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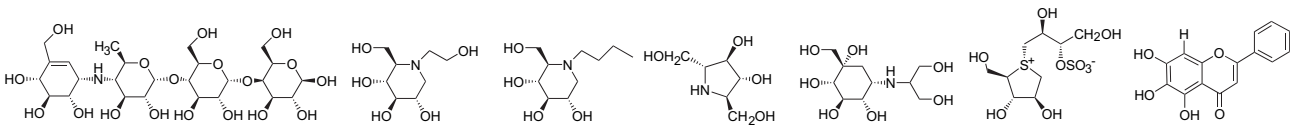
Contents

REPORT



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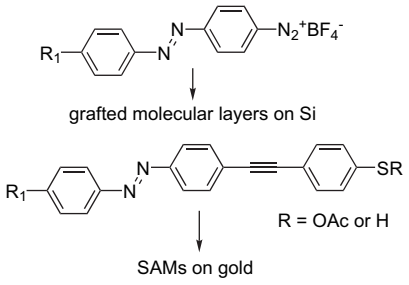
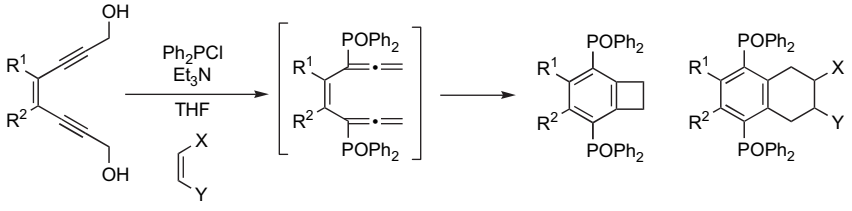
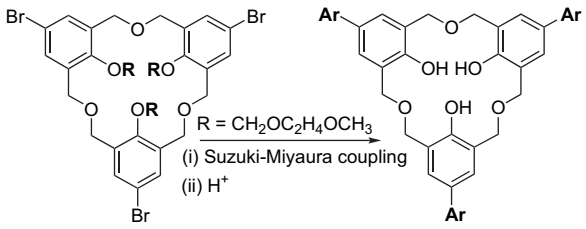
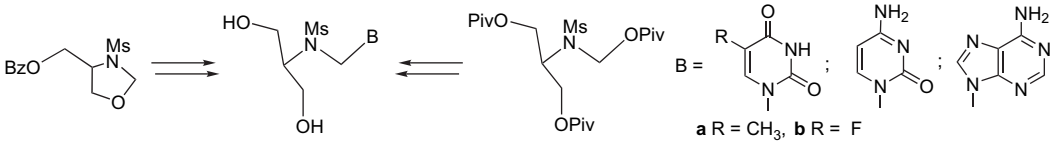
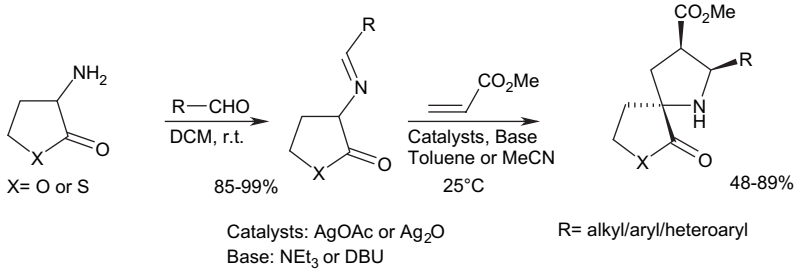


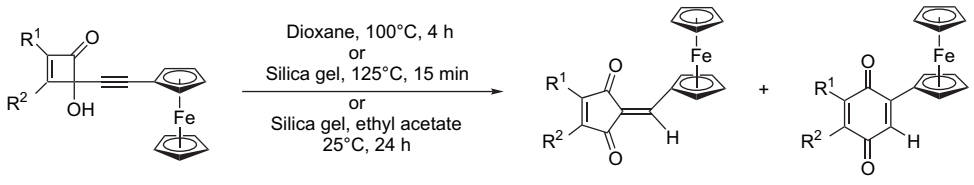
New non-symmetrical azobenzene derivatives have been synthesized as potential molecular electronics switching device candidates.

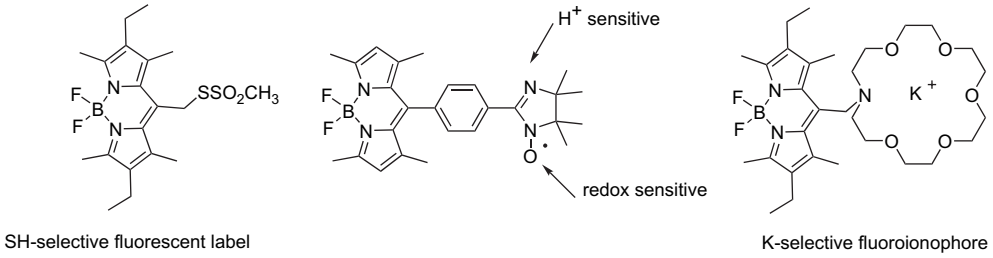


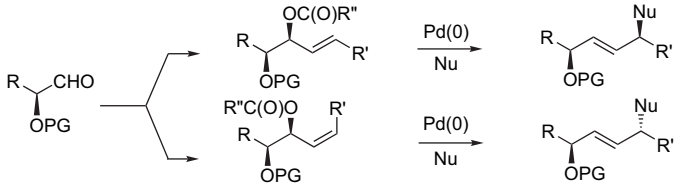


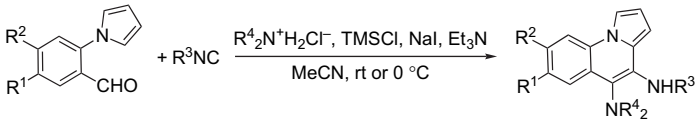


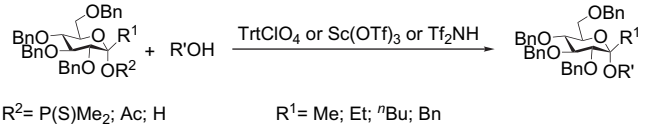


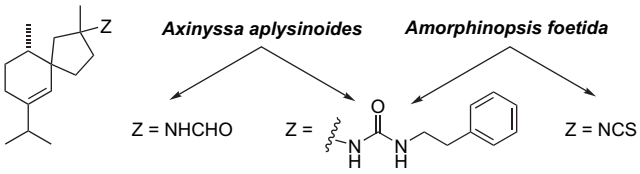


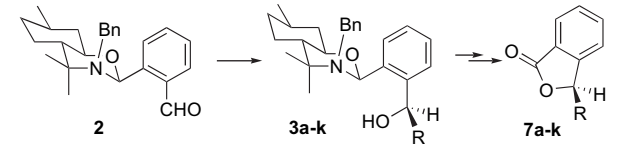




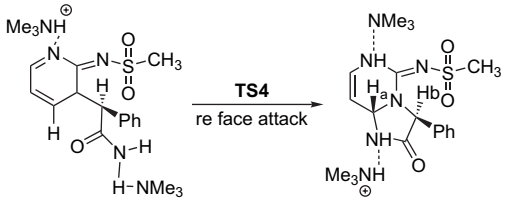


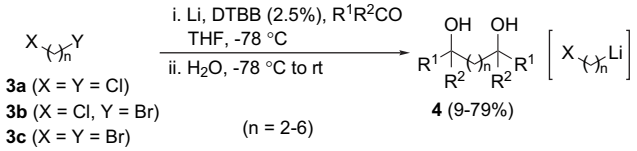


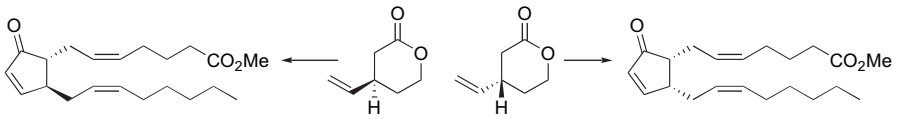




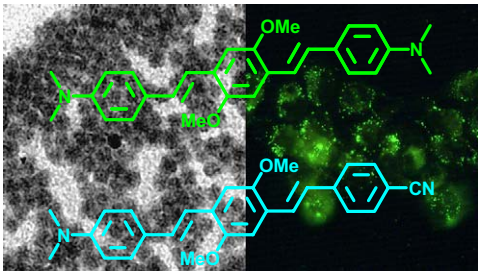
a	b	c	d	e	f	g	h	i	j	k
Me	Et	*i*-Pr	*n*-Bu	Ph	Bn	CH_2=CH-	CH_2=$CHCH_2$-	CF_3	-CH_2CO_2Et	-CF_2CO_2Et

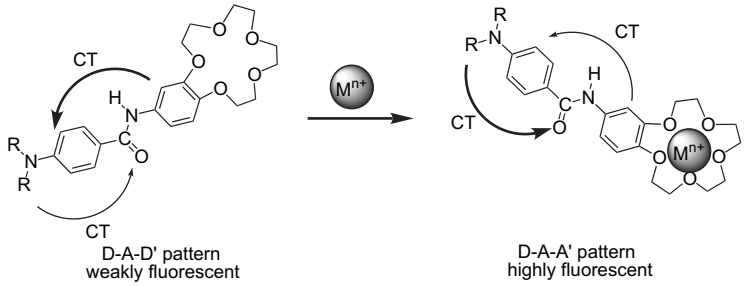


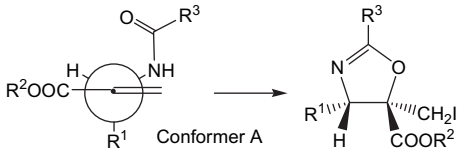




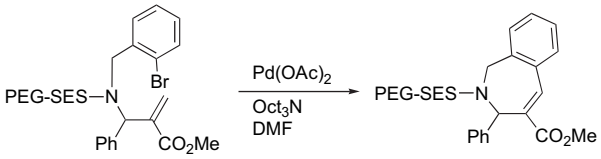
Organosilica nanoparticles doped with two-photon absorbing distyryl-benzene derivatives were prepared and studied as cell staining agents. They proved to be strong emitting, water soluble, two-photon absorbing reporters that can easily penetrate and stain living cells.







Near Attack Conformations leading to the *cis*-product



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α- and β-Glucosidase inhibitors: chemical structure and biological activity

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Abstract—Glycoside trimming enzymes are crucially important in a broad range of metabolic pathways, including glycoprotein and glycolipid processing and carbohydrate digestion in the intestinal tract. Amongst the large array of enzymes, glucosidases are postulated to be a powerful therapeutic target since they catalyze the cleavage of glycosidic bonds releasing glucose from the non-reducing end of an oligo- or polysaccharide chain involved in glycoprotein biosynthesis. Glucosidase inhibitors are currently of interest owing to their promising therapeutic potential in the treatment of disorders such as diabetes, human immunodeficiency virus (HIV) infection, metastatic cancer, and lysosomal storage diseases. Glucosidase inhibitors have also been useful in probing biochemical pathways and understanding structure–activity relationship patterns required for mimicking the enzyme transition state. Amongst the various types of glucosidase inhibitors, disaccharides, iminosugars, carbasugars, thiosugars, and non-sugar derivatives have received great attention. This review is aimed at highlighting the main chemical classes of glucosidase inhibitors, as well as their biological activities toward α- and β-glucosidases, but it is not intended to be an exhaustive review on the subject. Inhibition data on the compounds covered in this review are included in a tabular form as an Appendix, where the type of each glucosidase associated with a specific inhibitor is also given.

Contents

1. Introduction

Glucosidases are enzymes that catalyze the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates. Several glucosidases are specific for the cleavage of glycosidic bonds depending on the number, position, or configuration of the hydroxyl groups in the sugar molecule. Thus, α- and β-glucosidases are able to catalyze the cleavage of glycosidic bonds involving terminal glucose connected at the site of cleavage, respectively, through α- or β-linkages at the anomeric center. The transition state structure for the substrates of these enzymes has a pseudoaxial orientation of the C–O bond and a skew conformation, suggesting that the main differences between α- and β-glucosidases are concerned with positioning of the catalytic nucleophile and the

Keywords: α-Glucosidase; β-Glucosidase; Glucosidase inhibitor; Disaccharide; Iminosugar; Carbasugar; Thiosugar; Non-glycosidic bond.
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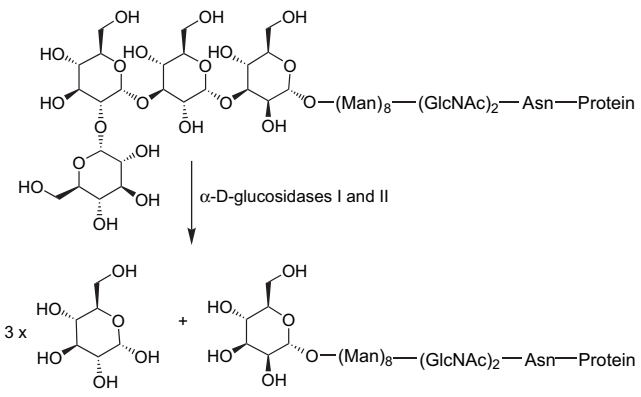
catalytic proton donor, represented by two carboxylic acids units.[1]

The activity of glucosidases is fundamental to several biochemical processes such as (i) degradation of diet polysaccharides to furnish monosaccharide units, which are then able to be metabolically absorbed and used by the organism, (ii) lysosomal glycoconjugate catabolism and glycoprotein processing, and (iii) biosynthesis of oligosaccharide units in glycoproteins or glycolipids.[2]

The generation of glycoproteins involves the cotranslational transference of the tetradeca-oligosaccharide $Glc_3Man_9GlcNAc_2$ from the dolichyl diphosphate (DolPP) to the *N*-asparagine of the nascent protein, by the action of the oligosaccharyl-transferase (OT) in the lumen of the reticulum endoplasmatic membrane.[3] The enzymes glucosidases I and II are involved in the key steps of trimming of this *N*-linked oligosaccharide by cleaving Glc(1-2)Glc and Glc(1-3)Glc linkages, respectively, liberating the three glucose terminal residues of the $Glc_3Man_9GlcNAc_2$ glycoprotein (Scheme 1).[4] Subsequently, this immature glycoprotein is processed by the concomitant action of glycosidases and transferases to give specific glycoconjugates, which play fundamental roles in the biological processes, such as the immune response, intercellular recognition (including fertilization), cellular differentiation, the stability and solubility of proteins, and in pathological processes, such as inflammation and cancer, since α-glucosidase may play a role in tumor cells for the metastatic process.[5]

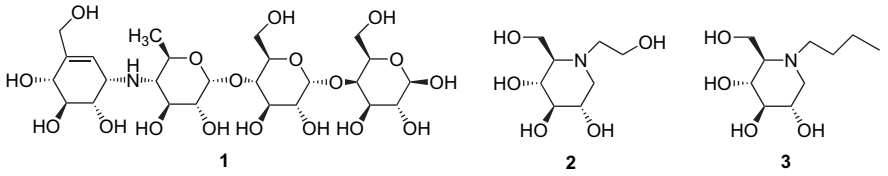


Scheme 1. Processing of the oligosaccharide ($Glc_3Man_9GlcNAc_2$) portion of the immature *N*-glycoprotein by the action of glucosidases I and II.

Inhibitors of glucosidases I and II have also been studied as potential anti-HIV agents.[6] The HIV viral envelope is composed of a bilipidic layer and a complex protein known as *env* that consists of glycoproteins gp41 (transmembrane) and gp120, the latter being displayed on the viral surface and anchored to gp41.[7] Glucosidases I and II participate in the formation of gp120, through processing of the $Glc_3Man_9GlcNAc_2$ *N*-linked glycoprotein, which is responsible for the recognition of the virus by CD4 receptors from T4 lymphocytes, in the initial process of viral infection. The modulation of the antigenicity of gp120 is dependent on the extension and variability of surface glycosylation and represents an interesting target to be explored in drug design.

The multiple functions of glucosidases in the organism warrant the search for potential therapeutic inhibitors to be used in diabetes,[8] obesity,[9] glycosphingolipid lysosomal storage disease,[10] HIV infections,[11] and tumors in general.[12]

Currently, three drugs are therapeutically used as anti-glucosidases: acarbose (**1**) (Precose®), miglitol (**2**) (Glyset®), and *N*-butyl-1-deoxynojirimycin (**3**) (Zavesca®). Drugs **1** and **2** are used in the treatment of non-insulin-dependent diabetes, type II, since they reduce the postprandial hyperglycemia by interfering with the digestion of dietary carbohydrates, while drug **3** is employed for the control of Gaucher's disease, related to disturbed lysosomal storage.



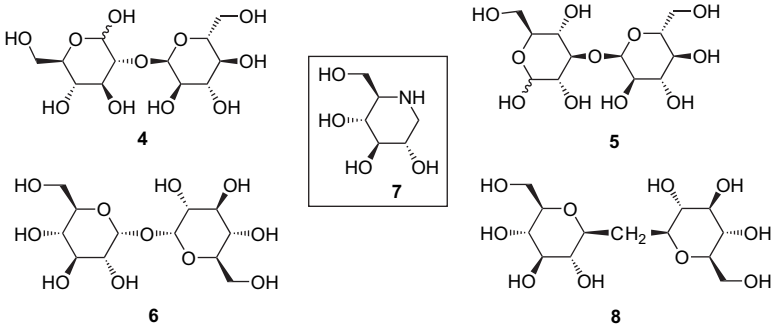
In support of the increasing interest in synthetic and natural glucosidase inhibitors as important tools for understanding biochemical processes and also as prospective therapeutic agents, this review describes the chemical structural diversity of the main α- and β-glucosidase inhibitors that comprise disaccharides, iminosugars, carbasugars, thiosugars, and non-glycosidic inhibitors, in addition to their corresponding biological activities.

