 (q, $J=7$ Hz, 2H), 5.63 (d, $J=7.8$ Hz, 1H), 7.25 (d, $J=8.0$ Hz, 2H), 7.40 (d, $J=8.0$ Hz, 2H), 9.47 (d, $J=7.8$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 14.2, 21.4, 65.4, 105.8, 129.0, 129.7, 130.7, 141.2, 177.1, 192.8; GC–MS *m/z* 190 (M+, 14), 189 (21), 175 (37), 161 (26), 147 (22), 131 (40), 119

(33), 91 (46), and 69 (100); HRMS (EI, M+) calcd for $C_{12}H_{14}O_2$ 190.0994, found 190.0985.

4.1.15. (*E*)-3-Ethoxy-3-(4′-methoxyphenyl)-2-propenal (5c). This compound was prepared from **1c** in 87% yield using the procedure described for the synthesis of **5a**. Kugelrohr distillation afforded an analytical sample: bp 210 °C (0.3 mmHg); IR (HATR) 2955, 2919, 2853, 2786, 2735, 1644, and 1588 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.19 (s, 3H), 3.89 (s, 3H), 5.66 (d, $J=7.7$ Hz, 1H), 7.27 (d, $J=8.0$ Hz, 2H), 7.40 (d, $J=8.0$ Hz, 2H), 9.49 (d, $J=7.7$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 21.3, 56.5, 105.4, 129.1, 129.7, 130.6, 141.4, 177.8, 192.9; GC–MS *m/z* 206 (M+, 53.8), 205 (51.1), 189 (15.4), 177 (51.1), 162 (41.6), 161 (38.2), 150 (26.2), 147 (21.5), 135 (100), 133 (61.8), 131 (35.5), 109 (49.2), 94 (14.8), 92 (20.3),89 (18.9), 77 (46.6), 69 (84.1), 63 (21.7), and 51 (14.2); HRMS (EI, M+) calcd for $C_{12}H_{14}O_3$ 206.0943, found 206.0947.

4.1.16. (*E*)-3-(4-Chlorophenyl)-3-ethoxy-2-propenal (5d). This compound was prepared from **1d** in 68% yield using the procedure described for the synthesis of **5a**: Kugelrohr distillation afforded an analytical sample: mp 62–64 °C; IR (HATR) 2981, 2930, 2853, 2781, 2735, 1660, and 1598 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (t, $J=7.0$ Hz, 3H), 4.09 (q, $J=7.0$ Hz, 2H), 5.66 (d, $J=7.8$ Hz, 1H), 7.45 (s, 4H), 9.45 (d, $J=7.8$ Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 65.5, 106.2, 128.8, 130.9, 131.9, 137.1, 175.7, 192.4; GC–MS *m/z* 212 (6.1), 211 (11.8), 210 (M+, 18), 209 (28), 193 (6), 183 (12), 181 (12), 165 (8), 154 (6), 141 (13), 139 (40), 131 (65), 111 (22), 101 (13), 89 (11), 75 (29), 71 (35), 69 (100), 51 (12), 50 (12); HRMS (EI, M+) calcd for $C_{11}H_{11}O_2Cl$ 210.0448, found 210.0451.

4.1.17. (*E*)-3-Ethoxy-3-(3′,4′-methylenedioxyphenyl)-2-propenal (5f). This compound was prepared from **1f** in 90% yield using the procedure described for the synthesis of **5a**: mp, 127–128 °C; IR (HATR) 3076, 2979, 2909, 2847, 1647, and 1581 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.47 (t, $J=7.1$ Hz, 3H), 4.07 (q, $J=7.0$ Hz, 2H), 5.61 (d, $J=7.7$ Hz, 1H), 6.05 (s, 2H), 6.87 (d, $J=8.2$ Hz, 1H), 6.99 (d, $J=1.6$ Hz, 1H), 7.02 (dd, $J=8.2$ Hz, 1H), 9.49 (d, $J=7.7$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 14.2, 65.4, 101.7, 105.8, 107.9, 109.6, 125.0, 127.3, 147.8, 149.9, 176.2, 192.6; GC–MS *m/z* 220 (M+, 47), 219 (23), 191 (16), 176 (17), 175 (15), 164 (9), 149 (32), 147 (25), 123 (17), 121 (19), 93 (14), 77 (13), 69 (100), and 51 (10); HRMS (EI, M+) calcd for $C_{12},H_{12}O_4$ 220.0735, found 220.0736.

4.1.18. 3,3-Dimethoxy-1-*p*-tolylpropan-1-one (6b). 1-Chloro-*p*-tolylpropenal (0.202 g, 0.0012 mol) was added to methanol (20 mL) at room temperature and the solution was refluxed for 23 h. Solid sodium bicarbonate (150 mg) was added and the methanol was removed with a rotary evaporator. Ethyl acetate (20 mL was added and the washed with 20 mL water. After drying ($MgSO_4$) the ethyl acetate was removed with a rotary evaporator leaving 148 mg (63%) of crude **6b**. A Kugelrohr distillation at120–130° (0.25 mmHg) provided an analytical sample: IR (HATR) 3036, 2924, 2827, 1677, 1603 cm^{-1}; ^1H NMR (300 MHz, CDCl3) δ 2.39 (s, 3H), 3.21 (d, $J=5.4$ Hz, 2H), 3.40 (s, 6H), 4.99 (t, $J=5.4$ Hz, 1H) 7.24 (d, $J=8.1$ Hz, 2H), 7.85 (d, $J=8.1$ Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.5, 42.4, 54.0, 102.2, 128.3, 129.2, 134.6, 144.0, 196.4; GC–MS *m/z* 208 (M+, 1), 193 (12), 161 (6), 119 (88), 91 (48), 85 (20), 75 (100), 65 (39); HRMS (EI, M^+) calcd for $C_{12}H_{16}O_3$ 208.1099, found 208.1092.

4.1.19. 3,3-Diethoxy-1-(*p*-tolyl) propan-1-one (7b). (*Z*)-3-Chloro-3-(*p*-tolyl)-2-propenal (**1e**) (208 mg, 0.0011 mol) was refluxed in 20 mL of absolute ethanol for 5.8 h. The resulting solution was cooled to room temperature. Solid sodium bicarbonate (103 mg) and 25 mL water were added and the resulting solution was extracted with 25 mL of ethyl acetate. After drying ($MgSO_4$) the ethyl acetate was removed in vacuo to leave 157 mg (57%) of **7b**. A Kugelrohr distillation at 180–185° (0.5 mmHg) provided an analytical sample: IR (HATR) 2955, 2920, 2850, 1674, 1604, 1118, 1052, and 579 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.21 (t, $J=7.0$ Hz, 6H), 2.43 (s, 3H), 3.29 (d, $J=5.5$ Hz, 2H), 3.61 (d ($J=9.2$ Hz) of q ($J=7.1$ Hz, 2H), 3.74 (d ($J=9.2$ Hz) of q, $J=7.0$ Hz, 2H), 5.13 (t, $J=5.5$ Hz, 1H), 7.28 (d, $J=8.2$ Hz, 2H), 7.88 (d, $J=8.2$ Hz, 2); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 15.3, 21.6, 43.7, 62.7, 100.6, 122.5, 129.2, 134.8, 143.9, 196.9; GC–MS *m/z* 236 (M+, 1), 207 (34), 119 (100), 103 (24), 91 (29), 75 (22), and 65 (13); HRMS (EI, M^+) calcd for $C_{14}H_{20}O_3$ 236.1412, found 236.1418.

4.1.20. 3-Morpholinyl-3-phenyl-2-propenal (8a) from 9a. A mixture of 10% NaOH (5 mL) and tetrahydrofuran (5 mL) was added to 1,3-di-4-morpholinyl-1-phenyl-2-propenylium hexafluorophosphate (**9a**) (0.300 g, 0.69 mmol) and stirred at room temperature for 24.5 h. Ethyl acetate was added (25 mL) and the aqueous layer was washed with water (10 mL) five times; emulsions were broken with brine. The organic layer was dried ($MgSO_4$) and the solvent was removed by high powered vacuum with no added heat leaving behind a pale yellow solid (**8a**). This was recrystallized in hot cyclohexane with 5 drops of ethyl acetate yielding fine cream colored crystals of 3-morpholinyl-3-phenyl-2-propenal (0.092 g, 61%); mp 105.5–106.5 °C; IR (HATR) 22959, 2909, 2843, 2753, and 1616 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.23 (br s, 4H), 3.73 (s broad, 4H), 5.50 (d, $J=8.2$ Hz, 1H), 7.33 (m, 2H), 7.47 (m, 3H), 8.83 (d, $J=8.2$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 47.1, 65.4, 103.8, 127.7, 128.6, 128.9, 132.5, 167.0, 190.9; GC–MS *m/z* 217 (M+, 25), 216 (20), 201 (15), 200 (100), 188 (11), 186 (10), 158 (21), 131 (12), 103 (37), 86 (22), and 77 (29); HRMS (EI, M+) calcd for $C_{13}H_{15}NO_2$ 217.1103, found 217.1104.

4.1.21. 3-(4′-Methylphenyl)-3-morpholinyl-2-propenal (8b) from 9b. This compound was prepared from **9b** in 68% yield using the procedure described for the synthesis of **8a**: an analytical sample was then recrystallized from boiling cyclohexane and 5 drops of ethyl acetate: mp 120.5–121.2 °C; IR (HATR) 2951, 2819, 2761, 2353, 1627, and1106 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.42 (s, 3H), 3.23 (br s, 4H), 3.73 (br s, 4H), 5.58 (d, $J=8.4$ Hz, 1H), 7.21 (d, $J=8.1$ Hz, 2H), 7.26 (d, $J=8.0$ Hz, 2H), 8.85 (d, $J=8.4$ Hz, 1H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 21.3, 48.2, 66.4, 105.0, 129.4, 129.6, 130.6, 140.1, 168.2, 192.0; GC–MS *m/z* 231 (M+, 25), 230 (20), 215 (15), 214 (100), 202 (10), 172 (17), 117 (33), 115 (33), 91 (16), and 86 (27);

HRMS (EI, M+) calcd for $C_{14}H_{17}NO_2$ 231.1259, found 231.1251.

4.1.22. 3-Morpholinyl-3-phenyl-2-propenal (8a) from 1a. 3-Chloro-3-phenyl-2-propenal (0.200 g, 1.2 mmol) was added to a mixture of 2 mL acetonitrile, 0.242 g of triethylamine and 1.0 equiv of morpholine (0.105 g, 1.2 mmol). The mixture was stirred overnight and the acetonitrile was removed in vacuo. The remaining mixture was then taken up in ethyl acetate (30 mL), washed with water and dried with $MgSO_4$. The ethyl acetate was removed in vacuo leaving 0.093 g (36%) of crude **8a**.

4.1.23. 3-(4′-Methylphenyl)-3-morpholinyl-2-propenal (8b) from 1b. This compound was prepared from **1b** in 81% yield using the procedure described for the synthesis of **8a**.