2. Disaccharides

The isolation of kojibiose (**4**) and nigerose (**5**), inhibitors of α-D-glucosidase I and α-D-glucosidase II,[13] respectively, opened up new perspectives for the development of novel drugs, especially of the pseudodisaccharide class, for the treatment of HIV infections. Kojibiose (**4**) containing α-(1→2) glycosidic bonds was isolated in 1953 from sake extracts and also from its primary source, koji, a product related to rice fermentation by *Aspergillus oryzae*.[14] On the other hand, acid hydrolysis of amylopectin produced nigerose (**5**), which was shown to have an α-(1→3) linkage.[15] The importance of nigerose and nigerosylmaltooligosaccharides has also been shown to influence the immune function and quality of life in the healthy elderly person as a supplemental syrup on food.[16]

Extracts of *Mormodica charantia* seeds and of *Grifola frondosa* fruits showing α-glucosidase inhibitory activity have also been investigated and D-(+)-trehalose (**6**) was identified as the active component.[17] Trehalose, constituted by two units of glucose linked by an α-(1→1) bond, is employed in the preparation of foods and manufacture of cosmetics and

was recently suggested as a drug to be used in the treatment of osteoporosis, since it was shown to increase trabecular density in rat tibias. Its inhibitory capacity compared with the model 1-deoxynojirimycin (**7**) indicated that, while a concentration of 1×10^{-7} M of **7** showed a 52% inhibition of α-glucosidase, trehalose at 2×10^{-3} M had only 42% inhibitory activity.[17]



C-Disaccharides constitute another class of glycosidic analogues with potential enzymic inhibitory activity. In these compounds, a *C*-glycosidic bond is substituted for the usual *O*-bond, but they are similar to disaccharides, and assume conformations similar to these natural substrates. Postema et al.[18] synthesized several of these compounds, one with an α-1,1 glucosidic linkage (**8**), moderately inhibiting β-glycosidases (K_i 126 μM) and this chemistry has been reviewed in a research monograph.[19]

3. Iminosugars

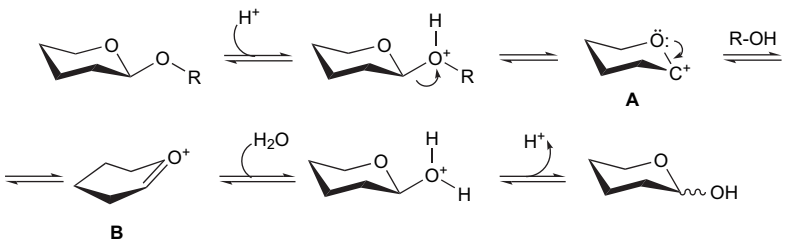
Iminosugars, isolated from plants or microorganisms, are considered to have a high potential therapeutic value and are of interest to be applied in the elucidation of biological recognition processes, due to their glucosidase inhibition properties.[20] The great potency and specificity of these inhibitors are related to their ability to mimic transition state pyranosidic or furanosidic units of natural glucosidase substrates. Significant competitive inhibition is observed with many inhibitors, suggesting that both conformational (shape) and electrostatic (charge) influences may be important in the active site binding.[1]

Iminosugars or polyhydroxylated alkaloids are low-molecular-weight compounds, able to inhibit glucosidases because they may mimic the conformation and charge of the oxycarbenium ion intermediate (**B**), but not of the carbocation (**A**) normally generated in the transition state with sp^2 character during the glycosidic bond cleavage (Scheme 2). The subsequent reaction results in overall retention or inversion of glucoside anomeric configuration, respectively, through double or single nucleophilic displacement.[1,21]

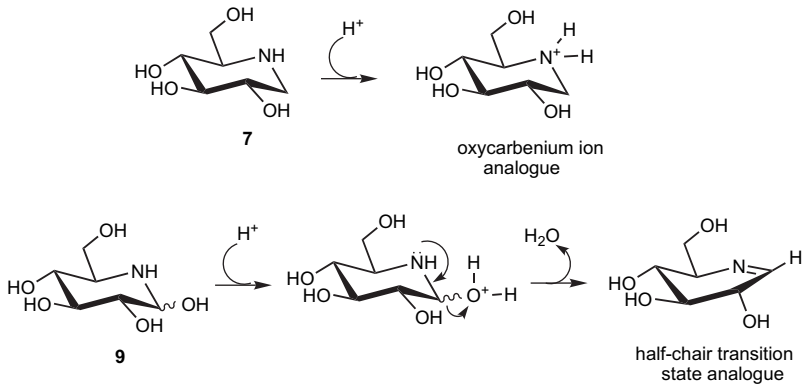
Considering that partial cleavage of the glycosidic bond intensifies the positive charge generated in the oxygen or anomeric carbon of the natural glycoside, substitution of one of the two atoms by protonated nitrogen will mimic, in the transition state, the charge in these centers.[22] In fact, the main characteristics consisting of stabilization of the positive charge on the nitrogen atom, trigonal anomeric center, half-chair conformation, and specific configuration of the hydroxyl ions are crucial for activity in these alkaloids.[23] Thus, relevant structural factors for glucosidase inhibition may be related to the charge and/or shape, defined by the hybridization and conformation of the pyranose ring in natural substrates or piperidine ring in inhibitors, like **7** and **9**. There is evidence suggesting that inhibitors in their natural basic form must be protonated to interact through ionic bonds with the carboxyl group in the active site, like **7** (Scheme 3).

Polyhydroxylated alkaloid structures containing at least two hydroxyl groups and one heterocyclic nitrogen atom may be mono or bicyclic and represented by rings consisting of piperidines, pyrrolidines, pyrrolizidines, indolizidines, or nor-tropanes.[24]

Nojirimycin (**9**) was discovered in 1966 as the first glucose analogue, which comprises endocyclic nitrogen in place of the oxygen pyranosidic atom. The polyhydroxypiperidine, initially isolated as an antibiotic from several strains of



Scheme 2. Carbocation (**A**) and oxycarbenium ion (**B**) intermediate formation during the cleavage of glycosidic bond catalyzed by β-glucosidases.



Scheme 3. Proposed mechanism for α-glucosidase inhibition by iminosugars, 1-deoxynojirimycin (**7**), and nojirimycin (**9**).

Bacillus, *Streptomyces*, and mulberry tree leaves, was shown to be a potent inhibitor of both α- and β-glucosidases of different origins. However, the presence of a hydroxyl group at C-1 adds instability that harms the biological assays. Reduction of **9** by catalytic hydrogenation or sodium borohydride provides analogue **7**, a more stable and a potent glucosidase inhibitor in vitro. Based on experiments involving cultured cells, compound **7** inhibited the formation of complex-type oligosaccharides, leading to the accumulation of $Glc_{1\text{-}3}Man_{7\text{-}9}GlcNAc_2$. However, its in vivo activity is only moderate, besides producing side effects.[25]

The search for more potent iminosugars has led to the preparation of *N*-alkylated analogues, like compounds **2** and **3** with anti-HIV activity, for the treatment of diabetes and Gaucher's disease and even with antiviral effects against B[26] and C hepatitis, bovine diarrhea (BVDV),[27] and dengue virus.[28] Chemical synthesis of *N*-alkylated compounds commonly involves reductive amination reactions between iminosugars and alkyl aldehydes in the presence of a reducing agent like sodium cyanoborohydride. The activity of 1-deoxynojirimycin (**7**) is pH dependent and inhibits glucosidase II more strongly than glucosidase I, while N-alkylation gives the opposite result, either for experiments involving purified enzymes or cell culture.[29] Interestingly, inversion of the configuration at C-5 in **7** to give 1-deoxy-L-idonojirimycin (**10**) leads to loss of the inhibitory activity toward yeast α-glucosidase shown by the former compound, which is a potent competitive inhibitor of this enzyme. Compound **10** shows only a non-competitive inhibition of the enzyme[30] and the fact that 1,5-dideoxy-1,5-iminoxylitol (**11**) has no effect on the activity of yeast α-glucosidase indicates that the 5-CH_2OH group in **7** plays an important role in promoting effective binding.[31]

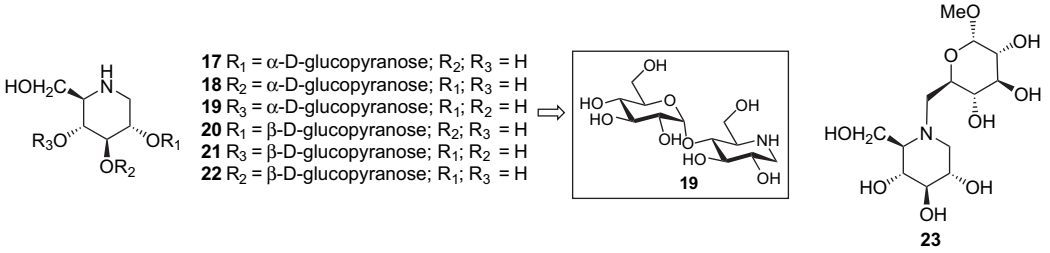
Polyhydroxypiperidine derivatives comprise the main class of glucosidase inhibitors, with a great variety of compounds of natural origin, isolated from fungi, bacteria, and plants, besides the synthetic derivatives, and several of them show high inhibitory constants for both α- and β-glucosidases. Recently, this class of compounds has attracted more attention owing to the inhibition of other enzymes such as glycosyltransferases, glycogen phosphorylase,[32] nucleoside phosphorylases,[33] and sugar-nucleotide mutases (UDP-Gal*p* mutase).[34]

α-Glucosidase inhibition studies in human hepatoblastoma cells (HepG2) using a series of *N*-alkylated iminosugars showed increasing activity with extension of the length of the linear alkyl chain and decreasing activity with the introduction of branching. However, in vitro assays with purified pork α-glucosidase I showed a decrease in the inhibitory activity, suggesting that these lipophilic alkylated groups may have a relevant role in cellular uptake.[35] Better results from the *N*-alkyl variations relied on a less lipophilic group instead of a linear long alkyl chain, revealing that derivatives incorporating an oxygen atom in an *N*-decyl side chain might be less cytotoxic and as potent as **3** against α-glucosidase. For instance, the *N*-7-oxadecyl-DNJ **12** had a strong effect toward α-glucosidase I, even though it was a weak inhibitor in cellular assays. Furthermore, compound **12** was able to inhibit HIV-1-induced syncytia formation and lymphocyte proliferation in vitro, along with a reduction profile in arthritis in rats.[36] Abolishing the basicity of the nitrogen by N-oxidation or N-acylation afforded less active products.[4]

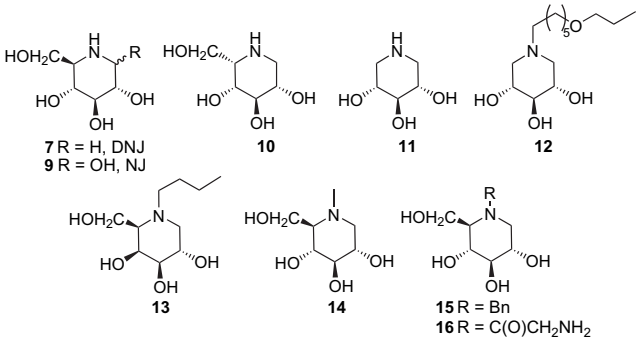
Ceramide glycosyltransferase inhibition occurred only with the *N*-alkyl derivatives of **7** containing at least three carbon atoms. In relation to the stereochemistry and functional groups of compounds similar to **7**, C-3 modifications induced loss of activity against α-glucosidases I and II, and ceramide glycosyltransferase, suggesting the common structural features for inhibition of these enzymes. The exclusive inhibition of a transferase by *N*-butyl-1-deoxygalactonojirimycin (NB-DGJ, **13**) is the only exception to this rule. In fact, compounds **3** and **13** are good inhibitors for ceramide-specific glucosyltransferase, either in vitro or in vivo, suggesting that they mimic the ceramide substrate, since there are similar stereochemical features for both α-glucosidase and ceramide glucosyltransferase inhibitors.[10]

The activity of compound **3** is extremely dependent on the positions and functions of groups in the piperidine ring, which should be adequately positioned to mimic the oxycarbenium ion intermediate in the enzyme active site. Thus, epimerization at C-2 or C-4 or methylation of the hydroxyl at C-3 decreases the activity, while modifications at C-1 and C-6 or at the *N*-alkyl group are tolerated.[10] Additionally, an investigation of the epimerization, deoxygenation, and conformation contributions of a series of seven polyhydroxylated piperidines toward their inhibitory activity was performed, leading to some structure-based evidence that 2-deoxygenation and/or 3-epimerization of **3** enhance the activity of rat intestinal lactase and bovine liver cytosolic β-galactosidase.[36]

Comparing compounds **3**, **7**, and *N*-methyl-1-deoxynojirimycin (**14**), there is no significant difference in their ring conformation, but a slight difference was detected in their conformations about the C-5–C-6 bond that is predominantly tg (trans/*gauche* H–H relationship) or gt for compound **7** and gg for **3** and **14**.[37] Moreover, the *N*-methyl derivative **14** is a more potent inhibitor of α-glucosidase I than is **7** and is also more active against the replication of the HIV virus.[6] An interesting activity was demonstrated by compound **14** and miglitol (**2**), owing to their protective effect against post-ischemia dysfunction of the left ventricle, by altering glycogenolysis through α-1,6-glucosidase



inhibition.[38] On the other hand, studying the interactions of the enzyme bound to *N*-^{13}C-methyl-deoxynojirimycin as a chemical probe, Hines et al.[39] prepared analogues **15** and **16** to explore the π–π stacking or cation–π interactions with the aromatic residues of the glucosidase active site. Although compound **15** showed no activity up to 3.0 mM, the *N*-glycyl derivative **16** was more potent than **14**.



The use of native or immobilized glucosidases in transglucosylation reactions with *N*-benzyloxycarbonyl-1-deoxynojirimycin as an acceptor molecule, led Asano et al.[40] to develop a strategy to prepare *N*-containing sugars illustrated by 2-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**17**), 3-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (**18**) and 4-*O*-α-D-glucopyranosyl-1-deoxynojirimycin (also named 4-*O*-α-D-glucopyranosylmoranoline, **19**),[41] and the corresponding anomers 2-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**20**) and 4-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**21**), by incubating with α-glucosidase and β-glucosidase, respectively. After cleavage of the *N*-benzyloxycarbonyl group of the product glycosides, the *N*-protective group being used to prevent complete loss of glucosidase activity during the enzymic synthesis, compounds **18** and **19** retained the activity of **7** toward rat digestive glucosidase, including sucrase and isomaltase, while **18** was a much stronger inhibitor in rice α-glucosidase experiments than **7** and the same effect was observed for **17** against trehalase. A series of disaccharides related to lactosamine and chitobiose also comprising a 1-deoxynojirimycin moiety were synthesized, but their activity toward glucosidase was not reported.[42]

Studies on 1-deoxynojirimycin-containing glycans isolated from *Morus alba* also gave the potent glucosidase inhibitors described above, along with 3-*O*-β-D-glucopyranosyl-1-deoxynojirimycin (**22**) and the corresponding inactive 6-*O*-β-derivative against maltase and weak inhibitor of sucrase (IC_{50} 940 μM). Isomer **19** was also isolated from natural sources such as *Scilla sibirica*.[43]

From this series of glycosylated deoxynojirimycins, it is worth mentioning compound **23**, namely MDL 73945, which showed potent inhibition against α-glucosidases, such as sucrase, maltase, and isomaltase, and which was approved for clinical trials to treat postprandial hyperglycemia in diabetes mellitus patients.[44] Despite the convenient introduction of a sugar residue into many positions of **7**, the results with compound **23** provide evidence that glycosylation on the nitrogen gives a more effective inhibitor.

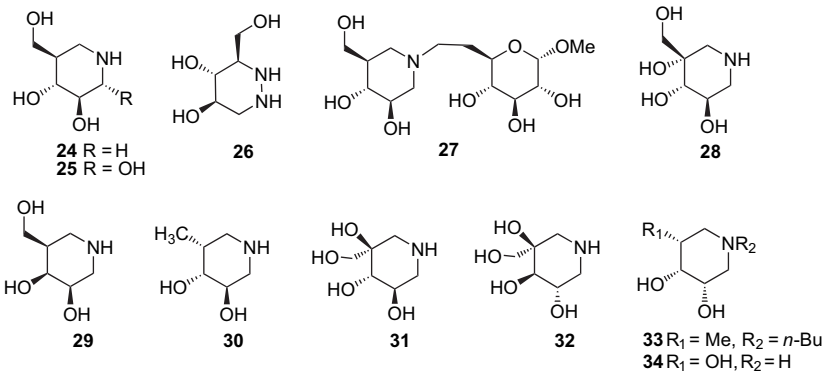
Some piperidinyl analogues are able to mimic the anomeric carbon's positive charge in the transition state, since the presence of the hydroxymethylene group is not essential to β-glucosidase inhibition, as demonstrated for dehydroxymethyl-1-deoxy-nojirimycin (**11**).[36]

These substances are called 1-azasugars and their main representative is isofagomine (**24**), which has the anomeric carbon and the ring oxygen of glucose replaced by nitrogen and carbon, respectively; the 2-OH group is also absent, but the remaining hydroxyl groups preserve the D-glucose configuration.[45] Compound **24** and derivatives, like **26**–**31**, lacking substituents on the α-carbon atom's neighboring nitrogen, are potent inhibitors, **24** being about 440 times more potent than **7** toward β-glucosidases, but only a moderate inhibitor of α-glucosidase.[46] Stereochemical alterations in ring-substituent groups may reduce activity, as illustrated by product **32**. Introducing a hydroxyl group at C-2, as shown in compound **25**, namely noeuromycin, afforded a stronger β-glucosidase inhibitor, probably due to an additional hydrogen bond interaction in the active site. Although compound **25** was prepared as a mixture of stereoisomers at C-2 (α/β 1:2), the activity was stronger than that of compounds **7**, **9**, and also **24**, presumably caused by the equatorial β-isomer. In contrast to isofagomine, compound **25** was also a potent inhibitor of α-glucosidase.[47]

Crystallographic studies on isofagomine derivatives in the endocellulase active site have provided evidence for the

presence of a protonated center, in the nitrogen-containing inhibitor bound with the active center, with a conformation similar to that of the ground state. Other studies, however, have revealed a variety of conformational distortions in the iminosugar target interactions. Although it is difficult to establish the true conformation assumed by these rings in an enzyme site, it seems clear that the binding of these derivatives is enthalpy favored and that protonation of the amino group contributes exothermically to this process.[22]

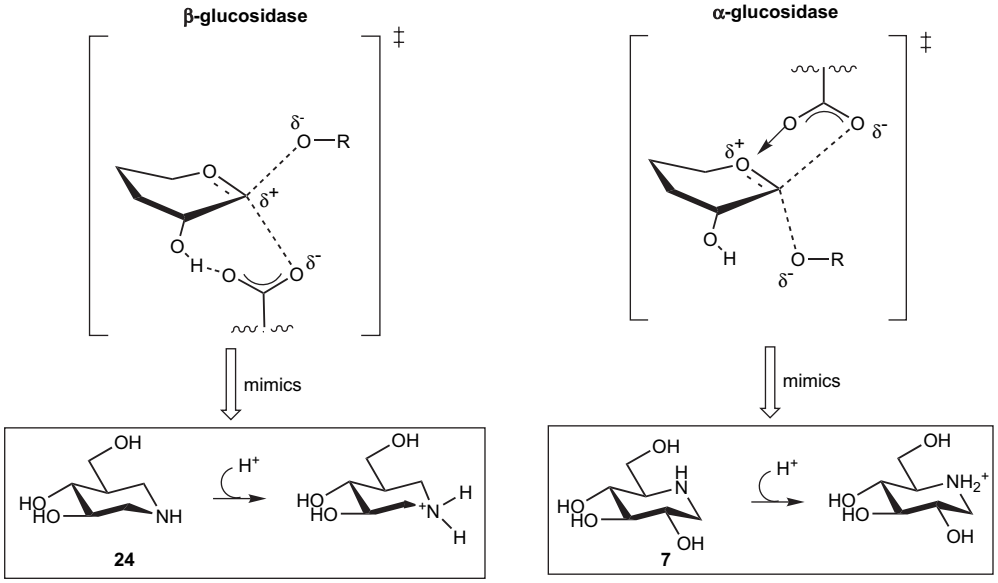
a *syn* interaction of the carboxyl nucleophile with the anomeric carbon, but, on β-glucosidase, the nucleophile interacts additionally with the hydroxyl group at C-2, favoring the carbocation formation, while, with α-glucosidase, this interaction occurs with the endocyclic oxygen, developing an oxycarbenium ion-like transition state.[21] Comparatively, deoxynojirimycin (**7**) may mimic the charge of the oxycarbenium ion, having potent anti-α-glucosidase activity, while isofagomine (**24**) mimics the charge developed on the anomeric carbon, leading to a more potent β-glucosidase



Concerning the specificities of the iminosugar inhibitor **7** and the azasugar, isofagomine (**24**), for α- and β-glucosidases, respectively, it is worth noting the similarities in mechanism of these inhibitors with that involving the corresponding natural substrate of each enzyme. According to kinetics and three-dimensional studies involving α- and β-glucosidases in the transition state, there is a difference in the oxycarbenium ion character between these enzymes. As shown in Scheme 4, the differing orientation at C-1 of the α- and β-anomeric group in the enzymic substrates will direct the best fit for interaction with the nucleophilic carboxyl oxygens in the active site. In both enzymes, there is

inhibitor. An interesting activity was observed for **24** consisting of potential inhibition of hepatic glycogen phosphorylase with an IC_{50} value of 0.7 μM, since this enzyme is involved in the suppression of the increased basal and glucagon-stimulated glycogenolyses in type II diabetes patients.[12]

Based on the assumption that the carbocation transition state might be more important for equatorial hydrolysis (β-glucosidase), while the oxycarbenium transition state is favored for axial hydrolysis (α-glucosidase), Bols[45] synthesized the hydrazine analogue of isofagomine, compound **26**, comprising both mimic functions. Comparatively, the inhibitory



Scheme 4. Comparison of mechanism of action of isofagomine (**24**) and deoxynojirimycin (**7**) with the corresponding substrates of β- and α-glucosidases, respectively, in their preferential transition state.

effect of **26** on α-glucosidase was stronger than that of compounds **7** and **24**, whereas on β-glucosidase, it was weaker than **24**, but more potent than **7**.

1-Azasugar analogues bearing an *N*-linked glucose residue, such as **27**, led to a stronger glucoamylase inhibitor than **24**, preserving high β-glucosidase activity. Alkylation at nitrogen in **27** afforded the ammonium derivative, which was not as active as the corresponding *N*-oxide compound.[45]

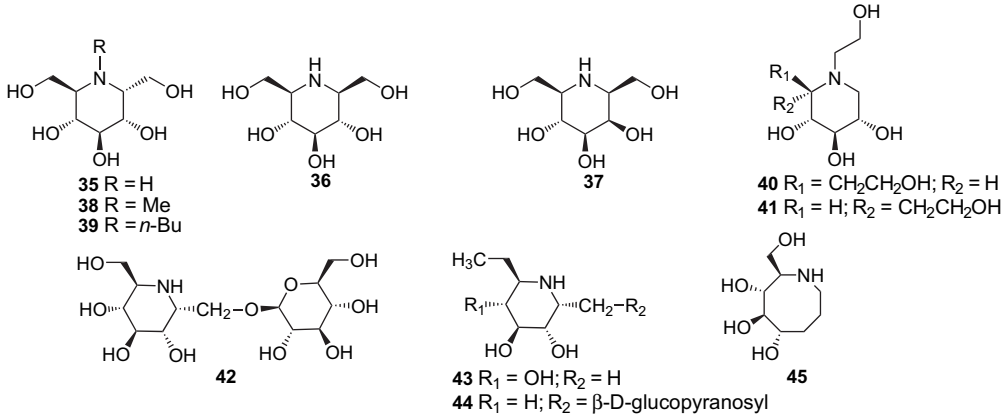
Some iminosugars that are not configurationally derived from glucose may also have high anti-glucosidase activity. Igarashi et al.,[48] in a search for new α-fucosidase inhibitors, synthesized compounds **33** and **34**, respectively, with reasonable and potent anti-glucosidase activity.

Nojirimycin homologues with a hydroxymethylene group at C-1 are named as α-homonojirimycin (**35**) and β-homonojirimycin (**36**) and were isolated from leaves of *Omphalea diandra*[49] and *Aglaonema treubii*,[50] respectively. The ability of **35** to inhibit digestive α-glucosidase was reported by Kite et al.[49] and compared to that of deoxynojirimycin (**7**), which suggested that the attachment of a hydroxymethyl group at the anomeric position of **7** gives better enzyme selectivity. Nevertheless, compound **36** was inactive as an inhibitor of almond β-glucosidase, but showed reasonable activity toward rice α-glucosidase.[51] In order to investigate the contribution of the chiral centers and conformation on glycosidase inhibition, Asano et al.[52] prepared a series of natural epimers of α-homonojirimycin and its *N*-alkylated derivatives and achieved some interesting results with β-homomannojirimycin (**37**) against α-glucosidases and α-L-fucosidase. Concerning the *N*-alkyl derivatives, *N*-methyl-α-homonojirimycin (**38**) was a better inhibitor of glucosidase I than **35** and the corresponding *N*-butyl-α-homonojirimycin (**39**), but, in contrast to **7**, all of them were inactive against HIV-1 replication. An approach to perform some modifications on miglitol (**2**) was also described involving the preparation of *N*-hydroxyethyl-D-*gluco*-1-deoxyhomonojirimycin (**40**) and *N*-hydroxyethyl-L-*ido*-1-deoxyhomonojirimycin (**41**), which differs in the configuration at C-5. Thus, the inhibition assays provide evidence that the introduction of an extended hydroxyethyl group in the α-position to the amino group, instead of a hydroxymethyl as observed in **7**, afforded better β-glucosidase inhibitors, as compared to α-glucosidases. The corresponding peracetylated derivative of **41** was also potent against β-glucosidase.[53]

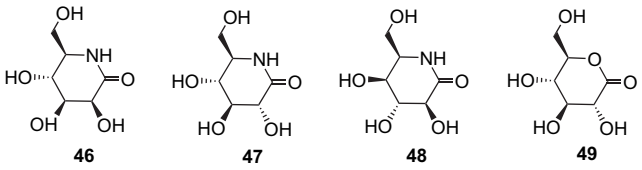
An analogue of homonojirimycin, 7-*O*-β-D-glucopyranosyl-α-homonojirimycin (**42**), was isolated from a methanolic extract of *Lobelia sessifolia*, and is a hybrid containing a polyhydroxylated piperidine system connected via an oxymethylenic bridge to a glucose unit. This compound has high α-glucosidase and trehalase inhibitory activities. Several other homonojirimycin derivatives were isolated from the roots of *Adenophora* spp., another member of the same family. Among the polyhydroxylated alkaloids obtained, such as adenophorine (**43**), the glucoside derivative of 5-deoxyadenophorine (**44**), along with compound **42**, showed potent inhibitory activity of α-glucosidases and, additionally, α-galactosidase.[54]

Godin et al.[55] recently described an expansion of the ring in piperidine derivatives to an eight-membered iminoalditol (**45**). ^{1}H NMR studies showed that this compound exists mainly in the boat–chair conformation, with the substituents assuming a pseudo-equatorial conformation. However, compound **45** was weakly inhibitory to α- and β-glucosidases (22.1 and 37%, respectively), probably due to the absence of OH substituents at C-6 and C-7, which would correspond to the OH groups at C-3 and C-2 in natural glycosides.

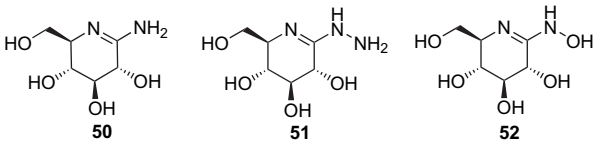
Glycono-δ-lactams, which also belong to the iminosugar class, were one of the first glycosidic groups found to have potent anti-β-glucosidase activity. Studies based on X-ray crystallographic analysis and molecular modeling of eight D-glycono-δ-lactams have been described by Nishimura et al.[56] These compounds follow some of the general rules for β-glucosidase inhibition, which are the half-chair conformation, simulation of the oxycarbenium ion intermediate, and the positive charge around the anomeric carbon. However, the *gluco*-configuration does not seem to be a requisite for high-potency glucosidase inhibition, since D-mannono-δ-lactam (**46**) was more active against β-glucosidase than D-glucono-δ-lactam (**47**), while D-idono-δ-lactam (**48**) showed a similar activity to **47**. It is possible that the carbonyl group in these compounds is topologically equivalent to the glycosidic oxygen atom in the high-energy transition state observed in β-glucosidases. The parent compound, D-glucono-δ-lactone (**49**), has also been described as a strong inhibitor of β-glucosidase, similar to the piperidine **9** and lactams **46**–**48**, and is more effective by a factor of 100 when compared to its inhibition of α-glucosidase.[57] The relative importance of the stereochemical and conformational similarities between **49** and the transition state in the



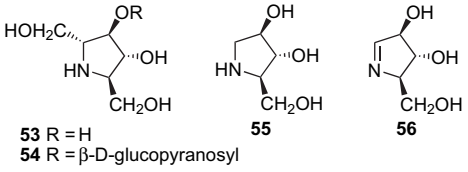
enzyme-catalyzed reaction has been argued about since 1968,[58] besides the influence of the polar oxy group that partially mimics the oxycarbenium ion intermediate, which may be stabilized by a carboxylate group at the active site involving charge–charge interactions.[57] Nevertheless, the true character of the transition state analogue is still under investigation.



Ganem et al.[59] prepared a variety of monosaccharide-like alkaloids containing anomeric centers hybridized to sp^2 carbon consisting of amidine (**50**), amidrazone (**51**), and amidoxime (**52**), obtained from nojirimycin (**9**), showing potent β-glucosidase inhibitory activity. The presence of nitrogen atoms in the *exo* and *endo* positions, 'circling' the anomeric carbon, combines the structural features of both families of iminosugars and glycosylamines. Owing to the common structural elements, these compounds may interact in the active site by similar H-bonding and electrostatic forces, even though they have different basicity properties. The authors concluded that the broad-spectrum of inhibition of **50**–**52** can be attributed largely to the flattened anomeric conformation adopted in the transition state binding, rather than through achieving the formal charge that mimics the oxycarbenium ion (**B**) (Scheme 2). On the other hand, Heightman and Vasella[1] suggested that the neutral inhibitor **52**, the inhibition of which is pH dependent, undergoes an enzymic protonation within the binding site and the basic derivatives **50** and **51**, which are protonated at pH values up to 7, interact most probably by electrostatic interaction.



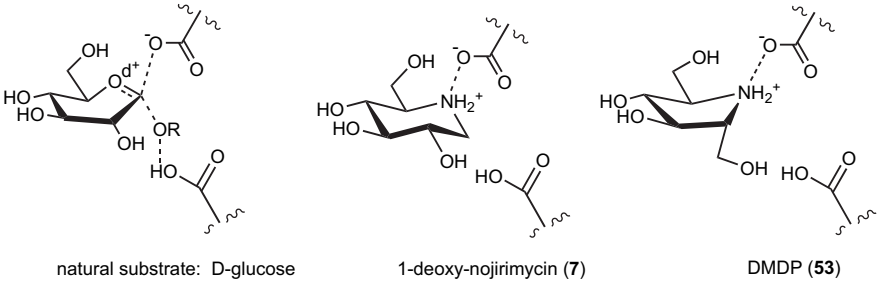
Analogues of pyrrolidine alkaloids include 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidines, for example, or 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **53**) having the all *R*-configuration, which can be regarded as a mimic of a natural β-D-fructofuranose unit. Compound **53** was first isolated from *Derris elliptica* and also from several plants and microorganisms, suggesting that it is a common metabolite in several species.[54] The relatively flat five-membered ring with a unique C_2 axis of symmetry makes compound **53** similar to the transition state observed during substrate hydrolyses. Additionally, its high affinity to glucosidases probably results from the spatial arrangement of the hydroxyl groups, similar to the natural substrate molecule, glucose (Scheme 5).[60] Standard substitution of the hydroxyl function of the hydroxymethylene group at C-1, exemplified by adding fluoro, amino, or methoxyl groups, decreases the activity, while introduction of *O*- or *N*-alkylated chains or an aryl group produces lipophilic derivatives with a potency similar or higher than that of the prototype **53**.[61] Introduction of methyl or hydroxymethylene groups at the hydroxymethylene carbon produces derivatives of reduced potency.[21]



The iminoalditol **53** is known as a potent reversible inhibitor of α-glucosidases I and II, almond β-glucosidase, bovine liver β-galactosidase, invertase, and PFP (pyrophosphate-D-fructose-phosphate-1-phosphotransferase), along with an interesting property, its ability to neutralize the activity of compound **7**. The ability of **53** to promote accumulation of $Glc_3Man_9GlcNAc_2$ *N*-linked oligosaccharides in the endoplasmic reticulum is regarded as a result of its strong inhibitory activity of processing by glucosidase I, and it showed significant anti-HIV activity.[37] An extra biological activity shown by DMDP is its toxicity observed toward several insects, demonstrating an antifeedant protector effect.[60]

Recently, 3-*O*-β-D-glucopyranosyl-DMDP (**54**) was isolated from the roots of *Stemona tuberosa* Lour, as a component of Thai traditional drugs used in China and Japan for various medicinal purposes. Despite the high activity shown by glycosylated-DNJ, including **18** and **19**, this derivative proved to be a weaker inhibitor of β-galactosidase than the parent compound **53**, and it was inactive toward β-glucosidase.[62]

Another active natural product comprising a pyrrolidine ring is illustrated by 1,4-dideoxy-1,4-imino-D-arabinitol (DAB-1, **55**), which was first isolated from *Angylocalyx boutiquenus* and showed a potent inhibitory activity along with a broad inhibitory spectrum toward mammalian

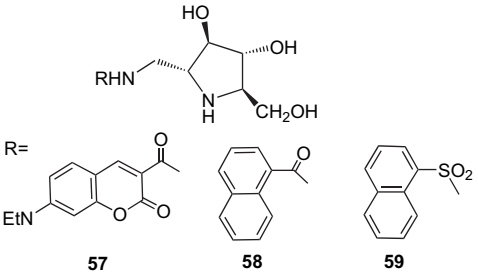


Scheme 5. Transition state in reaction of D-glucose with glucosidase, compared to those with inhibitors DNJ (**7**) and DMDP (**53**).

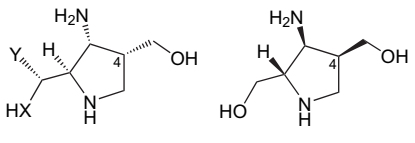
glucosidases, including α-glucosidase II, α-mannosidases I and II, intestinal isomaltase, and trehalase.[60] As proposed for **24**, compound **55** was also found to be a potent inhibitor of hepatic glycogen phosphorylase that could be used as a new antihyperglycemic agent for the treatment of type II diabetes (IC_{50}=1 μM).[12] However, the five-membered analogue **55** was less potent than compounds **7** and **53** as an anti-HIV agent, since the inhibition of α-glucosidase II is less correlated with anti-HIV activity.[37] Another pyrrolidine compound, namely nectrisine (**56**), isolated from the fungus *Nectria lucida* as an immunomodulator, has also received much attention, owing to its potent α-glucosidase inhibitory activity in vitro and in cultured cells by acting as a competitive inhibitor.[63]

Structure–activity relationship studies demonstrated that the introduction of amide groups comprising aromatic or aliphatic lipophilic chains at C-1 produces β-glucosidase inhibitors active in the nanomolar range. The coumarinic derivative bound by an amide bridge to the nucleus (**53**), exemplified by compound **57**, was the most potent pyrrolidine inhibitor of β-glucosidase (K_i=1.2 nM), while naphthyl derivatives bound by amide **58** and sulfonamide **59** bridges showed inhibition constants of 550 and 100 nM, respectively. On the other hand, introducing the *N*-dimethylamino group into the aromatic ring in **59** gave a sulfonamide derivative with strong inhibitory activity (K_i=2.4 nM). Unlike piperidine derivatives, introduction of the *N*-alkyl group did not increase the relative potency of **57** against the HIV virus.[61]

Falb et al.,[64] exploring intramolecular cycloaddition reactions between oximes and alkenes, from L- and D-amino acids as starting materials, synthesized compounds **60–62** and **63**, respectively. In these analogues, the hydroxyl group at C-4 in compounds such as **53** is replaced by a hydroxymethyl group, providing branched-chain azasugars. Additionally, they have a C-3 amino group introduced as an isostere of the hydroxyl group to keep the hydrogen bonding in the active site. These structural characteristics provide the necessary pattern for anti-glucosidase activity, as illustrated by the planar ring conformation and the possibility to stabilize a positive charge generated in the transition state. Among these derivatives, compound **63** was the best inhibitor of α-glucosidase.



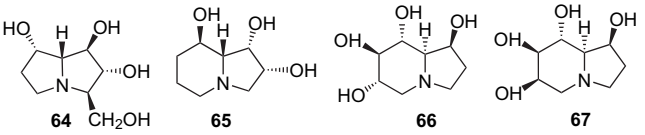
57 **58** **59**



60 X= O; Y= H
61 X= O; Y= Me
62 X= S; Y= H

63

Polyhydroxylated pyrrolizidine alkaloids, exemplified by australine (**64**, isolated from *Castanospermum australe*), are structurally related to the fusion of two pyrrolidine rings, with a common nitrogen atom at the junction. Unlike the symmetry of pyrrolidine derivative **53**, australine does not have a C_2 axis of symmetry, but it does provide a good pattern of competitive inhibition toward processing α-glucosidase and amyloglucosidase.[65]



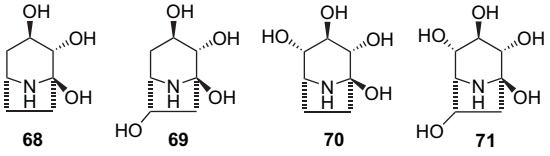
64 **65** **66** **67**

Fusion of the piperidine and pyrrolidine rings results in the indolizidine class of compounds comprising (−)-swainsonine (**65**), isolated from *Swainsona canescens*, *Astragalus lentiginosus*, *Ipomoea carnea*,[66] etc., and castanospermine (**66**), extracted initially from the seeds of *Castanospermum australe*,[67] the first alkaloids to exhibit strong inhibition toward α-mannosidases and α-glucosidases, respectively. Compound **66** may be regarded as a derivative of 1-deoxynojirimycin (**7**) on account of its similar structural and biological properties. The hydroxymethyl group of **7** is spatially locked by an ethylene bridge, which is linked to the nitrogen, generating a pyrrolidine ring. This rigidity could explain the preferred anti-glucosidase activity exhibited by **66** and its derivatives, as the ethylenic bridge helps to stabilize the transition state conformation, with participation of the enzyme acidic group.[11] The basic nitrogen in the indolizine class plays an important role in the inhibition, since experiments performed with castanospermine *N*-oxide involving almond β-glucosidase gave K_i values 500-fold larger than **66**.[21]

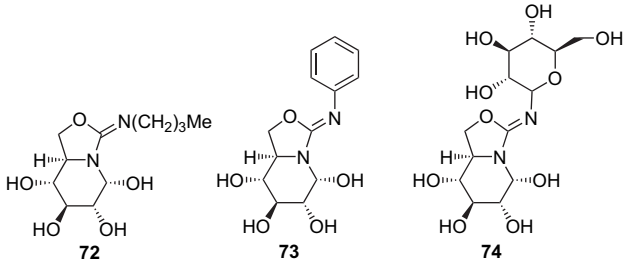
The octahydroindolizidine alkaloid **66** is one of the derivatives with the highest inhibitory effects on glucosidases, inhibiting both mammalian and plant α- and β-glucosidases, β-glucocerebrosidase, and rat intestinal sucrase. However, **58** has no effect on yeast glucosidase or β-*N*-acetyl-hexosaminidase. The corresponding diastereomer (6*R*), (+)-6-epicastanospermine (**67**), does not have the wide range of activity as **66**, even though it was a potent inhibitor of amyloglucosidases.[68] Structure–activity studies suggest that the bicyclic system of compound **66**, able to lock the bond corresponding to C-5–C-6 in hexopyranoses, provides a high specific enzymic activity when compared to the monocyclic compound **9**.[69] It seems that compound **66** may also interfere in the transport of free oligosaccharides (FOS) performed in the endoplasmic reticulum and cytosol during glycoprotein biosynthesis. The progressive pathology in patients with glucosidase I congenital deficiency may be included in this anomaly.[70] Furthermore, the use of castanospermine (**66**),[71] swainsonine (**65**),[72] and glucono-lactone (**49**)[73] screened on B16 melanoma cells led to the inhibition of experimental metastasis by blockage of protein glycosylation or oligosaccharide processing, whereas **66**[74] and **7**[75] inhibited virus

syncytium formation and HIV replication of human immunodeficiency virus.