4.1.24. 1,3-Di-4-morpholinyl-1-phenyl-2-propenylium hexafluorophosphate (9a). A mixture of 3-phenyl-3-chloro-2-propenal (**1a**) (0.200 g, 1.2 mmol) and morpholine (0.209 g, 2.4 mmol) in acetonitrile (3 mL) was stirred for 21 h at room temperature. The solvent was removed using a rotary evaporator leaving a foamy orange solid. Enough methanol was added to fully dissolve the solid (approximately 3 mL). Sodium hexafluorophosphate (0.605 g, 3.6 mmol) dissolved in methanol was added to the flask. The flask remained at room temperature for one hour and was then stored at 10 °C for 19 h. A white powder was collected by filtration and dried in an oven yielding 0.146 g (86%) dry 1,3-di-4-morpholinyl-1-phenyl-2-propenylium hexafluorophosphate: mp 213–214 °C; IR (HATR) 2986, 2863, 1609, 1537 and 1240 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.30 (m, 2H), 3.39 (m,2H), 3.68 (m,2H), 3.75 (m, 2H), 3.79 (br s, 2H), 3.88 (br s, 2H), 3.99 (br s, 4) 6.02 (d, $J=11.4$ Hz, 1H), 6.76 (d, $J=11.4$ Hz, 1H), 7.34 (m, 2H), 7.60 (m, 3H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 47.7, 49.5, 51.6, 55.5, 66.0, 66.5, 66.6, 66.8, 93.0, 119.9, 128.7, 129.7, 131.3, 160.9, 171.5; mass spec *m/z* 432 (M+, 4), 287 (15), 286 (14), 220 (27), 201 (17), 200 (100), 170 (28), and 115 (23); HRMS (EI, M+) calcd for $C_{17}H_{23}N_2O_2^+$ PF_6^- 432.4101, found 432.4108.

4.1.25. 1,3-Di-4-morpholinyl-1-(*p*-tolyl)-2-propenylium hexafluorophosphate (9b). This compound was prepared from **1b** in 90% yield using the procedure described for the synthesis of **9a**: recrystallization from methanol afforded an analytical sample: mp 213–214 °C; IR (HATR) 3647, 2981, 2868, 1619, 1542, 1240, and 1112 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 2.46 (s, 3H), 3.32 (t, $J=4.7$ Hz, 2H), 3.41 (t, $J=4.7$ Hz, 2H), 3.66 (t, $J=4.8$ Hz, 2H), 3.75 (q, $J=4.7$ Hz, 4H), 3.84 (t, $J=5.3$ Hz, 2H), 3.95 (s, 4H), 5.94 (d, $J=12.4$ Hz, lH), 6.81 (d, $J=9.9$ Hz, lH), 7.21 (d, $J=7.9$ Hz, 2H), 7.38 (d, $J=7.9$ Hz, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 21.5, 47.4, 49.3, 51.5, 55.4, 65.9, 66.3, 66.6, 66.8, 92.6, 128.1, 128.7, 130.3, 141.9, 160.9, 172.1; mass spec *m/z* 446 (M+, 3), 301 (15), 300 (17), 243 (16), 234 (22), 215 (19), 214 (100), 184 (42), 107 (40), and 57 (69); HRMS (EI, M+) calcd for $C_{18}H_{25}N_2O_2^+$ PF_6^- 446.1558, found 446.1555.

4.1.26. 1-(*p*-Methoxyphenyl)-1,3-di(4-morpholinyl)-2-propenylium hexafluorophosphate (9c). This compound was prepared from **1c** in 97% yield using the procedure described for the synthesis of **9a**: mp 206–209 °C; IR (HATR) 3000–3700, 2979, 2854, 1616, and 1545 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.35 (m, 2H), 3.42 (m,2H), 3.67 (m,2H), 3.77 (m, 4H), 3.88 (m, 2H), 3.91 (s, 3H), 3.97 (m, 4H), 5.97 (d, $J=11.6$ Hz, 1H), 6.83 (d, $J=11.6$ Hz, 1H) 7.08 (d, $J=8.0$ Hz, 1H), 7.26 (d, $J=8.0$ Hz, 1H); ^{13}CNMR (125.8 MHz, $CDCl_3$) δ 47.6, 49.6, 51.7, 55.4, 55.6, 66.0 66.4, 66.6, 66.9, 93.1, 115.1, 123.0, 130.7, 161.0, 162.0, 172.0; mass spec *m/z* 462 (M+, 1), 317 (12), 316 (32), 273 (20), 259 (53), 230 (94), 198 (37), 147 (46), 133 (39), 115 (36), 107 (90), 86 (59), 77 (41), and 57 (100); HRMS (EI, M+) calcd for $C_{18}H_{25}O_3N_2^+PF_6$ 462.1507, found 462.1522.

4.1.27. 1-(*p*-Chlorophenyl)-1,3-di-(4-morpholinyl)-2-propenylium hexafluorophosphate (9d). This compound was prepared from **1d** in 92% yield using the procedure described for the synthesis of **9a**: mp 182–188 °C; IR (HATR) 3000–3700 (broad), 2984, 2854, 1616, and 1540 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.01 (m, 2H), 3.44 (m, 2H), 3.68 (m, 2H), 3.77 (m, 4H), 3.89 (m, 2H), 3.96 (m, 4H), 5.96 (d, $J=11.9$ Hz, 1H), 6.78 (d, $J=11.9$ Hz, 1H), 7.33 (d, $J=8.4$ Hz, 2H), 7.58 (d, $J=8.4$ Hz, 2H); ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 47.6, 9.3, 51.5, 55.6, 65.8, 66.2, 66.5, 66.7, 92.8, 129.5, 130.1, 130.3, 137.8, 160.8, 170.5; mass spec *m/z* 466 (M+, 2), 321 (13), 320 (27), 277 (27), 263 (57), 234 (95), 204 (35), 151 (32), 115 (72), 107 (90), 86 (74), and 57 (100); HRMS (EI, M+) calcd for $C_{17}H_{22}0_2N_2Cl^+$ PF_6^- 466.1012, found 466.1029.

4.1.28. 1,3-Di-4-morpholinyl-1-(*p*-fluoro)phenyl-2-propenylium hexafluorophosphate (9e). This compound was prepared from **1e** in 80% yield using the procedure described for the synthesis of **9a**; mp 189–191 °C; IR (HATR) 2971, 2863, 1629, 1542, 1230, and 1112 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 3.43 (m, 2H), 3.63 (m, 2H), 3.73 (m, 4H), 3.80 (m, 2H), 3.85 (m, 2H), 3.90 (m, 2H), 4.02 (m, 2H), 6.21 (d, $J=12$ Hz, 1H), 7.28 (d, $J=12$ Hz, 1H), 7.39 (m, 2H), 7.57 (m, 2H); ^{13}C NMR (125.8 MHz, acetone-d_6) δ 47.6, 49.1, 51.4, 54.9, 65.6, 65.7, 66.5, 66.6, 92.2, 116.4 (d, $J=22.8$ Hz), 127.9 (d, $J=3.9$ Hz), 131.8 (d, $J=8.8$ Hz), 161.3, 164.1 (d, $J=250.1$ Hz), 170.8; mass spec *m/z* 305 (16), 304 (13), 247 (8), 238 (27), 219 (18), 218 (100), 188 (28), 133 (38), and 107 (33); HRMS (EI, M+) calcd for $C_{17}H_{22}N_2O_2F^+$ PF_6^- 450.1307, found 450.1301.

4.1.29. 1,3-Di-4-morpholinyl-1-(3′,4′-methylenedioxyphenyl)-2-propenylium hexafluorophosphate (9f). This compound was prepared from **1f** in 94% yield using the procedure described for the synthesis of **9a**: an analytical sample was recrystallized from methanol: mp 163–165 °C; IR (HATR) 3775, 2935, 2868, 1614, 1547, and 1245 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6) δ 3.48 (m, 2H), 3.78 (m, 12H), 3.99 (m, 2H), 6.15 (m, 3H), 6.97 (m, 2H), 7.06 (d, $J=7.7$ Hz, 1H), 7.40 (d, $J=12.0$ Hz, 1H); ^{13}C NMR (75.5 MHz, acetone-d_6) δ 65.6, 65.7, 66.5, 66.6, 92.1, 102.2, 108.8, 109.3, 92.1, 102.2, 108.8, 109, 123.9, 124.9, 148.5, 150.0, 161.4, 171.5; mass spec *m/z* 331 (4), 330 (8), 302 (6), 259 (7), 244 (45), 214 (29), and 107 (100); HRMS (EI, M+) calcd for $C_{18}H_{23}O_4N_2^+$ PF_6^- 476.1299, found 476.1281.

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Biphasic enantioselective partitioning studies using small-molecule chiral selectors

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Abstract—Enantioselective partitioning of racemic *N*-3,5-(dinitrobenzoyl)leucine or racemic naproxen was studied using a two-component chiral phase transfer approach. A combination of an achiral ion-pairing reagent and a chiral complexing agent (selector) is necessary to effect enantioselective partitioning between an aqueous bicarbonate solution and a nonpolar organic solvent. In these biphasic resolutions, the interplay between the ion-pairing reagent and the selector is essential for maximizing enantioselectivities. Furthermore, the lipophilicity of the ion-pairing reagent, the concentration of the ion-pairing reagent and selector, and the polarity of the organic solvent all exert a considerable influence on the biphasic process. In this manuscript, we conduct optimization studies through analysis of solvent, concentration and ion-pairing effects. Conclusions concerning the mechanistic rationale behind enantioselective partitioning are given.

1. Introduction

Owing to the dramatic increase in sales of single enantiomer drugs over the past decade, the development of new chiral technologies continues to be a very active area of research in the pharmaceutical and specialty chemicals industries. Strategies aimed at the preparative resolution of racemic compounds are amongst the most important means of producing drugs in single enantiomer form.[1] Traditional methods employ crystallization[2] and preparative chromatographic separations.[3] Despite their widespread usage in industrial scale-up processes, these techniques are limited to batch or sequential processes.[4] From an economic standpoint, a continuous separation process is ideal. Indeed, simulated counter-current processes have found growing application in enantioselective separation processes. In particular, simulated moving-bed (SMB) technology has become an important means of performing preparative chiral separations.[5] Nonetheless, SMB often requires extremely thorough optimization procedures and can be quite costly.

An alternative approach to continuous enantioselective separation processes involves using membrane devices.[6] Many of the drawbacks associated with conventional separation strategies such as low substrate throughput and solvent conservation can be circumvented with enantioselective membrane-based separations. Several examples have been demonstrated using chiral selectors that are soluble in one of two liquid phases separated by a semipermeable membrane. The chiral selector mediates transfer of a racemic compound across the membrane by associating with each enantiomer of the racemate, generating diastereomeric complexes. Owing to the nature and directionality of the intermolecular interactions involved, these complexes may be energetically nondegenerate. As a result, the selector is capable of influencing the position of equilibrium of each enantiomer between the respective phases. In general, the more highly complexed enantiomer is preferentially extracted into the phase containing the selector. A variety of chiral selectors have been employed in enantioselective membrane separations including tartaric acid derivatives,[7] chiral amine hydrochlorides,[8] chiral ionophores,[9] chiral crown ethers,[10] polyamino acid derivatives,[11] cyclodextrins,[12] quinine-based compounds[13] and hydroxyproline derived compounds.[14]

Chiral selectors synthesized from small organic molecules developed in our laboratories are capable of separating a variety of enantiomers when used as chromatographic chiral stationary phases (CSPs).[15] These selectors have been designed using first principles through consideration of the minimum requirements that necessitate strong chiral recognition. The majority of selectors incorporate a combination of hydrogen bonding and π–π interactions. Acting in conjunction, these intermolecular forces can exert a significant level of stereochemical control. Many designed

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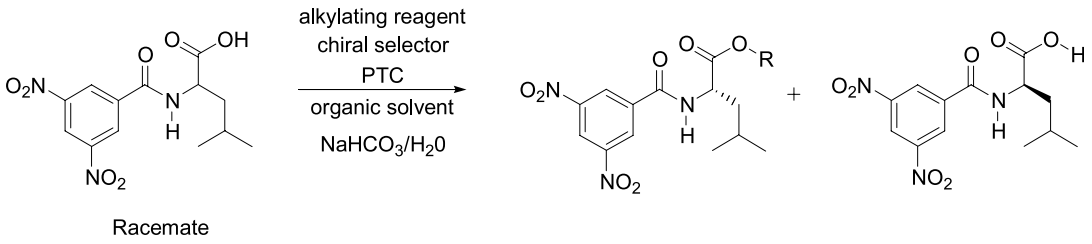
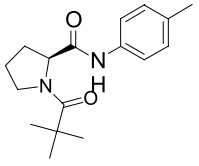


Scheme 1.