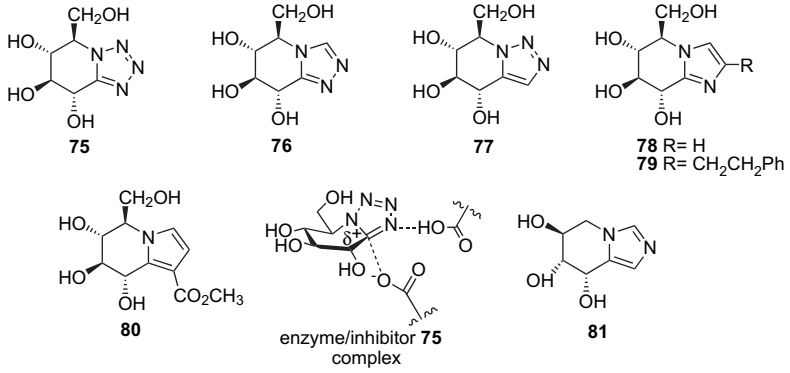
Another class of natural products contains the bicyclic system of nor-tropane, and these compounds exhibit potential β-glucosidase-inhibiting properties. Such compounds with the nor-tropane structure are called calystegins (**68–71**) and are isolated from many plants, such as *Calystegia sepium*, *Ipomoea carnea*, and *Physalis alkekengi* var. *francheti*. They are classified according to the number of hydroxyl groups present in the bicyclic system, as illustrated by calystegins A_3 (**68**), B_1 (**69**), B_2 (**70**), and C_1 (**71**). Despite the structural variation, all calystegins have a hydroxyl group at the ring junction, in the α-position relative to the nitrogen atom, generating an amino-hemiacetal function and an ethano bridge across the 1,5-positions.[76] Initially known by their unique involvement in plant–bacterium relationships, the potential use of calystegins B complex (27% of **69** and 73% of **70**) has been extended by Molyneaux et al.[77] as a competitive inhibitor of β-glucosidase and α-galactosidase, while **71** was shown to exhibit strong inhibition only on β-glucosidase.



Recently, Garcia-Moreno et al.[69] reported a new class of hybrid compounds, derivatives of indolizidines, such as castanospermine and trehazolins. The structure of this new class of azasugars was designed to replace the sp^3 amino nitrogen by the pseudo-amide nitrogen (as in urea, thiourea, and carbamate) with higher sp^2 character. This structural change should increase the anomeric effect in the proposed aminoacetal center and keep the original conformation and configuration in aqueous media that is not observed in classical iminosugars. The isourea-type products synthesized include compounds **72–74** comprising structural characteristics of polyhydroxyindolizidines and, in **74** (trehazolin), which is a natural inhibitor of trehalase, comprising exocyclic nitrogen susceptible to further modifications that should include the introduction of different substituents to modulate enzyme specificity. In fact, the inhibitory activity was greatly influenced by the nature of these substituents and pH, leading to an increase in activity or preferred inhibition between α- or β-glucosidase.



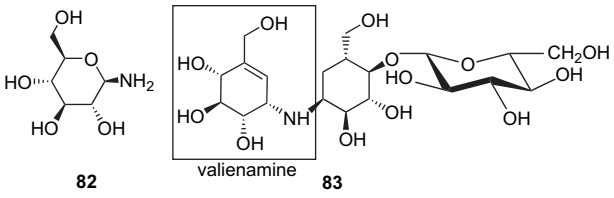
Other bicyclic systems with the piperidine nucleus fused to a tetrazole ring **75**, a triazole ring **76**, **77**, a pyrazole ring **78**, **79**, and a pyrrole ring **80** have been synthesized to afford a half-chair conformation of the six-membered ring as a result of its fusion with the heterocycle, thereby mimicking the transition state of glucosidases. A higher degree of selective anti-β-glucosidase activity was achieved in derivatives containing a nitrogen atom adjacent to the anomeric center in the iminosugar, as in compounds **76**, **78**, and **79**. This suggests that this nitrogen atom, corresponding to the oxygen of the *O*-glycosidic linkage, is important for interaction in the active site by hydrogen bonding with a carboxylic acid group, which in turn intensifies the anomeric center's positive charge, as illustrated in the enzyme/inhibitor complex for **75**, favoring interaction with a carboxylate group. This model requires a coplanar ring arrangement of the tetrazole nucleus and the carboxylate group in addition to the presence of flexible hydrophobic aglycon mimics to provide unspecific interactions in the binding site. These factors could explain, at a molecular level, the strong activity of compound **79**, a tetrahydroimidazo[1,2-*a*]pyridine, the most potent β-glucosidase inhibitor of this group, which contains an extra hydrophobic 2-phenylethyl group.[1,78] It is worth mentioning that changes in the nitrogen position and in the number and conformation of the hydroxyl groups to give compound **81** provided a weak inhibitor of yeast α-glucosidase.[79]



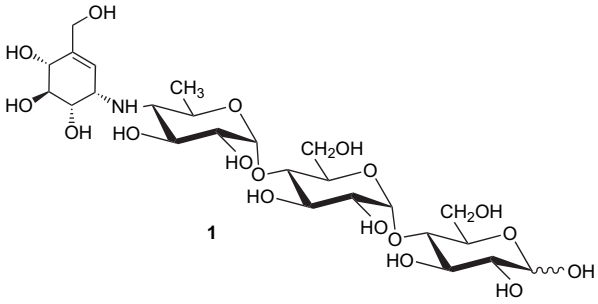
4. Carbasugars and pseudoaminosugars

The aminoglycoside, 1-amino-1-deoxy-D-glucose (**82**), having an unstable hemi-aminal structure, was the first glucosidase inhibitor to be described having a basic nitrogen group.[80] Its inhibitory activity was enhanced by N-alkylation, due to an additional interaction with a hydrophobic

pocket of the enzyme.[81] However, the greater interest in this class of compounds lies with the pseudoaminosugars, in which the oxygen atom of the pyranose ring is substituted by carbon, the oxygen of the glycosidic bond is replaced by nitrogen, and the overall configuration is similar to D-glucose. They exist in a monomeric form or condensed to other cyclic units, as illustrated by the main representatives, acarbose (**1**) and validamycin A (**83**).[82] The more complex structures consist of an unsaturated cyclitol linked via a nitrogen bridge to the other cyclic units. The aminocyclitol, valienamine (**84**), is probably biosynthesized from 2-*epi*-5-*epi*-valiolone (**85**) by *Streptomyces hygroscopicus* var. *limoneus*.[83] Valienamine (**84**) and validamine (**86**) were obtained by microbiological degradation of validoxylamine A (**87**) by *Pseudomonas denitrificans* and *Flavobacterium saccharophilum* or chemically by treatment with *N*-bromosuccinimide (NBS), as well as being synthesized in both racemic and enantiomerically pure forms.[84]



82 **83**



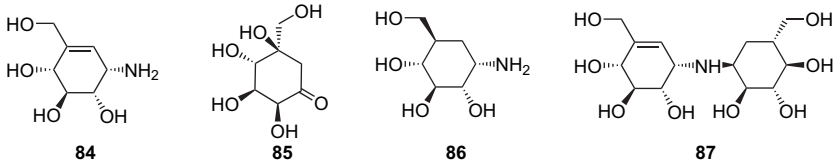
1

Validamycin A (**83**) is the main component isolated from *S. hygroscopicus* var. *limoneus* and it represents a pseudotrisaccharide consisting of a unit of **84** connected by a nitrogen bridge to validamine (**86**), as in validoxylamine A (**87**),[85] which is itself linked to D-glucose. However, instead of being a potent α-glucosidase inhibitor, **83** showed antibiotic activity against *Rhizoctonia solani* and *Pellicularia sasakii*, and is also used in agriculture to control sheath blight disease of rice plants caused by the fungus *R. solani*.[82]

sp. SE-50.[88] Catalytic hydrogenation of **1** afforded fragments consisting of trisaccharide derivatives, which are devoid of inhibitory activity on α-amylase or sucrase, suggesting the importance of the valienamine unit (**84**). Besides the valienyl residue, the pseudotetrasaccharide (**1**) contains a 4-amino-4,6-dideoxy-glucose unit and two glucose residues (forming maltose). The combined first two residues form a pseudodisaccharide called acarviosin and, connected by an *N*-glycosidic bond, are able to mimic the *O*-glycosidic bond of natural substrates.[82]

Valiolamine (**88**), hydroxyvalidamine (**89**), and voglibose (**90**) have similar absolute configurations to α-D-glucose, and these derivatives are strong α- and β-glucosidase inhibitors having a considerable potential for use in the treatment of cancer or AIDS.[24] Compound **84** exhibited strong inhibitory activity against α-glucosidase and α-glucoamylase from *Rhizopus* and a moderate to weak effect on almond β- and α-amylases, respectively. Extension of this work by Kameda et al.[89] showed that **84** is also an antibiotic against *Bacillus* sp. On the other hand, assays involving porcine intestinal sucrase, maltase, and isomaltase revealed that **88** was a more effective compound than the other aminocyclitols.[82]

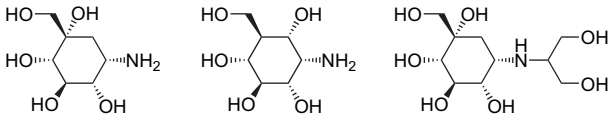
Voglibose (Basen®, **90**), which also has a high inhibitory activity against sucrase and maltase, has been employed in Japan for the treatment of diabetes since 1994. In recent studies based on α-glucosidase inhibitory activity, it was shown to be 20 to 30 times more potent than **1**, thus increasing glucose tolerance by inhibiting its digestion and absorption in the intestine, especially after meals.[90] Additionally, the use of **90** led to less adverse effects including flatulency and abdominal distention, as shown in a random comparative study.[91]



84 **85** **86** **87**

Amongst the members of the aminocyclitol family of natural products, acarbose (**1**)[86] is one of the most important clinical derivatives, being effective in the micromolar range against bacterial, fungal, and plant glucosidases, including sucrase, maltase, dextrinase, and glucoamylase, but to a lesser extent against α-amylase.[77] Treatment of diabetic patients with compound **1** to decrease postprandial hyperglycemia has been related to a reduction in frequency of myocardial infarction (49%) and hypertension (34%) and to an interference in physiopathological and atherothrombotic processes by mechanisms probably involving fibrinogen and a positive modulation of the hemostatic balance.[87]

Acarbose (**1**) has been produced as a secondary metabolite on a large scale from fermentation cultures of *Actinoplanes*



88 **89** **90**

Other compounds related to **1** are adiposin-1 (**91**) and trestatin B (**92**). Compound **91** and adiposin-2 (which has an additional D-glucose unit at the reducing end of adiposin) were isolated from *Streptomyces calvus* TM-521, and **91** can be regarded as a pseudotrisaccharide, differing from acarbose by replacing its 4-amino-4,6-dideoxy-glucose unit by a group derived from 4-amino-4-deoxy-glucose. Along with its inhibitory effect against human α-amylase and disaccharidases from porcine small intestine, this derivative exhibited antibacterial and anti-phytopathogenic fungal activities.[92] As pointed out for compound **1**, compound **92**, isolated as a complex mixture along with trestatins A and C from *Streptomyces dimorphogenes*, provides the pharmacophore with high affinity for the glucosidase active center, being primarily responsible for the main interactions leading to inhibition.[93] Other natural products of C_7N aminocyclitols comprise amilostatins, oligostatins, oxirane pseudooligosaccharides, salbostatin, piralomycin, and epoxyquinomicins.[82]

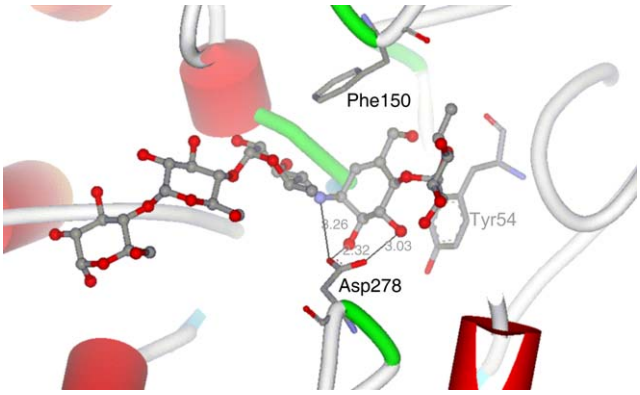
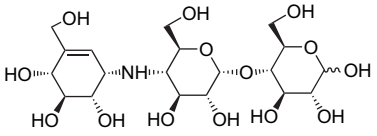


Figure 1. Complex of modified acarbose (**1**) in the catalytic site of *T. maritima* 4-α-glucantransferase (code PDB 1LWJ), showing interactions of the cyclitol unit and the bridge nitrogen of modified **1** with residues in the active site of the enzyme.[95]



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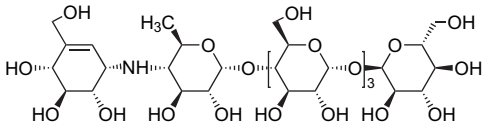
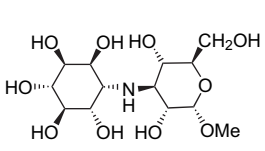


92

Because of the non-availability in the Protein Data Bank (PDB) of three-dimensional structures of α-glucosidase enzymes commonly used in biological assays, such as the native or complexed protein of *Saccharomyces cerevisiae*, we recently constructed a model of a glucosidase homologue based on a 4-α-glucanotransferase of *Thermotoga maritima* (PDB code 1LWJ), the latter showing a high sequential identity with the glucosidase of *S. cerevisiae*.[94] In the *T. maritima* 4-α-glucantransferase complex,[95] containing modified acarbose (a pentasaccharide form), the glycosidic nitrogen bridge interacts strongly with the conserved residue Asp278 by hydrogen bonding (corresponding to Asp349 in the glucosidase model of *S. cerevisiae*) through a distance of 2.73 Å. Based on the overview of this acarbose complex, it is also possible to visualize the main interactions of the cyclitol unit of modified **1** comprising the hydrogen bonding between the C-2 hydroxyl group with the Asp349 residue, and a similar linkage involving the hydroxyl at C-3 and His 348, close to Asp 349. Additionally, the cyclitol primary hydroxyl interacts with residues Asp 214 and His 111 (close to the ε nitrogen of imidazole). It is also possible to observe the flanking of the carbasugar system by aromatic residues Phe 150 and Tyr 54, corresponding to residues Phe 177 and Tyr 71, respectively, in the *S. cerevisiae* glucosidase model (Fig. 1).

In order to provide additional information about the structural requirements for anti-glucosidase activity, a pseudodisaccharide **93** was synthesized, which mimics the valienamine unit **84** and simultaneously preserves the structural similarity of two glycosidic units, cleaved by glucosidase II, of the natural oligosaccharide [$Glc_3Man_9GlcNAc_2$] of the immature glycoprotein (Scheme 1). However, assays performed with baker's yeast α-glucosidase demonstrated that inhibition promoted by **93** was only 20% when compared to the activity of 1-deoxynojirimycin (**7**) and it showed no inhibition activity against glucosidases I and II using rat liver microsome possessing activity in these enzymes.[96]

A series of amino-(hydroxymethyl)cyclopentanetriols, illustrated by compounds **94** and **95**, showed strong inhibition against glucosidase, the strength of which depends on the configuration of the amino group, since, in a protonated form, it may mimic the exocyclic oxygen of the protonated substrate. Compound **94** preserving the *gluco*-configuration exhibited similar K_i values for both α- and β-glucosidases,[97] while, surprisingly, the corresponding *galacto*-configured **95** was a more potent inhibitor toward β-glucosidase.[98]



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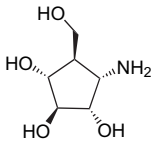

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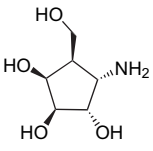
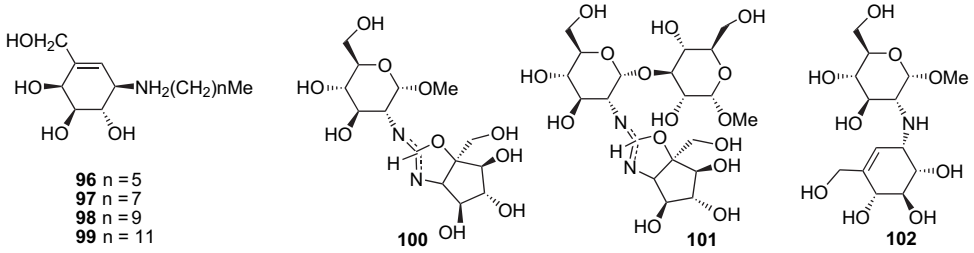


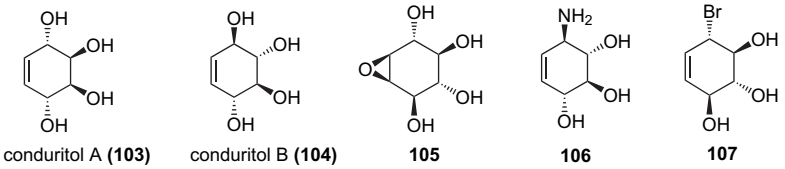
95

Ogawa et al.[99] synthesized β-galactose analogues of valienamine **96**–**99**, which exhibited considerable anti-β-glucosidase activity and anti-α- and β-galactosidase effects, due to the introduction of an *N*-alkyl group. The longest *N*-alkyl chain in compound **99** provided the best β-glucosidase inhibitor. However, compound **97** has received much attention as a potential drug for the treatment of GM_1-gangliosidosis and β-galactosidosis. The same group obtained two kojibiose-type analogues, pseudodisaccharide (**100**) and pseudotrisaccharide (**101**), both containing a 5-amino-1-hydroxymethyl-1,2,3,4-cyclopentanetetrol residue. A further analogue **102**, which contained valienamine linked to C-2 glucose via nitrogen, was also synthesized. Compounds **100** and **101** were potent inhibitors of baker's yeast α-glucosidase with IC_{50} values of 0.012 and 0.18 μM, respectively.