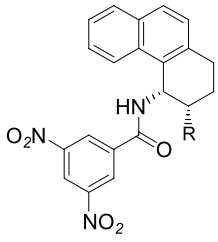
chiral selectors show high levels of enantiodiscrimination for pharmaceutically important compounds and are thus amenable to preparative chromatographic and membrane-based separation processes. For instance, a chiral selector has been used in a hollow fiber membrane system (Sepracor) to separate the enantiomers of *N*-protected amino acids.[16] The approach relies on using the chiral selector in combination with an achiral phase transfer catalyst (PTC) to achieve appreciable rates of extraction across the membrane. Recently, we have extended the methodology to performing biphasic kinetic resolutions by coupling the enantioselective transport to a subsequent chemical reaction, a process referred to as two-component chiral phase transfer catalysis (Scheme 1).[17] Although these kinetic resolutions are performed as batch processes, they are potentially extendable to membrane type reactors. Herein, we report on the various factors that contribute to enantiodiscrimination in two-component chiral phase transfer catalysis, the ultimate goal being optimization for potential enantioselective membrane extraction systems, membrane reactors, and process-scale chiral organocatalytic reactions.

2. Results and discussion

Success of the kinetic resolutions in two-component chiral phase transfer catalysis stems primarily from the enantioselective partitioning step, although secondary effects such as differences in reaction rates between the complexed and uncomplexed enantiomer contribute slightly.[17] Therefore, our primary focus involved optimizing the enantioselective extraction step. To this end, a chiral selector ((*S*)-**1**, (*S*)-**2a** or (3*S*,4*S*)-**2b**) was dissolved in an organic solvent and mixed with an aqueous sodium bicarbonate solution containing either racemic *N*-(3,5-dinitrobenzoyl)leucine, (±)-**3**, or racemic naproxen, (±)-**4**, in the presence of a suitable achiral ion-pairing reagent. Three symmetrical ion-pairing reagents were employed in this study, namely tetrahexylammonium chloride (THAC) tetrahexylammonium bromide (THAB) and tetrabutylammonium chloride (TBAC). The simple partitioning studies were designed to study the effects of selector concentration, racemate

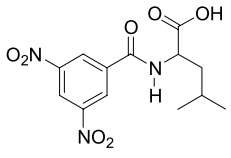


(*S*)-**1**

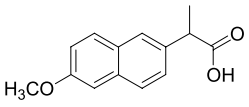


(*S*)-**2a**: R = H

(*3S, 4S*)-**2b**: R = allyl



(±)-**3**



(±)-**4**

Table 1. Biphasic Enantioselective Partitioning of (±)-**3** in the Presence of (*S*)-**1**[a]

EntrySolvent	Solvent	(*S*)-**1** (equiv)	[(*S*)-**1**] (M)	$N^+(alkyl)_4X^-$	$N^+(alkyl)_4X^-$ (mol%)	% Extracted[b]	%ee (*S*)[c]
1	CH_2Cl_2	1.0	0.008	$N^+(hexyl)_4Cl^-$	20	20.2	27
2	CH_2Cl_2	1.0	0.025	$N^+(hexyl)_4Cl^-$	20	19.6	50
3	CH_2Cl_2	1.0	0.10	$N^+(hexyl)_4Cl^-$	20	19.5	70
4	CH_2Cl_2	1.0	0.30	$N^+(hexyl)_4Cl^-$	20	21.0	84
5	CCl_4	1.0	0.008	$N^+(hexyl)_4Cl^-$	20	19.5	76
6	CCl_4	1.0	0.025	$N^+(hexyl)_4Cl^-$	20	19.7	93
7	CCl_4	1.0	0.10	$N^+(hexyl)_4Cl^-$	20	21.0	96
8	CCl_4	0.25	0.002	$N^+(hexyl)_4Cl^-$	20	19.4	50
9	CCl_4	0.25	0.006	$N^+(hexyl)_4Cl^-$	20	20.0	57
10	CCl_4	0.25	0.025	$N^+(hexyl)_4Cl^-$	20	19.5	67
11	CCl_4	1.0	0.025	$N^+(hexyl)_4Cl^-$	40	38.0	73
12	CCl_4	1.0	0.025	$N^+(hexyl)_4Br^-$	40	37.5	74
13	CCl_4	1.0	0.025	$N^+(butyl)_4Cl^-$	40	17.0	91
14	Hexane	1.0	0.025	$N^+(hexyl)_4Cl^-$	40	19.5	90

[a] Standard conditions entailed vigorously mixing 0.10 mmol (1.0 M equiv) of (±)-**3** in 4.0 mL of saturated $NaHCO_3$ with an organic solution containing selector (*S*)-**1** and the indicated ion-pairing reagent at room temperature. Layers were separated and **3** was derivatized with excess phenacyl bromide.
[b] Determined by HPLC internal standard analysis (see Section 4).
[c] Refers to %ee of the compound extracted into the organic layer. Determined by chiral HPLC (see Section 4).

concentration, solvent and ion-pairing reagent on enantioselective partitioning.

Results of enantioselective partitioning of (±)-**3** in the presence of chiral selector (*S*)-**1** are displayed in Table 1. A general schematic of the equilibrium process is shown in Figure 1. In all cases, 1 M equiv of (±)-**3** was dissolved in an aqueous bicarbonate solution and mixed with an organic solvent containing the selector and a tetraalkylammonium halide. Mixtures were shaken vigorously and allowed to equilibrate. The layers were separated and analyzed. Equilibration occurs rapidly once the layers are mixed and results are independent of the contact time between layers. The concentration of (±)-**3** in the aqueous layer was kept constant at 0.025 M for all enantioselective extractions shown in Table 1. The natures of the organic solvent and tetraalkylammonium salt as well as the concentration of selector (*S*)-**1** were varied from one run to the next.

In entries 1–4 of Table 1, methylene chloride (CH_2Cl_2) was used as the extraction solvent. In each case, 1 M equiv of the chiral selector (*S*)-**1** and 20 mol% THAC are present in the organic layer. Changing the effective concentration of selector (and THAC) in the organic layer was accomplished by diluting with methylene chloride. The results from these partitioning studies reveal that the quantity of **3** extracted into CH_2Cl_2 is generally equivalent to the mole% of THAC in the solution. The selector by itself is incapable of effecting transfer between layers. The enantiomeric excess of the extracted **3** is highly dependent on the concentration of selector in the CH_2Cl_2. A threefold increase in enantioselectivity is observed as selector concentration is increased from 0.008 to 0.30 M.

The same general trends are observed with carbon tetrachloride (CCl_4) as the extraction solvent. However, the polarity of the organic solvent also has a considerable influence on enantioselective bias. Association constants for selector/substrate complexes are maximum in nonpolar solvents where there is less competitive solvation from the

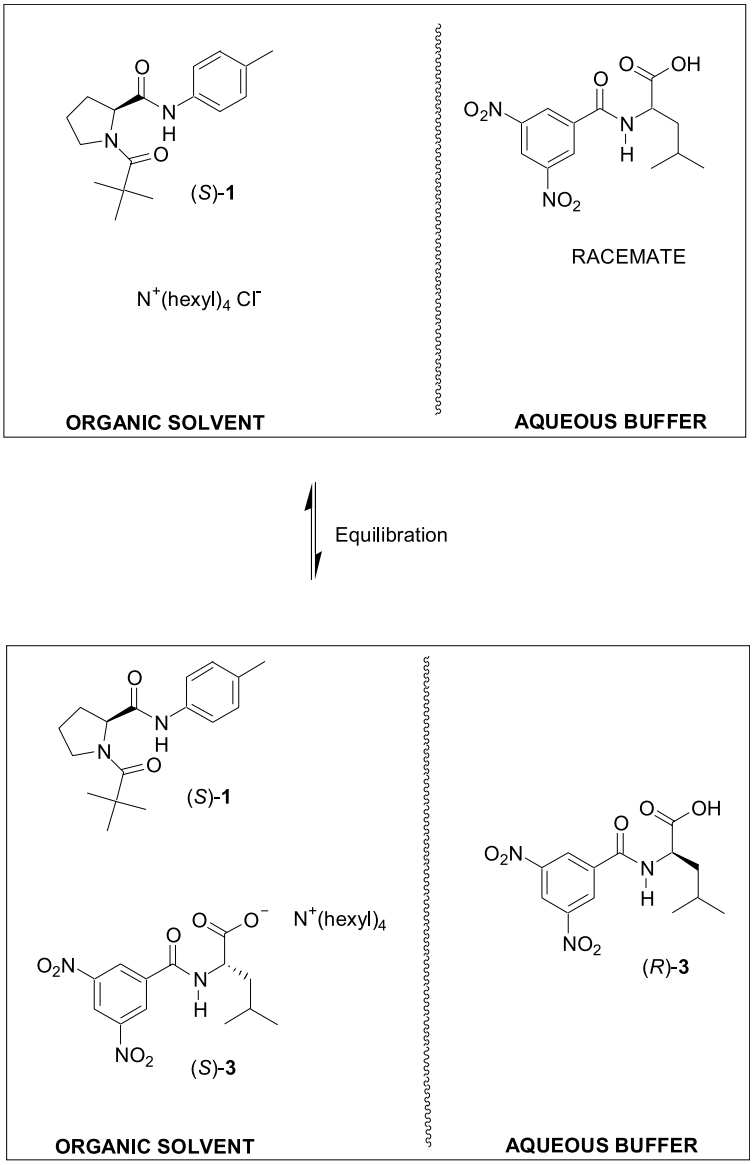


Figure 1. Schematic of the two-component enantioselective partitioning process.

medium. Hence, extractions performed in CH_2Cl_2 are far less selective than those performed in CCl_4. For instance, comparing entry 2 with entry 6 in Table 1, an increase from 50%ee (19.6% extracted) to 93%ee (19.7% extracted) is observed when the extraction solvent is changed from CH_2Cl_2 to CCl_4 under otherwise identical conditions. The ratio of racemate/selector in the biphasic solution also has a significant influence on enantioselective partitioning. As seen in entries 6 and 10 in Table 1, decreasing the number of molar equivalents of selector (*S*)-**1** from 1.0 to 0.25 while keeping the selector concentration constant (by adjusting the amount of CCl_4) gives significantly lower enantioselectivities of extraction. Presumably, in the later case, an increased amount of achiral extraction ensues as the selector is effectively 'tied-up' through its strong association with a single enantiomer of the racemate. Changing the counterion of the achiral ion-pairing reagent from chloride to bromide has little influence on the enantioselective partitioning process, as seen in entries 11 and 12 of Table 1.

To analyze the influence of changing the mol% of achiral ion-pairing reagent, it is necessary to define the stereoselectivity factor (*s*) of enantioselective partitioning. In a kinetic resolution, the *s* factor represents the ratio of rate constants between the enantiomers and is given by the following equation:

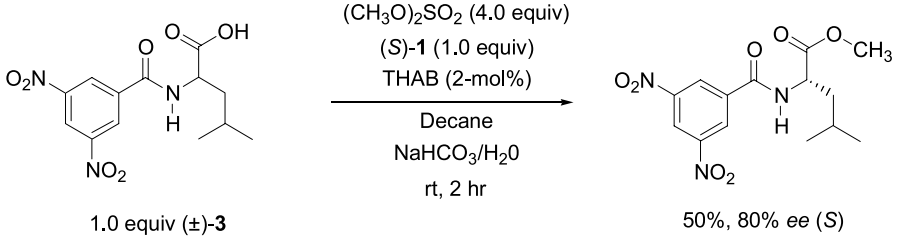
$$s = \ln[1 - C(1 + \mathrm{ee}\prime)]/\ln[1 - C(1 - \mathrm{ee}\prime)]$$

where ee′ is the enantiomeric excess of product at conversion C.[18] The *s* factor is expected to remain constant throughout the course of a kinetic resolution unless secondary effects (e.g., product inhibition) contribute to the selectivity of the process. In the present case, the *s* factor for enantioselective partitioning may be defined as the relative ratio of partitioning coefficients of the enantiomers between the respective layers. The above equation still applies, accept that the term *C* corresponds to the total amount extracted into the organic layer. As displayed in Table 1, increasing the mol% of THAC from 20% (entry 6) to 40% (entry 11), the total quantity of **3** extracted increases from 19.7 to 38% and the ee decreases from 93 to 73%, corresponding to an *s* factor of 35 and 10, respectively. The decrease in *s* factor most likely results from a greater degree of achiral partitioning with increased amounts of THAC, although the influence of ion-pair aggregation may also be a factor.