However, products **100**–**102** did not possess activity against rat sucrase and isomaltase or rat liver processing α-glucosidase I.[100]



Unsaturated cyclitols, the conduritols, comprise a 1,2,3,4-cyclohexenetetrol unit, differing from each other by the configurations of the hydroxyl groups around the ring. These polyhydroxylated cyclohexenoid derivatives have attracted great synthetic interest, due to their potential use as therapeutic agents in diabetes, viral infection, cancer, and other diseases. Several conduritol derivatives exhibited antifeedant, antibiotic, antileukemic, and growth-modulation activity.[101] The first representative of the conduritols was isolated by Kübler[102] in 1908 from the bark of the vine *Marsdenia condurango*, and investigation of its structure by Dangschat and Fisher,[103] and again by Kern et al.,[104] showed it to be conduritol A (**103**).



The conduritol family consisting of conduritols A–F includes 10 isomeric forms, since two are *meso* (A and D) and four are D,L pairs (B, C, E, and F).[105] Of these, only conduritol F is found in small quantities in green plants, while **103** is restricted to specific tropical plant families and, notably, it can be isolated from *Gymnena sylvestre*, a shrub, which is the basis of a popular medicine used in India and Asia against diabetes for 2000 years.[106] The four other conduritols are isomeric products obtained by synthesis and are not found in nature.[101,107] These compounds are also synthetic precursors for the preparation of cyclitols of biological interest, exemplified by inositol phosphate, quercitols, cyclophellitol, pseudosugars, pancratistatine, licoridinei, aminoglycosidic antibiotics, sugar amino acid analogues, etc.[101]

Conduritol A (**103**) is particularly important in this class of compounds because of its ability to inhibit intestinal glucose absorption and its potential use as an agent in the treatment of obesity and diabetes. Although the mechanism of its action remains unclear, Miyatake et al.[108] showed that the hypoglycemic effect of **103** prevented diabetic rats developing cataracts as a consequence of the inhibition of lens aldose reductase, the enzyme that converts aldoses to sugar alcohols. Furthermore, the hypoglycemic activity of **103** was also shown by conduritol B (**104**) having the all trans hydroxyl configuration and it was also able to modulate insulin release from isolated pancreatic islets.[109] The preferred half-chair conformation adopted by the cyclohexene ring may favor interaction at the active site. Reduction of the double bond considerably decreased the enzymic activity, but changes in the hydroxyl configuration also had an effect.

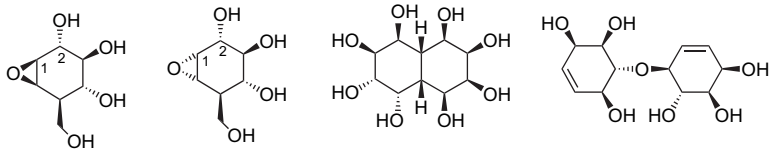
Conduritol B epoxide (1L- and 1D-1,2-anhydroinositols, **105**) is a potent glucosidase inhibitor. For instance, the lower reactivity of the oxirane of **105**, due to the electron-withdrawing effect of the hydroxyl group, makes it more resistant to hydration, allowing an effective interaction that requires protonation and nucleophilic attack by two distinct carboxyl groups at the enzyme active site. Exploring the glucocerebrosidase inhibitory activity, Kanfer et al.[110] and Das et al.[111] studied the effect of aminoconduritol (**106**) in animal models and cells for Gaucher's disease. Compound **106** was able to completely inhibit the glucocerebrosidase from rat peritoneal macrophages in a concentration of 10 and 100 μM for 16 and 2 h of incubation, respectively. However, **106** showed no inhibition against yeast and rice α-glucosidases, amyloglucosidase or almond, and *Caldocellum saccharolyticum* β-glucosidases.[112]

Based on the studies of active site-directed inactivation, it was demonstrated that conduritol epoxide is a potent inhibitor against many different sources of β-glucosidase, while its activity toward α-glucosidase is remarkably lower, suggesting that the trans-diaxial orientation may play a role in the selective inhibition. Even though both conduritol B epoxide (**105**) and bromoconduritol B (**107**)[113] are irreversible glucosidase inhibitors, when covalently linked to the enzyme, **107** has the ability to promote the accumulation of $Glc_1Man_9GlcNAc_2$ by the inhibition of α-glucosidase I or II from rat liver. The same result was observed when virus-infected cells were treated with **107**. Thus, while the position of the acidic group in the β-enzyme favors interaction with **105** (Scheme 6), in the α-enzyme an initial

intermediary epoxide is formed by a hydroxyl intramolecular attack on the neighboring carbon, releasing the halogen leaving group (Scheme 7), and the resulting epoxide then reacts, inactivating the enzyme.[114]

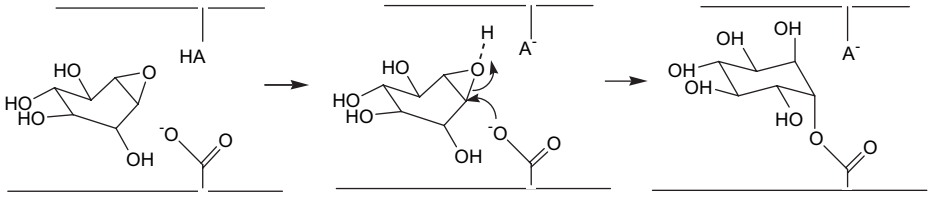
Cyclophellitol (**108**), (1*S*,2*R*,3*S*,4*R*,5*R*,6*R*)-5-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptane-2,3,4-triol, a conduritol B epoxide analogue, was isolated from a culture filtrate of the fungus *Phellinus* sp. and can be regarded as a carbasugar analogue of D-glucopyranose.[115] Compound **108** is one of the most potent and specific competitive and irreversible inhibitors of almond and Molt-4 lysate β-glucosidases, being around 3, 50, and 14 times more active than nojirimycin (**9**), 1-deoxynojirimicyn (**7**), and castanospermine (**66**), respectively.[116] Moreover, the activity toward Molt-4 β-glucocerebrosidase was higher than that of **66**, **7**, and **9**, and **108** did not show cytotoxicity in cultured cells, both properties being required for the treatment of Gaucher's disease. However, compound **108** has little chance of being used as an anti-HIV agent, since Atsumi et al.[116] showed that it had no effect on infected CEM cell lines and MT-4 cells, suggesting that the anti-HIV activity observed for **7** and **66** may be a result of their α-glucosidase inhibition. As an extension of this work, performed by the same group,[117] the non-natural epoxide diastereomer of **108**, (1*R*,6*S*)-cyclophellitol (**109**), was synthesized and it showed potent inhibition only against baker's yeast α-glucosidase. An explanation might be that the orientation of the C1–O positions, that is recognized by both enzymes, involves a pseudo-equatorial orientation in **108** and pseudoaxial in **109** mimicking the natural substrate of the corresponding β- and α-glucosidases, respectively.

In a search for potential glycosidase inhibitors, Mehta and Ramesh[118] prepared new types of cyclitols consisting of two conduritols fused in the double-bond position, thereby simulating the conformational restriction of conduritols. The octahydroxydecahydronaphthalene (**110**) exhibited selective inhibitory activity against α-glucosidase that was higher than **7**, but was inactive against β-glucosidase. Further work involving a diastereomer of **110** had revealed that the substituent configurations is an important feature for enzyme inhibition, since this diastereomer showed no significant inhibition up to millimolar concentrations.

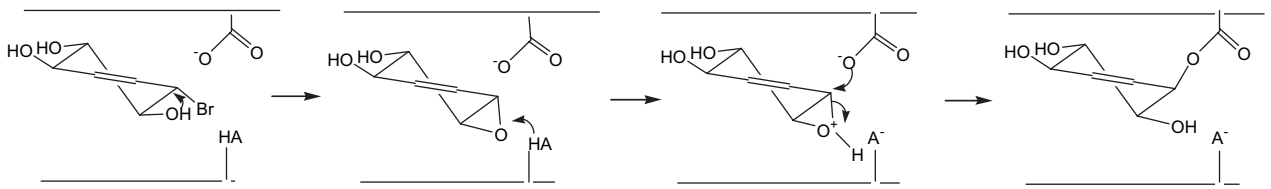


A new class of derivatives that may be used as α-glucosidase inhibitors is the oligoinositols. Work by Freeman and Hudlicky[119] with the oligoinositol of conduritol F (**111**) showed that this compound is active because it mimics the enzyme transition state. In contrast, the corresponding oligomer of *muco*-inositol did not exhibit the same effect when evaluated against many glycosidases.

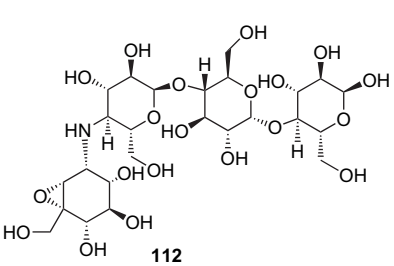
CKD-711 (**112**) is a novel pseudotetrasaccharide inhibitor of α-glucosidase that was isolated from culture of *Streptomyces* sp. as a component of CK4416 (**113**). According to Kwon et al.,[120] its structure consists of an epoxide C_7N aminocyclitol unit connected to three linear glucose molecules. Based on a comparative screening, compound **112** was as active as acarbose (**1**) against intestinal sucrase and maltase, showed a two-fold lower activity than acarbose (**1**) against α-amylase, and exhibited no toxicity. Consequently, **112** might cause less side effects such as flatulence, abdominal pain, and diarrhea that are observed on strong α-amylase inhibition.[121]



Scheme 6. Proposed mechanism for the reaction of conduritol epoxides like **105** in the active site of β-glucosidases.



Scheme 7. Proposed mechanism for the reaction of bromoconduritol (**107**) in the active site of α-glucosidase.



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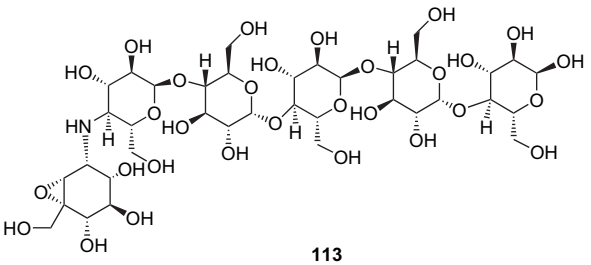


113

5. Thiosugars

Replacement of the oxygen atom in the ring of a carbohydrate by sulfur affords thiosugars, a class of compounds comprising derivatives with strong anti-α-glucosidase activity. The naturally occurring salacinol (**114**) is a remarkable member of this class, consisting of a thiocyclopentane with a trivalent sulfur atom in the ring (sulfonium ion) and an *O*-sulfate as part of an erythritol side chain, which is able to act as a counterion. Its structure was established by X-ray crystallographic analysis, showing it to have a spiro-like configuration with the 1-deoxy-4-thioarabinofuranosyl cation, bearing a resemblance with the iminoalditol **55**, linked through sulfur to a 1′-deoxyerythrosyl-3′-sulfate anion. Compound **114** was isolated by Yoshikawa et al.[122] from an aqueous extract of the roots and stems of *Salacia reticulata* Wight, traditionally used in India and Sri Lanka for the treatment of diabetes, and it has been synthesized.[123] Salacinol (**114**) produced a strong inhibition for the increase of serum glucose levels in in vivo screening, along with competitive inhibition against intestinal α-glucosidases such as maltase, sucrase, and isomaltase, in which the activity against isomaltase was higher than that of acarbose (**1**). Kotalanol (**115**), a derivative of 1,2,3-trihydroxy-propyl-salacinol (**114**), also consists of an inner salt sulfonium-sulfate structure and has been isolated from *Salacia reticulate*, and showed more potent inhibitory activity against sucrase than **114** and **1**.[124]

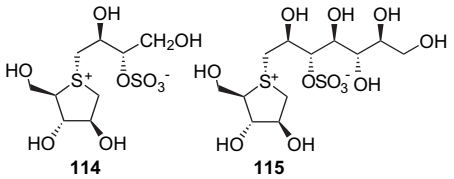
With the aim of gaining information on structure–activity relationships, the azacyclic version of **114** was synthesized in which the thiosugar sulfur was replaced by nitrogen to give compound **116**. Inhibition assays using intestinal α-glucosidase indicated that it was less potent against maltase and isomaltase, compared to **114** and **55**, but was as potent as **55** against sucrase.[125] Despite the low activity of the natural sulfonium ion toward glucoamylase, product **116** exhibited a 10-fold higher inhibition value than **114**.[126] Furthermore, substitution of the sulfonium ion of **114** by selenium produced a derivative **117** with activity against glucoamylase G2 and with a K_i of 0.7 mM, but there was no activity against pancreatic α-amylase.[127]

Several salacinol analogues have been synthesized with varying stereochemistry at one or more stereogenic centers by modifying substituents and ring size or by replacing the sulfur in the sulfonium ion by nitrogen or selenium. Pinto et al.[22] synthesized a series of pyranosil compounds (**118a–122a**) and the corresponding isomers (**118b–122b**), in which an L-erythritol sulfated side chain was attached to the ring N, S, or Se atom. The objective was to investigate if changes to this group, acting as a counterion to ammonium or selenium salt analogues, might increase the in vivo stability or biomembrane permeability. However, the activity of these salacinol analogues with six-membered rings against glucoamylase G2 was weak or absent, indicating the importance of the five-membered ring incorporated in salacinol.

The synthesis of heteroanalogues of disaccharides was also described using the isostere strategy of changing the pyranose oxygen and/or the glycosidic oxygen atoms for carbon, sulfur, selenium, or nitrogen. From this series, compounds **123–126** are worthy of mention, owing to their competitive inhibition of glucoamylase G2. The activity of **126** was due, apparently, to the α-anomer, but the compound was, in fact, isolated as an α/β mixture.[128]

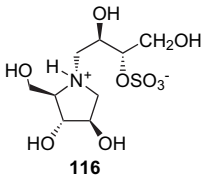
A bicyclic analogue of castanospermine (**66**), carrying a positively charged sulfonium salt in place of the nitrogen, led to **127** with a permanent and stable positive charge that might improve interaction at the active site.[129] Screening of inhibition with three glucosidase enzymes, glucoamylase G2, porcine pancreatic α-amylase, and barley α-amylase, showed that **127** was a slightly better inhibitor than **114** against the former enzyme.[130]

Expanded-ring derivatives containing ring sulfur or nitrogen, the tetra-hydroxythiepanes (**128–133**)[131] and the tetra-hydroxyazepane (**134**),[132] showed competitive anti-α- and β-glucosidase activities. This property has been attributed to the flexibility of the seven-membered ring, which allows mimicking of the natural substrate transition state. Thiepane derivatives **130** and **132** were moderately active against α-glucosidase, but inactive against β-glucosidases.

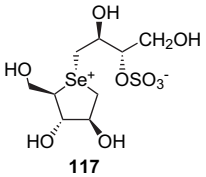


114

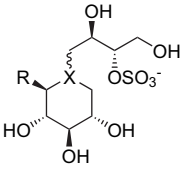
115



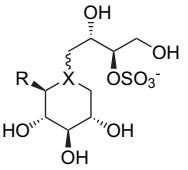
116



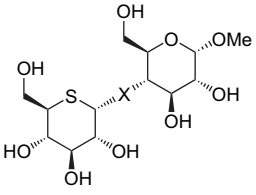
117



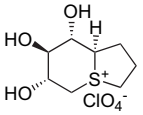
118a X= NH, R= H
119a X= NH, R= CH_2OH
120a X= S, R= H
121a X= S, R= CH_2OH
122a X= Se, R= H



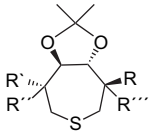
118b X= NH, R= H
119b X= NH, R= CH_2OH
120b X= S, R= H
121b X= S, R= CH_2OH
122b X= Se, R= H



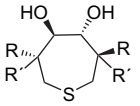
123 X= O
124 X= S
125 X= Se
126 X= N



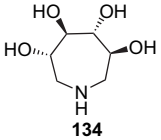
127



128 R, R′= N_3, R′′, R′′′= H
129 R= N_3, R′= H, R′′= N_3, R′′′= H
130 R, R′= NH_2, R′′, R′′′= H
131 R= NH_2, R′= H, R′′= NH_2, R′′′= H



132 R= N_3, R′= H
133 R= NH_2, R′= H

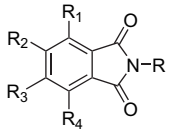


134

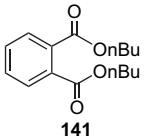
6. Non-glycosidic derivatives

There is great structural diversity among glucosidase inhibitors, which are not based on a sugar scaffold.

Based on pharmacological studies involving thalidomide, it was found that tetrachlorophthalimide derivatives (**135**–**140**) exhibited potent α-glucosidase inhibition. The compounds have hydrophobic groups such as halogen and *N*-alkyl side chains, and structure–activity relationship studies revealed the importance of hydrophobic *N*-substituents and the positive influence of electron-withdrawing groups attached to the aromatic ring, as exemplified by **135** and **137**, which were stronger inhibitors than **7** toward α-glucosidase (from Wako Pure Chemical Industries).[133] Interestingly, dibutyl phthalate (**141**) (isolated from *Streptomyces melanosporofaciens*) is also described as a non-competitive α-glucosidase inhibitor.[134]

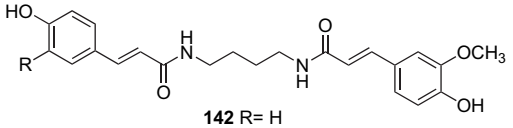


135 R= $(CH_2)_4Ph$, R_1 to R_4= Cl
136 R= $(CH_2)_2Ph$, R_1 to R_4= Cl
137 R= Ph, R_1 to R_4= Cl
138 R= $(CH_2)_3Ph$, R_1 to R_4= Cl
139 R= Ph, R_1= NO_2, R_3 to R_4= H
140 R= Ph, R_2= NO_2, R_1, R_3, R_4= H



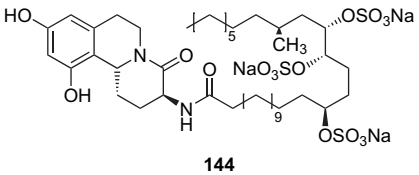
141

Bisamides were recently isolated as side products of corn starch processing. The compounds were active in reducing glucose levels occurring after meals. This class is represented by *N*-*p*-coumaroyl-*N*′-feruloylputrescine (**142**) and *N*-*N*′-diferuloylputrescine (**143**). Compound **142** is more potent in reversible α-glucosidase inhibition assays. Studies on structure–activity relationship indicated that the phenyl hydroxyl group is fundamental for the activity of these compounds.[135]

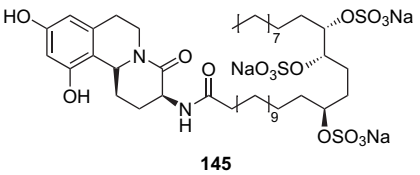


142 R= H
143 R= OCH_3

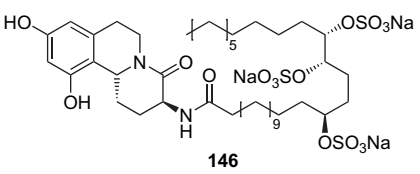
Currently, several natural compounds from marine sources have been described as potent inhibitors of α-glucosidase. Takada et al.,[136] isolated from a hydrophilic extract of the sea sponge *Penares schulzei* three tetrahydroisoquinolinic alkaloids containing two amido functions and a C_{28} sulfated fatty acid, which belong to a new class of compounds named schulzeines, the three compounds being schulzeine A (**144**), B (**145**), and C (**146**). The IC_{50} values of these compounds against yeast α-glucosidase varied from 48 to 170 nM and those analogues devoid of the sulfate group still had inhibitory activity, indicated that these groups are not fundamental for their activity. Schulzeines are structurally similar to another class of compounds, also isolated from the *Penaria* sp. family, the penarolide sulfates A_1 (**147**) and A_2 (**148**), bearing a proline unit inserted into a macrolide trisulfate system and having potent anti-yeast α-glucosidase activity (IC_{50}=1.2 and 1.5 μg/mM, respectively). Recently, Nakao et al.[137] have isolated from the same sponge penasulfate A (**149**), a compound 10 times more potent than **147** and **148** against yeast α-glucosidase.



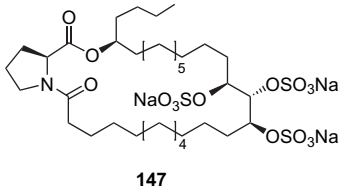
144



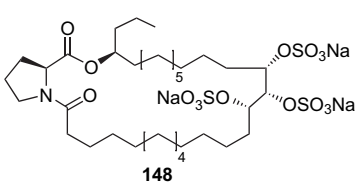
145



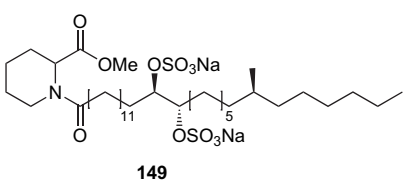
146



147

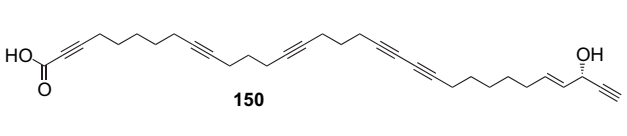


148

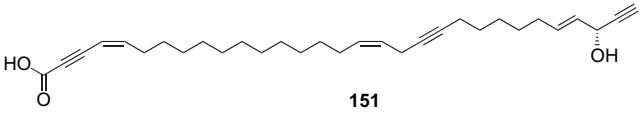


149

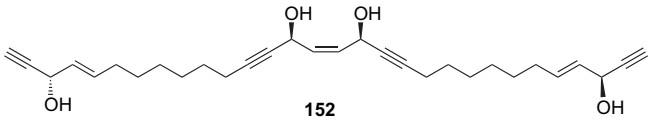
Polyacetylenic compounds (**150**–**152**), isolated from marine sponges on the coast of Japan, showed potent anti-α-glucosidase activity, besides other interesting biological activities. Callyspongynic acid (**150**) is a carboxylic derivative in this class of substances isolated from the sponge *Callyspongia truncata* by Nakao et al.[138] Other compounds were also active, such as corticatic acid A (**151**) from *Petrosia corticata* and petrosynol (**152**) from *Petrosia* sp. The inactivity of the polyacetylene, callytetrayne (**153**) (also isolated from *C. truncata*), and the products of methylation of the carboxylic acid groups of **150** and **151**, suggests the importance of the carboxylic acid group and the allyl/propargyl alcohol functionality in generating the inhibitory properties of these compounds.[139]



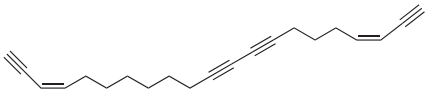
150



151

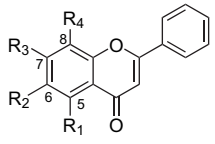


152



153

Baicalein (**154**), a 5,6,7-trihydroxyflavone isolated from marjoram leaves of *Origanum majorana*, is a potent α-glucosidase inhibitor. Compound **154** is a unique flavone bearing a 6-hydroxy group together with the 5,7-dihydroxy substituents. Structure–activity relationship studies with different derivatives **155**–**160** indicated that loss of hydroxyls from positions 5, 6, and 7 significantly reduced the activity, and introduction of an electron-withdrawing or -donating group in R suggests an unfavorable steric influence in the enzyme interaction (Appendix).[140]



154 R_1, R_2, R_3= OH; R_4= H
155 R_1, R_3= OH; R_2, R_4= H
156 R_1, R_2, R_3= OH; R_4= F
157 R_1, R_2, R_3, R_4= OH
158 R_1, R_2, R_3= OH; R_4= NH_2
159 R_1= OH; R_2, R_3, R_4= H
160 R_1, R_3, R_4= H; R_2= OH

7. Concluding remarks

Glucosidase inhibitors have proved useful to reduce postprandial hyperglycemia by suppressing the absorption of glucose, being effective for the treatment of type II diabetes and obesity. Current interest in these compounds has been extended to a diverse range of diseases including lysosomal storage disorders and cancer, and special attention has been given to those compounds with anti-HIV activity. Isolation of suitable glucosidase inhibitors from natural sources or their chemical synthesis provides biochemical tools for the elucidation of enzyme mechanistic activity through the use of kinetic data combined with variations in potential inhibitor structural information. Such knowledge is fundamental to the discovery of lead compounds, because of their promising therapeutic potential.

The polyhydroxy glucosidase inhibitors are a widely diverse class of compounds often isolated from plants and microorganisms and they have significant therapeutic use or potential. Some carbocyclic compounds include potent HIV inhibitors, such as conduritol epoxides and aminoconduritols, while conduritol A analogues modulate the release of insulin. Thiosugars, either synthesized or isolated from natural sources, have also been investigated as inhibitors and have widened the structural diversity of compounds available from natural sources. Compounds with no obvious structural similarity to a carbohydrate skeleton are a new class of inhibitors and the elucidation of their mechanism of action may add new insights in the search for new therapeutic agents.

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Appendix. Data on the inhibition of activity produced in various α- and β-glucosidases by compounds 1–160

	α-Glucosidase inhibition (μM)				β-Glucosidase inhibition (μM)			
Compound	IC_{50}		K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
1	0.47[a] 0.06[c] 108.5[f] 5.9[g]		0.99[b] 1.1×10^{-6}[c] 9.0×10^{-3}[d] 46.3[e]	38–1.0[h]		NI		8, 12, 21, 22, 82
2			0.086[b] 0.36[e] 0.21[h]	61–1.0[b] 40–1.0[d] 0–200.0[h] 43–2300[q]		NI		8
3	15.0[i] 5.3[j] 0.57[k]		3.7[i]					10, 29
4				12–1000[i] 91–1000[k]				13
5				53–2000[i] 7–2000[k]				13
6				45–2000[b]				17
7	0.22[b] 0.096[d] 0.13[e] 9.6[f] 4.6[i]	0.4[j] 0.36[l] 0.05[m] 12.6[n] 330[o]	1.3[i] 12.6[f] 0.01[m] 14.6[f]	52–0.1[b]	153[a′] 81[c′] 520[d′]	300 (pH 5.0)[a′] 47 (pH 6.8)[a′] 2.7[b′] 210[e′] 180[f′] 25[g′]		1, 17, 21, 29, 30, 36, 40, 60, 82
8			NI			126[a′]		18
9	0.56[b] 0.76[d] 0.25[e] 6.3[f] 1.7[l]		6.3[f] 0.01[m] 330[n]			0.89[a′] 0.36[b′] 4.5[f′]		1, 21, 47, 82
10								
11			NI[f]			430[a′]	35–1000[a′]	31
12	0.28[k]							36
13	940[e] 1000[j]		NI[f,i]			(540 as DGJ)[a′]		36, 59
14	20[i] 1.5[j]		5.8[i] 54[f]					29, 39
15			NI					39
16			23[n]					39
17	2.4[b] 24[l]				1000[a′] 230[h′]			40
18	0.35[b] 5.2[l]				NI[a′] NI[h′]			40
19	22[b] 2.3[l]				80[a′] 50[h′]			40, 41
20	0.79[b] 4.0[l]				NI[a′] NI[h′]			40
21	2.5[b] 24[l]				NI[a′] 560[h′]			40
22	0.31[b] 1.7[l] 30[m]				NI[a′] NI[h′]			43
23	0.2[b] 5.0[d] 8.0[e] 1.0[l]							44
24			86[f] 3.7[d] 7.2[p]			0.11[a′]		45, 47
25			0.022[f] 0.025[p]			0.069[a′]		47
26	3.9[f] 1.06[p]				0.65 (pH 6.8)[a′] 0.76 (5.0)[a′] 1.09 (pH 7.5)[a′]			45
27	59[f] 100[p] 0.063[c]				2.3[a′]			45
28	NI[f]				420[a′]			45
29	NI[f]				0.19[a′]			45
30						30[i′]		46
31						96[i′]		46

(*continued*)

Appendix. (*continued*)

	α-Glucosidase inhibition (μM)				β-Glucosidase inhibition (μM)			
Compound	IC_{50}		K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
32						580[i′]		46
33					80[a′]			48
34					8.8[a′]			48
35	0.17[b]	8.4[i]						52
	0.04[m]	0.26[j]						
	0.70[e]	0.34[l]						
36	7.2[b]	15[l]			NI			52
	8.2[j]	8.4[m]						
37	3.0[b]	4.6[l]						52
	5.0[j]	3.2[m]						
38	0.02[b]	1.0[j]						52
	2.8[e]	0.17[l]						
	50[i]	0.06[m]						
39	3.0[b]	2.2[j]						52
	100[e]	4.8[l]						
	100[i]	4.2[m]						
40					4.25[a′]			53
41					2.11[a′]			53
42	3.3[b]	2.3[l]			NI			54
	0.27[e]	0.25[m]						
43	110[b]	280[l]			NI			54
	110[e]	21[m]						
44	2.4[b]	6.1[l]			NI			54
	2.1[e]	0.49[m]						
45				NI[b]			37–1×10[3a′]	55
				22.1–1000[f]				
				33.9–1000[e]				
				28.8–1000[l]				
				33–1000[m]				
46	NI[o]				0.79[a′]	0.51[a′]		56
47	NI[o]				62[a′]	51[a′]		56
48	NI[o]				160[a′]	85[a′]		56
49			3×10[3c]			0.20[a′]		21, 57
						9.5×10[−3b′]		
						0.015[j′]		
50						10±2[a′]		12, 59
51						8.4±0.9[a′]		12, 59
52						13.8±3 (pH 5.6)[a′]		12, 59
53	20.1[a]		0.73–7[f]		2.4–13[a′]	1.7–50[a′]		60, 61
	NI[b]				7.8[c′]	57[b′]		
	91[c]				11[h′]	44[g′]		
	3.3[f]				34[d′]			
	NI[i]				25[k′]			
	92[j]							
	290[l]							
	200–300[m]							
	0.059[n]							
	3.6–15[o]							
54	NI[r]							62
55	0.18[f]	20[i]			965[a′]	280[a′]		60
	7.0[o]	100[j]			200[c′]			
	5.8[e]	55[l]			NI[d′]			
56	0.048[f]				41[a′]			63
57					1.2×10[−3k′]			
58					0.55[k′]			61
59					0.1[k′]			61
60			150[o]		NI[a′]			64
61			240[o]		1220[a′]			64
62			NI[o]		1120[a′]			64
63			18[o]					64
64	5.8[d]							1, 65
65 IC_{50}=0.001 Jack beans α-mannosidase					NI[l′]			66
66			≥1500[f]			1.5[a′]		21, 60
			0.015[m]			0.9[b′]		
			0.1[j]			25[g′]		
			0.55×10[−3b]					
67 (+)-6-Epicastanospermine	20[c]							68

(*continued*)

Appendix. (*continued*)

	α-Glucosidase inhibition (μM)			β-Glucosidase inhibition (μM)			
Compound	IC_{50}	K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
68	NI[o] NI[m]			37[h′] 26[a′]	20[a′] 12[h′]		76
69	NI[o] NI[r]			4[a′] 1[h′]	1.8[a′] 0.43[h′]		76
70	NI[o] 75[m]			2.6[a′] 2.4[h′]	1.2[a′] 0.55[h′]		76
71	NI[o] 420[m]			0.82[a′] 0.86[h′]	0.45[a′] 0.29[h′]		76
72		57[f] NI[c]			30 (pH 5.5)[a′] 15 (pH 7.3)[a′] 27[m′]		69
73		57[f] NI[c]			23 (pH 5.5)[a′] 23 (pH 7.3)[a′] 244[m′]		69
74		17[f] NI[c]			212 (pH 5.5)[a′] 157[m′]		69
75					150[a′]		1
76					19[a′]		1
77					>8000[a′]		1
78					0.1[a′]		1
79					0.1×10^{-3}[h′]		78
80					300[a′] 410 (pH 6.8)[h′]		1
81		580[o]					79
82		32[f]			310[a′] 240[e′] 65[f′] 2000[c′] 1.7[b′]		21
83 Anti-fungal antibiotic		NI			NI		82
84	18[f] 53[a] 340[r] 1000[s] $>10\times10^{3}$[g] 6800[t]	300[b] 960[l] 890[d] 760[e]		8800[a′] $>10\times10^{3}$[n′]			84
85 2-*epi*-5-*epi*-Valiolone	NG			NG			83, 88
86	7.5[a] 580[f] 110[r] $>10\times10^{3}$[t] $>10\times10^{3}$[g] 130[s]	32[b] 180[l] 160[d] 88[e]		1500[a′] $>10\times10^{3}$[n′]			84
87 Validoxylamine A IC_{50}=0.0024 (pig kidney trehalase)		NI[b] NI[l]					85
88	0.049[a] 190[f] 2.2[r] $>10\times10^{3}$[t] $>10\times10^{3}$[g] 2.7[s]	0.32[b] 2.9[l] 1.2[d] 0.91[e]		8100[a′] $>10\times10^{3}$[n′]			84
89	420[a] 360[f] 8300[r] $>10\times10^{3}$[t] $>10\times10^{3}$[g] $>10\times10^{3}$[s]			7400[a′] $>10\times10^{3}$[n′]			84
90	0.0046[a] 0.015[r]						12, 90
91			2.0 Unit/mg[a] 265 Unit/mg[g] 123 Unit/mg[q]				92
92	0.61[g] 31[u] NI[f]			NI[n′] NI[a′]			93
93		~1500[f] NI[k] NI[i]					96

(*continued*)

Appendix. (*continued*)

	α-Glucosidase inhibition (μM)				β-Glucosidase inhibition (μM)			
Compound	IC_{50}		K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
94			1.6[v] 0.6[w] 85[m]			6.5[a′] 1.5[h′]		97
95						2.2[a′] 0.17[h′]		98
96					1.2[a′]			99
97					3.1[a′]			99
98					2.5[a′]			99
99					0.87[a′]			99
100	0.012[o] NI[b]	NI[e] NI[k]						100
101	0.18[o] NI[b]	NI[e] NI[k]						100
102	3.1[o] NI[b]	NI[e] NI[k]						100
103				45–100[x]				109
104				41–100[x]				109
105			25×10^{3}[f] $>50\times10^{3}$[y] $>10\times10^{3}$[z]			17×10^{3}[f′] 4×10^{3}[b′] $\geq0.6\times10^{3}$[f′] 4.1×10^{3}[e′]		21
106			NI[f] NI[m] NI[c]			NI[a′] NI[h′]		112
107				$48.4–5\times10^{3}$[z1]				113
108				15–568[f]	4.5[a′]		99–568[a′]	115, 116
109	5.7[o]				NI[a′]			117
110			12[o]			NI[a′]		118
111			60[z]			NI[i′]		119
112	2.5[r] 0.5[a] 78[g]							121
113	NG					NG		82
114	2.5[b] 9.6[l] 1.76[e]		0.92[l] 0.95[b] 1.40[e] 10±2[g] 15±1[z] 1700[z2]					122, 125, 130
115								124
116	306[l] 44[b] 136[e]							125, 126
117			NI[g] 720[z2] NI[z3]					127
118			**118b**: 70×10^{3}[z2]					22
119			NI[z2]					22
120			NI[z2]					22
121			**121a**: 70×10^{3}[z2]					22
122			NI[z2]					22
123			1340±0.06[z2]					128
124			2040±0.42[z2]					128
125			796±0.03[z2]					128
126			4±0.3[z2]					128
127			1320[c] NI[z3] NI[g]					130
128			35[o]			NI[a′]		131
129			170[o]			NI[a′]		131
130			410[o]			190[a′]		131
131			280[o]			800[a′]		131
132				10–0.5[o]		NI[a′]		131
133			760[o]			NI[a′]		131
134			4.8[z4]	97–1000[z4]		17[a′]	86–1000[a′]	132
135	2.0[z5]							133
136	6.0[z5]							133
137	2.6[z5]				17.9[a′]			133
138	4.5[z5]							133
139	25.9[z5]							133

(*continued*)

Appendix. (*continued*)

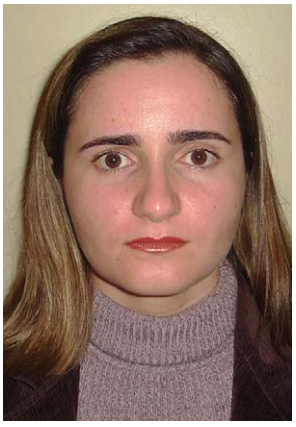
	α-Glucosidase inhibition (μM)			β-Glucosidase inhibition (μM)			
Compound	IC_{50}	K_i	% Inhibition-concentration	IC_{50}	K_i	% Inhibition-concentration	Ref.
140	23.7[z5]						133
141	4.0[f]	3.9[f]					134
142	~2000[f]						135
143			17–2000[f]				135
144	0.048–0.17[f]						136
145	0.048–0.17[f]						136
146	0.048–0.17[f]						136
147	1.33[f]						137
148	1.66[f]						137
149	4.4[o]						138
150	0.53[z5]				NI[a′]		139
151	0.34[z5]						139
152	8.8[z5]						139
153	NI[z5]						139
154	45[b]						140
155	NI[b]						140
156	86[b]						140
157	960[b]						140
158	1000[b]						140
159	NI[b]						140
160	NI[b]						140