To mitigate the effects of achiral background partitioning, one may adjust the lipophilicity of the ion-pairing reagent such that the selector is necessary to effect transfer between the aqueous and organic layers. Under standard partitioning conditions (Table 1) but in the absence of a chiral selector, enantiomeric ion-pairs formed between TBAC and (±)-**3** partition almost exclusively into the aqueous layer. On the other hand, in the presence of selector (*S*)-**1**, a significant quantity of **3** is extracted into the CCl_4 (e.g., entry 13 in Table 1). This result indicates that the association constant between selector (*S*)-**1** and (*S*)-**3** is sufficiently large as to perturb the partitioning equilibrium that exists without selector present. The diastereomeric associates likely form at the biphasic interface as two polar groups of (*S*)-**3** lose their hydrophilicity owing to strong hydrogen bonding interactions with the selector. Furthermore, a multipoint π–π interaction provides the essential third point of contact and ensures strong complexation necessary for extraction into the organic layer.

Background extraction is also reduced significantly by using hydrocarbon solvents such as hexane (entry 14 of Table 1) or decane, although symmetrical ion-pairing reagents used in this study have limited solubility in both hydrocarbon and aqueous solutions. From an environmental standpoint, hydrocarbon solvents are far more ideal than halogenated solvents such as CCl_4.As a result, we explored the use of decane as a solvent in the two-component chiral phase transfer catalysis (Scheme 1). With phenacyl halides as alkylating reagents, reactions proceed very slowly unless significant quantities of ion-pairing reagent are added. Simple methyl or ethyl esters of 3 can be prepared by the two-component chiral phase transfer methodology. Although methyl halides are not reactive in the two-component systems, dimethyl sulfate ($(CH_3O)_2SO_2$)) is a highly reactive methylating reagent capable of converting **3** into its corresponding ester quite rapidly with significant enantioselectivity (Scheme 2). Notably, the reaction is accomplished in a hydrocarbon solvent (decane) using a catalytic amount of THAB. However, competitive hydrolysis of dimethyl sulfate restricts one to use greater than stoichiometric quantities of this electrophile. As shown in Scheme 2, four equivalents of dimethyl sulfate are required for 50% of **3** to be esterified.

Chromatographic separation of underivatized naproxen, (±)-**4**, may be accomplished through using a CSP derived from (*S*)-**2a** or (3*S*,4*S*)-**2b**. Separation factors are modest, ranging from 2.25 to 2.95 depending on the means by which the selector is tethered to the silica support.[19] Enantiomers of naproxen may also be separated by the selector mediated partitioning process described above. Data for the biphasic enantioselective partitioning of 0.10 mmol (1.0 M equiv) of (±)-**4** in the presence of 0.10 mmol of selector (*S*)-**2a** or (3*S*,4*S*)-**2b** and 20-mol% THAC is given in Table 2. Unlike biphasic extractions of **3** discussed above, the quantity of **4** extracted into the organic layer not only depends on the



Scheme 2.

Table 2. Biphasic enantioselective partitioning of (±)-**4** with 20-mol% THAC[a]

Entry	Solvent	Selector	[Selector] (M)	[(±)-**4**] (M)	% Extracted[b]	%ee (*S*)[c]	*s*[d]
1	CH_2Cl_2	(*S*)-**2a**	0.025	0.025	20.8	18	1.5
2	CH_2Cl_2	(*S*)-**2a**	0.10	0.025	11.6	33	2.2
3	CH_2Cl_2	(3*S*,4*S*)-**2b**	0.025	0.025	20.1	33	2.1
4	CH_2Cl_2	(3*S*,4*S*)-**2b**	0.10	0.025	13.5	51	3.4
5	CCl_2/CH_2Cl_2[e]	(3*S*,4*S*)-**2b**	0.025	0.025	16.8	50	3.3
6	CH_2Cl_2	(3*S*,4*S*)-**2b**	0.10	0.10	18.0	45	2.9
7	CCl_2/CH_2Cl_2[e]	(3*S*,4*S*)-**2b**	0.025	0.10	17.0	50	3.3

[a] Standard conditions entailed vigorously mixing 0.10 mmol (1.0 M equiv) of (±)-**4** in 4 mL of saturated $NaHCO_3$ with an organic solvent containing the indicated chiral selector and 20 mol% THAC at room temperature. Layers were separated and **4** was derivatized with excess dimethyl sulfate.
[b] Determined by HPLC internal standard analysis (see Section 4).
[c] Determined by chiral HPLC (see Section 4).
[d] Stereoselectivity factor.
[e] Used a 3:1 ratio of CCl_4/CH_2Cl_2.

mol% of THAC in solution but also the amount of solvent in the respective layers. Generally, the quantity extracted is equal to or less than 20%. Dilution of the organic layer or concentration of the aqueous layer results in a larger quantity of naproxen ion-pairs extracted. For comparative purposes, the stereoselectivity factor (*s*) for each experiment was calculated. Comparing entry 1 to entry 2 or entry 3 to entry 4 of Table 2, one observes that more concentrated solutions of the selector result in less total naproxen extracted but with higher *s* factors. The *cis*-**3** substituted selector (3*S*,4*S*)-**2b** gives higher *s* factors than (*S*)-**2a** for these partitioning studies, a result consistent with chromatographic studies (table 2; entry 1,2 vs 3,4).[20] Ideally, one should develop a more soluble analogue of (3*S*,4*S*)-**2b** by increasing the bulk on the second stereocenter of the cyclohexane ring, hence making it possible to perform these enantioselective partitionings in more nonpolar or concentrated solvent systems.