NI: no inhibition; NG: not given.
[a] *α-Glucosidase*: porcine intestinal sucrase.
[b] *α-Glucosidase*: rat intestinal sucrase.
[c] *α-Glucosidase*: *Aspergillus niger* glucoamylase.
[d] *α-Glucosidase*: rat intestinal glucoamylase.
[e] *α-Glucosidase*: rat intestinal isomaltase.
[f] *α-Glucosidase*: yeast α-glucosidase.
[g] *α-Glucosidase*: porcine pancreas α-amylase.
[h] *α-Glucosidase*: rat pancreas α-amylase.
[i] *α-Glucosidase*: glucosidase II rat liver ERII.
[j] *α-Glucosidase*: rat liver lysosomal α-glucosidase.
[k] *α-Glucosidase*: rat liver α-glucosidase I.
[l] *α-Glucosidase*: rat intestinal maltase.
[m] *α-Glucosidase*: rice α-glucosidase.
[n] *α-Glucosidase*: brewers' yeast α-glucosidase.
[o] *α-Glucosidase*: baker's yeast α-glucosidase.
[p] *α-Glucosidase*: yeast isomaltase.
[q] *α-Glucosidase*: human pancreatic α-amylase.
[r] *α-Glucosidase*: porcine maltase.
[s] *α-Glucosidase*: porcine isomaltase.
[t] *α-Glucosidase*: glucoamylase (*Rhizopus* sp.).
[u] *α-Glucosidase*: *A. oryzae* α-amylase.
[v] *α-Glucosidase*: yeast maltase.
[w] *α-Glucosidase*: bakers yeast isomaltase.
[x] *α-Glucosidase*: modulation of insulin release from pancreatic islets.
[y] *α-Glucosidase*: rabbit intestine sucrase.
[z] *α-Glucosidase*: rabbit intestine isomaltase.
[z1] *α-Glucosidase*: whitefly α-glucosidase.
[z2] *α-Glucosidase*: glucoamylase G2.
[z3] *α-Glucosidase*: barley α-amylase (AMY1).
[z4] *α-Glucosidase*: *Bacillus stearothermophilus*.
[z5] *α-Glucosidase*: not specified.
[a′] *β-Glucosidase*: sweet almond.
[b′] *β-Glucosidase*: *Aspergillus wentii*.
[c′] *β-Glucosidase*: bitter almond.
[d′] *β-Glucosidase*: rat intestine cellobiose.
[e′] *β-Glucosidase*: calf liver cytosol.
[f′] *β-Glucosidase*: calf spleen lysosome.
[g′] *β-Glucosidase*: bovine kidney.
[h′] *β-Glucosidase*: *Caldocellum saccharolyticum*.
[i′] *β-Glucosidase*: not specified.
[j′] *β-Glucosidase*: human liver cytosolic.
[k′] *β-Glucosidase*: *Agrobacterium* sp.
[l′] *β-Glucosidase*: rat epididymis.
[m′] *β-Glucosidase*: bovine liver.
[n′] *β-Glucosidase*: β-amylase (sweet potato).

Biographical sketch

Ivone Carvalho received her B.Sc., Master, and Ph.D degrees from the University of São Paulo (USP), completing her doctoral thesis on synthesis of natural products in 1991 under the guidance of Professor Maurício Gomes Constantino. In 1995, she joined Professor A. H. Haines's group at University of East Anglia as a postdoctoral researcher to work on the synthesis of pseudodisaccharides as potential α-glucosidase inhibitors. She then returned to Great Britain in 2000 to complete another postdoctoral period in Professor R. A. Field's group at the University of St Andrews in Scotland, where she worked on the synthesis of glycopeptides to study the mechanism and function of parasite enzymes. She is currently working as an Associate Professor of Medicinal Chemistry at Faculty of Pharmaceutical Sciences of Ribeirão Preto/USP and her research interests concentrate on the design and synthesis of potential bioactive compounds.

Eduardo Borges de Melo was born in Riolândia, State of São Paulo, Brazil. He completed his Master Degree in 2001 under the supervision of Professor I. Carvalho at the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, where he worked on the synthesis of ketoconduritols with potential glucosidase inhibitors. In 2002, he became assistant teacher of Pharmaceutical Chemistry at Pharmacy School of State University of Western Parana (UNIOESTE). In 2005, he joined Professor M. M. C. Ferreira's research group at State University of Campinas (UNICAMP) as a Ph.D student to work with molecular modeling and structure–activity relationship methods applied to design of HIV-integrase inhibitors.



Adriane da Silveira Gomes was born in Iporá, State of Goiás, Brazil. She graduated from University of Goiás in 2002 before moving to Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, where she is carrying out her Ph.D studies under the supervision of Professor Dr Ivone Carvalho since 2003. Her research has focused on the synthesis of glucosidase inhibitors of biological interest.

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Syntheses of new functionalized azobenzenes for potential molecular electronic devices

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Abstract—New non-symmetrical azobenzene derivatives have been synthesized as potential molecular electronic switching device candidates. The Oxone® mediated oxidation of anilines provided nitroso-functionalized arenes, which were then condensed with substituted anilines to provide a series of azobenzene derivatives that could be further converted into oligo(phenylene ethynylene)s or diazonium salts. The resulting thiolacetates, thiols, or diazonium salts are capable of forming molecular layers on the surface of gold or silicon, thereby paving the way for molecular electronics testing.

1. Introduction

Molecular electronics is a promising area of research that focuses on a bottom-up strategy to fabricate nanoscopic electronic devices based on self-assembled monolayers (SAMs) of organic molecules on surfaces.[1] One of the classes of compounds we have concentrated on is a series of oligo(phenylene ethynylene) (OPE) derivatives that have enabled the study of molecular switching processes in electronic devices.[1,2]

Alternatively, the self-assembly of photo-responsive molecules onto the substrates creates the possibility of using switching devices as photo-responsive components in optical storage and molecular recognition applications.[3] Azobenzene derivatives are interesting molecular device candidates that have been widely investigated due to their non-degradative reversible trans–cis photoisomerization during repeated switching cycles; azobenzenes also possess useful electrochemical activity.[4] Accordingly, the isomerization of azobenzene derivatives has been observed under the influence of an external electric field at the single molecule level.[5]

Previously we have shown that symmetrical azobenzene OPEs bearing protected thiol end groups form SAMs on metal surfaces.[2e] We envisioned that the synthesis of non-symmetrical azobenzene derivatives could be realized by changing the substituents (R_1 and R_2 in **A**, Fig. 1) of

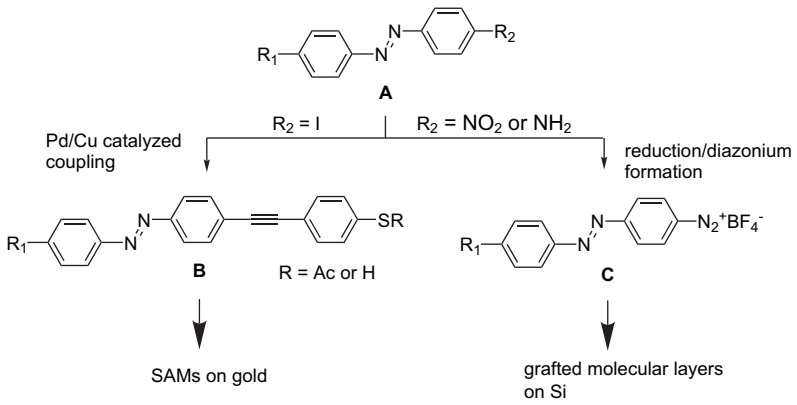

Figure 1. Synthesis of azobenzene derivatives **B** (for self-assembly on gold) or **C** (for grafting of molecular layers on silicon) from disubstituted azobenzene **A**.

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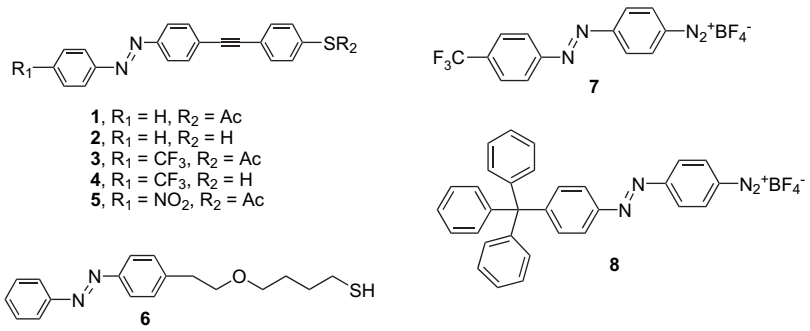


Figure 2. The structures of the type **B** azobenzene OPEs **1**–**5**, azobenzene alkanethiol **6** and type **C** azobenzene diazonium salts **7** and **8** synthesized in this work.

a disubstituted azobenzene. The resulting products would lead to an azobenzene OPE thiol/thioacetate that could form SAMs on many metal surfaces through compound **B**[1,2] or alternatively to an azobenzene diazonium salt **C** for grafting of molecular layers on semiconductor surfaces (Fig. 2).[6]

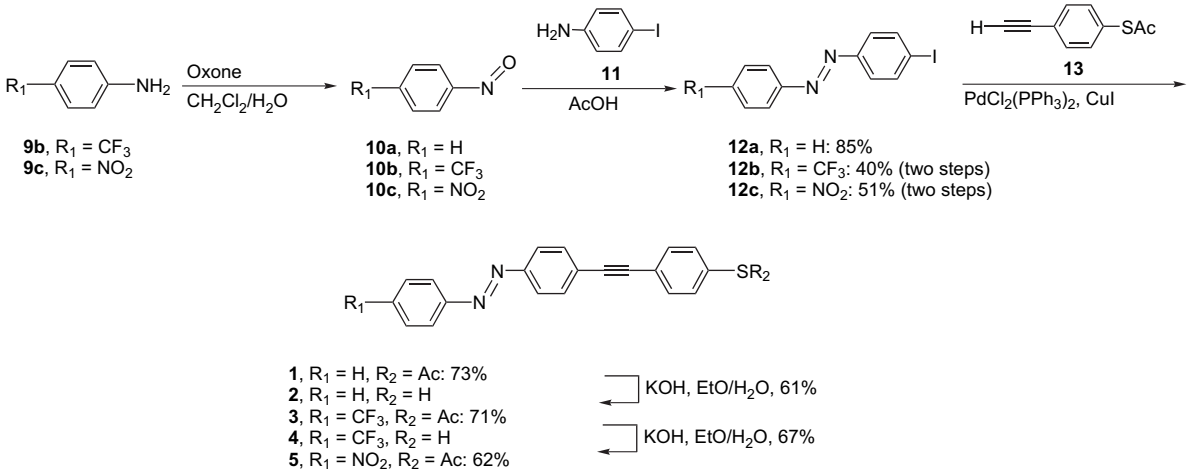
2. Results

2.1. Syntheses of azobenzene derivatives of type B

Functionalized azobenzene OPEs bearing free thiol or protected thiol end groups are preferred for formation of SAMs on gold substrates. Therefore the azobenzene OPEs **1**–**5** were prepared. After some preliminary experiments, the Oxone® mediated oxidation of anilines **9b**,**c** to provide nitrosoarenes **10a**–**c**,[7] followed by condensation with iodoaniline **11**, was found to be an efficient path to prepare a series of azobenzene derivatives. For example, the nitrosobenzene **10a** (Scheme 1, prepared from aniline[7]) was treated with **11** in acetic acid to afford the iodoazobenzene **12a**. This was coupled with thioacetate **13**[1,8] to give the protected azobenzene OPE **1**. The thioacetate **1** was carefully treated with aqueous potassium hydroxide in ethanol under an oxygen-free atmosphere (to minimize the oxidative coupling of the thiol) to provide the azobenzene OPE thiol **2** in 61% yield.

It is known that the relative order of the formed SAMs and the photo responsiveness of the molecules are dependent on the substituent at the *para* position of the azobenzene moieties comprising the SAM.[9] Therefore *para*-substituted azobenzene OPEs **3**–**5** were prepared. It was expected that the SAMs formed from **3** or **4** would show changes in wettability depending on whether the molecules were in their trans (hydrophobic) or cis (hydrophilic) forms. Since the trans and cis isomers also have different dipole moments,[10] it is possible that isomerization of the molecules in the SAMs could change the electrical properties of the SAM. With that in mind, we prepared **3** and **4** (Scheme 1). Commercially available 4-trifluoroaniline **9b** was smoothly oxidized using Oxone® to afford the nitrosobenzene **10b**, which was subsequently condensed with **11** in acetic acid to give the iodoazobenzene **12b**. Coupling to **13** gave **3**, which was converted to the corresponding thiol **4** as previously described.

In a similar fashion, the nitro-substituted azobenzene OPE **5**, which might show enhanced switching characteristics due to the electron-withdrawing nature of the nitro group, was prepared by oxidation of **9c** to **10c** using Oxone®, followed by condensation with **11** to give **12c**. The Oxone® mediated oxidation is noteworthy because nitrosobenzenes containing electron-withdrawing groups at the *para* position are difficult to prepare on a large scale.[7] Compound **12c** was subsequently coupled with the thioacetate **13** to afford the nitroazobenzene OPE **5**. Compounds **3**–**5** are currently being tested by our collaborators using scanning tunneling microscopy (STM) to detect switching effects at the single molecular level.[2f–g]



Scheme 1. Synthesis of the azobenzene OPEs **1**–**5**.

Azobenzenes bearing an alkanethiol functional group have been used to study molecular photo-switching at the nanoscale in SAMs.[4] To test single molecular switching by STM we prepared the azobenzene alkanethiol **6**. Compound **10a** was condensed with hydroxyethyl aniline (**14**) in acetic acid. The resulting alcohol was alkylated using dibromobutane in the presence of excess NaH in DMF. The alkylation, which was sluggish at room temperature, afforded the bromide **16**. Subsequent conversion of the bromide into the thiol **6** was accomplished by addition of thiourea followed by treatment with aqueous potassium hydroxide (Scheme 2).

2.2. Syntheses of azobenzene derivatives of type C

The formation of grafted molecular layers on silicon is important due to the possibility of integrating molecular electronics into existing silicon microelectronic architectures.[11] The Si–C bond is both thermodynamically and kinetically stable due to the high bond strength and low polarity of the bond.[11a] Covalent attachment of arenes via aryldiazonium salts to hydride-passivated Si⟨111⟩ and Si⟨100⟩ has been successfully demonstrated.[6] Until now few methods concerning the preparation of azobenzene molecular layers on silicon surfaces have been reported.[11b] Therefore we synthesized new functionalized azobenzene diazonium salts for grafting azobenzene derivatives onto Si substrates.

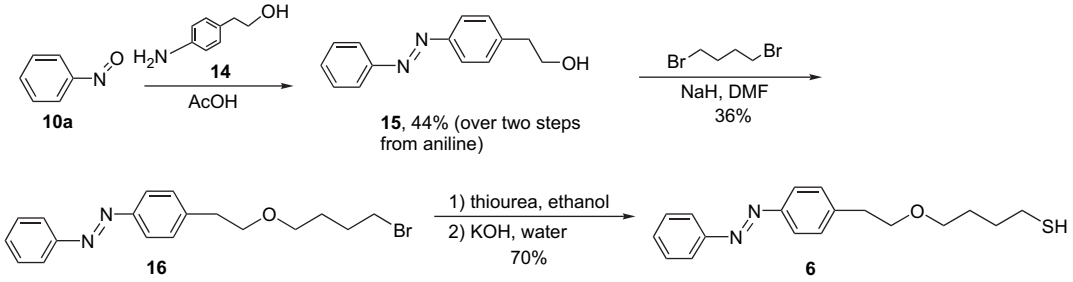
4-Trifluoromethylnitrosobenzene (**10b**) was condensed with *p*-phenylenediamine to afford **18**. The resultant amine was converted into the desired diazonium salt **7** upon treatment with boron triflouride-diethyl etherate and *tert*-butylnitrite (Scheme 3).

The tritylazobenzene diazonium salt **8** was prepared in a similar manner (Scheme 4). Commercially available 4-nitroaniline **19** was oxidized with Oxone® to afford 4-nitronitrosobenzene **20**. Without purification the labile nitroso compound was directly submitted to an acetic acid mediated condensation reaction with 4-tritylaniline (**21**) to provide 4-tritylnitroazobenzene (**22**) in 82% yield over two steps. Reduction of the nitro group to the corresponding amine **23** in the presence of the diazo moiety was accomplished by treatment with sodium sulfide in a mixture of refluxing dioxane, ethanol and water.[12] The amine **23** was subsequently converted into the air stable diazonium salt **8** upon the treatment with boron triflouride-diethyl etherate and *tert*-butylnitrite.

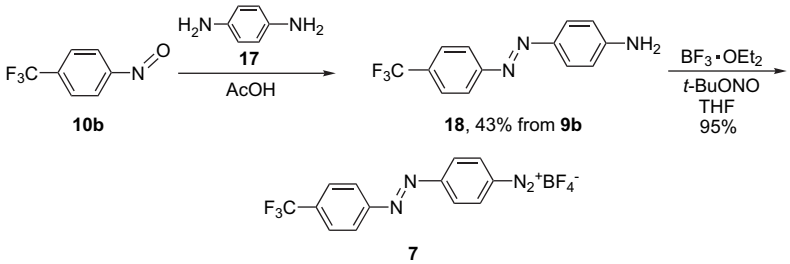
Additional examples of the synthesis of disubstituted azobenzenes are shown in Table 1. Oxidation of 4-substituted anilines by Oxone® followed by condensation of the resulting nitrosobenzenes with 4-substituted anilines provides 4,4′-disubstituted azobenzenes in 40–93% yields. The excellent yield of 4′-bromo-4-iodoazobenzene (entry 8) is particularly noteworthy because the compound could be useful for non-symmetric azobenzene OPE synthesis.[13] The azobenzenes formed were generally insoluble in acetic acid; a different work-up procedure was used for those products that were soluble (see Section 4). Formation of the corresponding nitrosobenzene from 4-trimethylsilylethynylaniline followed by coupling with **11** to provide the corresponding 4′-trimethylsilyliodoazobenzene could be useful for more advanced azobenzene OPE oligomers (entry 9).