3. Conclusions

The biphasic enantioselective partitioning studies and kinetic resolutions described in this paper may have a significant practical value. The method provides a simple and convenient way to resolve a variety of enantiomers, provided that a suitable chiral complexing agent is available. Two such examples were demonstrated, resolution of *N*-(3,5-dinitrobenzoyl)leucine and resolution of naproxen. In the former case, high levels of enantiodiscrimination were achieved both for enantioselective partitioning and kinetic resolutions, even for the simple batch experiments described. Furthermore, results for separation of naproxen by a single-stage batch extraction process are encouraging although enantioselectivities are far below that needed for practical separation of enantiomers. The same will likely be true of many racemate/selector combinations. Hence, a continuous process involving multiple membrane units (staging) will be needed for such cases. Indeed, Ding and co-workers have developed an enantioselective hollow-fiber membrane approach using countercurrent flow of the two phases leading to complete separation of enantiomers, even for a selector with an undesirably low enantioselectivity.[14] This type of enantioselective membrane approach can clearly be applied to two-component chiral phase transfer separations and reactions. Furthermore, this methodology provides a simple means of assessing models of chiral recognition. This should greatly facilitate the production of future CSPs and chiral organocatalysts.

4. Experimental

The racemates and chiral selectors used in this study were prepared in a manner described by the original workers.[19,21] Selector (3*S*,4*S*)-**2b** was obtained from Merck. All ion-pairing reagents, phenacyl bromide and dimethyl sulfate were purchased from Aldrich. Carbon tetrachloride was purchased from Fisher. HPLC was conducted using the following equipment: injectors (Beckman, Rheodyne), pumps (Rainin Rabbit-HPX, Beckman 100B, Alcott 760), variable wavelength (λ) UV-detector (Linear UVIS 200), recorder/integrator (HP 3390A). Chromatographic runs were recorded at ambient temperature with the flow rate 2 mL/min. Dimensions of all analytical HPLC columns were 24 cm×4.6 mm.

4.1. Typical procedure for enantioselective partitioning

A solution containing (±)-**3** (0.10 mmol, 32.5 mg) in 4 mL saturated $NaHCO_3$ was added to 4 mL CH_2Cl_2 containing (*S*)-**1** (0.10 mmol, 28.8 mg) and THAC (0.02 mmol, 7.8 mg). The solution was added to a screw-capped scintillation vial, shaken vigorously for 1 min and allowed to settle for an additional 3 min. The contents were placed in a separatory funnel and the layers were separated. The organic layer was dried over magnesium sulfate. The extracted ion-pairs of **3** were converted to their phenacyl ester derivatives by the addition of excess phenacyl bromide (0.12 mmol, 23.9 mg). Enantioselective partitioning of (±)-**4** were carried out in similar fashion except that enantioenriched extracted ion-pairs of **4** were converted to their methyl ester derivatives by the addition of excess dimethyl sulfate. Residual dimethyl sulfate was hydrolyzed with a saturated solution of $NaHCO_3$.

4.2. HPLC analysis

Enantiomeric excess was determined by chiral HPLC by analyzing the ester derivatives of the extracted ion-pairs of **3** and **4**. In the former case the enantiomers of the phenacyl ester of **3** were separated using (D)-Leucine CSP (mobile phase 15% 2-propanol in hexane, λ=270 nm) available from Regis Technologies. Enantiomers of methyl esters of **4** were separated using an (*R*,*R*)-Whelk-O1 CSP (mobile

phase 10% 2-propanol in hexane, $\lambda = 283$ nm) available from Regis Technologies. In all cases, absolute configurations were assigned by comparison with authentic samples.

Quantities extracted were determined by HPLC using internal standard analysis. The chiral selector was used as an internal standard. Calibration curves for each study were generated by preparing stock solutions of the chiral selector with the appropriate racemic ester derivative of the compound being studied (i.e., the phenacyl ester derivative of **3** or the methyl ester derivative of **4**) at various concentrations. Linear plots were obtained in all cases. To determine the amounts extracted, aliquots from the worked-up organic layer were injected and compared to the calibration data.

4.3. Procedure for biphasic reaction between (±)-3 and dimethyl sulfate in the presence of selector (*S*)-1 (Scheme 2)

To a 10 mL round bottom flask equipped with magnetic stir bar was added 1.0 mol equiv of (±)-**3** (0.10 mmol, 32.5 mg) dissolved in saturated $NaHCO_3$. To this solution was added 3.0 mL of decane containing 1.0 mol equiv (*S*)-**1** (0.10 mmol, 28.8 mg) and 0.1 mL of CH_2Cl_2 containing 2-mol% THAB (0.002 mmol, 0.87 mg). The biphasic mixture was stirred magnetically and 0.1 mL of CH_2Cl_2 containing 4.0 mol equiv dimethyl sulfate (0.40 mmol 50.5 mg) was added drop wise. Aliquots were assayed periodically by chiral HPLC using a (*R*, *R*)-Whelk-O1 column (12% 2-propanol in hexane, $\lambda = 283$ nm) available from Regis Technologies. Conversion was measured by internal standard analysis (see above). Production of ester stopped after approximately 2 h. At this point, close to 50% conversion had been reached. Addition of more dimethyl sulfate leads to additional conversion. Workup was carried out as follows: the organic layer was diluted with CH_2Cl_2, and the aqueous layer was diluted by adding H_2O. The layers were separated and the organic layer was washed sequentially with 1 M HCl, saturated NaCl and water. The solution was dried over sodium sulfate and concentrated under reduced pressure to yield a mixture of product ester, THAB and (*S*)-**3**. Separation of the mixture was accomplished by flash column chromatography (SiO_2, hexane/ethyl acetate).

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