3. Summary

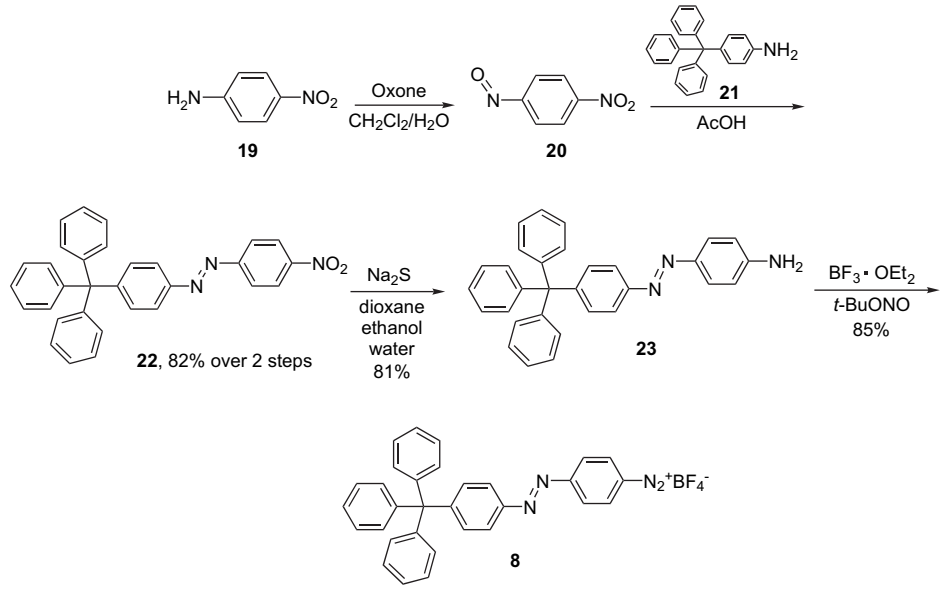
In summary, the methodology detailed here provides versatile synthetic pathways to azobenzene derivatives that could be used as potential molecular electronic or photo-responsive nanoscale devices. Work is currently underway to evaluate these photo-responsive azobenzene derivatives for their effectiveness as molecular switches integrated in nanoscale devices.



Scheme 2. Synthesis of the azobenzene alkanethiol **6**.

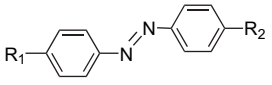


Scheme 3. Synthesis of the trifluoromethyldiazobenzene diazonium salt **7**.



Scheme 4. Synthesis of the trityldiazobenzene diazonium salt **8**.

Table 1. Synthesis of the *para*-disubstituted azobenzenes



Entry	Product	R_1	R_2	Yield (%)[a]
1	**12a**	H	I	85
2	**12b**	CF_3	I	40
3	**12c**	NO_2	I	51
4	**15**	H	$(CH_2)_2OH$	44
5	**18**	CF_3	NH_2	43
6	**22**	NO_2	Trityl	82
7	**24**	Br	H	74
8	**25**	Br	I	93
9	**26**	TMS—≡	I	35
10	**27**	NO_2	$(CH_2)_2OH$	72

[a] All yields are based on the two-step reaction sequence from the aniline except entry 1.

4. Experimental

4.1. General synthetic methods

Unless stated otherwise, reactions were performed in oven-dried, nitrogen flushed glassware equipped with a magnetic stir bar using freshly distilled solvents. Reagent grade tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Triethylamine (TEA) was distilled from calcium hydride. Trimethylsilylacetylene (TMSA) was donated by FAR Research Inc. and Petra Chemical. Compound **13** was prepared as described previously.[1] All other commercially available reagents were used as received. Unless otherwise noted, reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Flash chromatography was performed with the indicated solvent systems using silica gel grade 60 (230–400 mesh). NMR chemical shifts are reported in parts per million downfield from tetramethylsilane (TMS) or relative to the known value of residual solvent signal. Mass spectroscopy was performed at the Rice University or University of South Carolina Mass Spectroscopy Laboratory. Melting point values are uncorrected. Compounds were named using the Struct=Name algorithm in ChemDraw 9.0 developed by CambridgeSoft.

4.2. General procedure for the coupling of a terminal alkyne with an aryl halide utilizing a palladium–copper cross-coupling (Castro–Stephens/Sonogashira protocol)[8]

To a screw cap tube or a round-bottom flask were added the aryl halide, bis(triphenylphosphine)palladium(II) dichloride (5 mol % based on aryl halide), and copper(I) iodide (10 mol % based on aryl halide). The vessel was sealed with a rubber septum, evacuated, and backfilled with nitrogen (3×). A cosolvent of THF was added followed by the amine base. The terminal alkyne was then added followed by replacing the septum with a screw cap when a tube was used and the reaction was heated if necessary. TLC was used to follow the progress of the reaction, and when complete, the reaction vessel was cooled to room temperature. The mixture was quenched with water or a saturated solution of NH_4Cl. The organic layer was diluted with organic solvent and washed with brine (3×). The combined aqueous layers were extracted with organic solvent (3×), and the combined organic layers were dried over anhydrous $MgSO_4$. The slurry was filtered, and the solvent was removed from the filtrate in vacuo, followed by further purification of the residue as indicated.

4.3. General synthesis of *para*-disubstituted azobenzenes[7]

To a solution of the substituted aniline in CH_2Cl_2 was added Oxone® (2.00 equiv) dissolved in water. The mixture was stirred under nitrogen at room temperature until TLC monitoring indicated complete consumption of the starting material (2–24 h). The color of the solution generally turned to green as the corresponding nitrosoarene formed. After separation of the layers, the aqueous layer was extracted with

CH_2Cl_2 (3×). The combined organic layers were washed with 1 N HCl, saturated sodium bicarbonate solution, water, brine and dried with $MgSO_4$. After filtration, removal of the solvent from the filtrate in vacuo yielded the corresponding labile nitrosoarene, which was submitted to the next condensation step without further purification. To the nitrosoarene dissolved in acetic acid was added the substituted aniline (1.00 equiv). The resulting mixture was stirred at room temperature for 24–48 h. The precipitate was separated by filtration and the collected solid was washed with acetic acid and water and dried in a desiccator over P_2O_5 under reduced pressure. The compound was further purified by chromatography as necessary. For the acetic acid-soluble azobenzenes, saturated $NaHCO_3$ was added slowly to precipitate the product. The mixture was extracted with CH_2Cl_2 (3×). The combined extracts were dried with magnesium sulfate. After filtration the solvent was removed from the filtrate and the residue was chromatographed on silica gel.

4.3.1. (*E*)-2-(4-Iodophenyl)-1-phenyldiazene (12a). Following the general azobenzene synthesis procedure, a solution of nitrosobenzene **10a** (510 mg, 4.76 mmol) and 4-iodoaniline **11** (935 mg, 4.33 mmol) in acetic acid (30 mL) was stirred for 24 h. The product **12a** (1.13 g, 85%) was a yellow solid: mp 89–90 °C; FTIR (KBr) 3064, 1950, 1561, 1472 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, J=7.8, 1.7 Hz, 2H), 7.88 (d, J=8.4 Hz, 2H), 7.66 (d, J=8.4 Hz, 2H), 7.54–7.47 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.7, 152.2, 138.6 (2C), 131.6, 129.4 (2C), 124.7 (2C), 123.2 (2C), 97.9; HRMS calcd for $C_{12}H_9IN_2$ 307.9810, found 307.9805.

4.3.2. (*E*)-*S*-4-((4-(Phenyldiazenyl)phenyl)ethynyl)-phenyl ethanethioate (1). Following the general coupling procedure, **12a** (259 mg, 0.841 mmol) was coupled to **13**[1] (163 mg, 0.925 mmol) using $PdCl_2(PPh_3)_2$ (23 mg, 0.034 mmol), CuI (13 mg, 0.067 mmol), TEA (1 mL), and THF (5 mL). Column chromatography of the crude product on silica gel (40% CH_2Cl_2 in hexanes) afforded **1** as a yellow solid (219 mg, 73%): mp 185–186 °C; FTIR (KBr) 3064, 2924, 2202, 1926, 1689, 1592, 1491, 1437, 1351, 1297, 1106 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94–7.91 (m, 4H), 7.67 (d, J=8.4 Hz, 2H), 7.58 (d, J=8.1 Hz, 2H), 7.54–7.46 (m, 3H), 7.41 (d, J=8.1 Hz, 2H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 152.6, 152.0, 134.3 (2C), 132.5 (2C), 132.2 (2C), 131.3, 129.1 (2C), 128.5, 125.5, 124.2, 123.0 (4C), 91.1, 90.8, 30.3; HRMS calcd for $C_{24}H_{24}N_2OS$ 356.0983, found 356.0972.

4.3.3. (*E*)-4-((4-(Phenyldiazenyl)phenyl)ethynyl)benzenethiol (2). A solution of ethanol (2 mL) and water (2 mL) was purged with N_2 for 40 min and then KOH (47 mg, 0.842 mmol) was added. The solution was purged again with N_2 for 20 min. The thioacetate **1** (90 mg, 0.252 mmol) was added and the solution was purged again with N_2 for 10 min. The reaction mixture was heated at reflux under N_2 for 30 min, and then was carefully neutralized with 1 N HCl under N_2. The orange solid was filtered, washed repeatedly with water under N_2 and dried in vacuo. Purification on silica gel using 40% CH_2Cl_2 in hexanes afforded the thiol **2** (48 mg, 61%) as an orange solid: mp 161–195 °C (broad range likely due to oxidative decomposition); FTIR (KBr) 3437, 3060, 2924, 2559, 2206, 1584, 1495, 1398, 1301 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96–7.93 (m, 4H), 7.68 (d, J=8.6 Hz, 2H), 7.55–7.46 (m, 3H), 7.36 (d, J=8.4 Hz, 2H), 7.25 (d, J=8.4 Hz, 2H), 3.54 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 152.0, 132.6 (2C), 132.5 (2C), 132.4, 131.5, 129.4 (2C), 129.2 (2C), 126.1, 123.18 (2C), 123.17 (2C), 120.3, 91.6, 89.8; HRMS calcd for $C_{22}H_{22}N_2S$ 314.8777, found 314.8779.

4.3.4. (*E*)-1-(4-Iodophenyl)-2-(4-(trifluoromethyl)phenyl)diazene (12b). Following the general synthesis of azobenzenes procedure, to a solution of **9b** (1.50 g, 9.31 mmol) in CH_2Cl_2 (20 mL) was added a solution of Oxone® (11.5 g, 18.6 mmol) in H_2O (20 mL). The crude product (2.0 g) was dissolved in acetic acid (50 mL) and **11** (2.00 g, 9.31 mmol) was added. The resulting product **12b** was isolated as an orange solid (1.40 g, 40%): mp 150–151 °C; FTIR (KBr) 3076, 1942, 1577, 1476, 1336 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J=8.2 Hz, 2H), 7.91 (d, J=8.6 Hz, 2H), 7.79 (d, J=8.2 Hz, 2H), 7.69 (d, J=8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.4, 151.9, 138.7 (2C), 132.7 (q, $^2J_{CF}$=32.5 Hz), 126.6 (2C, q, $^3J_{CF}$=3.7 Hz), 124.9 (2C), 124.1 (q, $^1J_{CF}$=272.4 Hz), 123.3 (2C), 99.0; HRMS calcd for $C_{13}H_8F_3IN_2$ 375.9682, found 375.9684.

4.3.5. (*E*)-*S*-4-((4-((4-(Trifluoromethyl)phenyl)diazenyl)-phenyl)ethynyl)phenyl ethanethioate (3). Following the general coupling procedure, the iodoazobenzene **12b** (250 mg, 0.665 mmol) was coupled to **13** (129 mg, 0.731 mmol) using $PdCl_2(PPh_3)_2$ (19 mg, 0.027 mmol), CuI (10 mg, 0.053 mmol), TEA (1 mL), and THF (5 mL). The crude product was purified using column chromatography on silica gel (50% CH_2Cl_2 in hexanes) to afford **3** (201 mg, 71%) as an orange solid: mp 184–187 °C; FTIR (KBr) 3103, 2920, 2843, 2206, 1918, 1705, 1588, 1499, 1406, 1316 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, J=8.2 Hz, 2H), 7.97 (d, J=8.7 Hz, 2H), 7.80 (d, J=8.2 Hz, 2H), 7.71 (d, J=8.7 Hz, 2H), 7.60 (d, J=8.5 Hz, 2H), 7.44 (d, J=8.5 Hz, 2H), 2.46 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 193.6, 154.6, 151.9, 134.5 (2C), 132.8 (2C), 132.5 (2C), 128.8, 126.7, 126.6 (2C, q, $^3J_{CF}$=3.8 Hz), 124.2, 124.1 (q, $^1J_{CF}$=272.4 Hz), 123.5 (2C), 123.3 (2C), 91.8, 90.8, 30.6 ($^2J_{CF}$ coupling of the aromatic carbon at the *ipso*-position from CF_3 group was not clearly observed due to the low intensity of the corresponding quartet signal); HRMS calcd for $C_{23}H_{15}F_3N_2OS$ 424.0857, found 424.0857.

4.3.6. (*E*)-4-((4-((4-(Trifluoromethyl)phenyl)diazenyl)-phenyl)ethynyl)benzenethiol (4). A solution of ethanol (2 mL) and water (2 mL) was purged with N_2 for 30 min and KOH (66 mg, 1.2 mmol) was added. The solution was purged with N_2 for 20 min. The thioacetate **3** (90 mg, 0.236 mmol) was added and the solution was purged again with N_2 for 10 min. It was then heated at reflux under N_2 for 30 min. The reaction mixture was carefully neutralized with 1 N HCl under N_2. The yellow solid was filtered, washed repeatedly with water under N_2, and dried in a desiccator in vacuo to afford the thiol **4** (60 mg, 67%) as an orange solid: mp 184–187 °C; FTIR (KBr) 3289, 3044, 2919, 1953, 1736, 1605, 1443, 1301 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (d, J=8.2 Hz, 2H), 7.96 (d, J=8.5 Hz, 2H), 7.80 (d, J=8.2 Hz, 2H), 7.68 (d, J=8.5 Hz, 2H), 7.44 (d, J=8.3 Hz, 2H), 7.27 (d, J=8.3 Hz, 2H), 3.56 (s, 1H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 154.6, 151.7, 132.8 (2C), 132.6, 132.5 (2C), 129.1 (2C), 127.0, 126.6 (2C, q, $^3J_{CF}$=3.8 Hz), 123.5 (2C), 123.3 (2C), 120.1, 92.2, 89.7; (Carbon signals with $^1J_{CF}$ and $^2J_{CF}$ couplings were not observed due to the low intensity of the quartet signals caused by limited solubility of the product.) HRMS calcd for $C_{21}H_{13}F_3N_2S$ 382.0753, found 382.0751.

4.3.7. (*E*)-1-(4-Iodophenyl)-2-(4-nitrophenyl)diazene (12c). Following the general procedure for synthesis of azobenzenes, to a solution of 4-nitroaniline **9c** (2.00 g, 14.5 mmol) in CH_2Cl_2 (40 mL) was added a solution of Oxone® (17.8 g, 29.0 mmol) in H_2O (150 mL). The crude intermediate was dissolved in acetic acid (50 mL) and then 4-iodoaniline (3.18 g, 14.5 mmol) was added. Purification of the product on silica gel (50% CH_2Cl_2 in hexanes) gave **12c** (2.60 g, 51%) as an orange solid containing a small amount of cis isomer (<5%) according to the ^{1}H NMR analysis: mp 237–243 °C; FTIR (KBr) 3099, 2928, 1926, 1596, 1515, 1468, 1332 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J*=9.1 Hz, 2H), 8.05 (d, *J*=9.1 Hz, 2H), 7.93 (d, *J*=8.7 Hz, 2H), 7.71 (d, *J*=8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.7, 151.9, 149.1, 138.9 (2C), 125.1 (2C), 125.0 (2C), 123.8 (2C), 99.9; HRMS calcd for $C_{12}H_8IN_3O_2$ 352.9661, found 352.9664.

4.3.8. (*E*)-4-((4-((4-Nitrophenyl)diazenyl)phenyl)ethynyl)benzenethiol (5). Following the general coupling procedure, **12c** (96 mg, 0.26 mmol) was coupled to **13** (55 mg, 0.31 mmol) using $PdCl_2(PPh_3)_2$ (17 mg, 0.025 mmol), CuI (8 mg, 0.042 mmol), TEA (1 mL), and THF (5 mL). Column chromatography of the crude product on silica gel (50% CH_2Cl_2 in hexanes) afforded **5** as an orange solid (66 mg, 62%): mp 184–185 °C; FTIR (KBr) 3103, 2924, 2854, 2214, 1709, 1522, 1491, 1340 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J*=8.9 Hz, 2H), 8.06 (d, *J*=8.9 Hz, 2H), 7.98 (d, *J*=8.5 Hz, 2H), 7.71 (d, *J*=8.5 Hz, 2H), 7.60 (d, *J*=8.3 Hz, 2H), 7.43 (d, *J*=8.3 Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.5, 155.9, 151.8, 149.1, 134.5 (2C), 132.9 (2C), 132.5 (2C), 129.0, 127.4, 125.0 (2C), 124.1, 123.8 (4C), 92.3, 90.7, 30.6; HRMS calcd for $C_{22}H_{15}N_3O_3S$ 401.0822, found 401.0834.

4.3.9. (*E*)-2-(4-(Phenyldiazenyl)phenyl)ethanol (15). Following the general azobenzene procedure, to a solution of aniline (1.05 g, 11.3 mmol) in CH_2Cl_2 (20 mL) was added a solution of Oxone® (13.9 g, 22.6 mmol) in H_2O (100 mL). The crude product was dissolved in acetic acid (100 mL) and 4-(hydroxyethyl)aniline (**14**) (1.55 g, 11.3 mmol) was added. After purification on silica gel (30% EtOAc in hexane), isolation of the product gave **15** (1.15 g, 44%) as an orange solid: mp 81–83 °C; FTIR (KBr) 3303, 3051, 2933, 2857, 1486, 1443, 1590, 1519, 1341 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93–7.88 (m, 4H), 7.53–7.48 (m, 3H), 7.40 (d, *J*=8.5 Hz, 2H), 3.94 (t, *J*=6.5 Hz, 2H), 2.97 (t, *J*=6.5 Hz, 2H), 1.60 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 151.7, 142.2, 131.1, 130.0 (2C), 129.3 (2C), 123.3 (2C), 123.0 (2C), 63.7, 39.3; HRMS calcd for $C_{14}H_{14}N_2O$ 401.0822, found 401.0834.

4.3.10. (*E*)-1-(4-(2-(4-Bromobutoxy)ethyl)phenyl)-2-phenyldiazene (16). To a suspension of NaH (60% dispersion in mineral oil) in 7 mL of DMF was added dropwise at 0 °C the azobenzene ethanol **15** (600 mg, 2.65 mmol). NaI (40 mg, 0.26 mmol) and dibromobutane (2.30 g, 10.7 mmol) were then added to the solution. The reaction mixture was stirred for 32 h at room temperature. Since a significant amount of the starting alcohol was present in the mixture by TLC analysis, additional portions of NaH (212 mg, 5.30 mmol), dibromobutane (1.72 g, 7.96 mmol), and DMF (3 mL) were added to the solution. The reaction mixture was further stirred at room temperature for 20 h. Water was carefully added to quench the excess NaH after cooling the mixture to 0 °C. The aqueous layer was separated and extracted with CH_2Cl_2 (3×). The extracts were washed with brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel. Elution with 10% EtOAc in hexanes gave the bromide **16** (352 mg, 36%) as an orange semi-solid: FTIR (KBr) 3049, 2932, 2850, 1950, 1600, 1478, 1433, 1347, 1243 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J*=8.2 Hz, 2H), 7.87 (d, *J*=8.3 Hz, 2H), 7.53–7.47 (m, 3H), 7.38 (d, *J*=8.3 Hz, 2H), 3.69 (t, *J*=7.0 Hz, 2H), 3.49 (t, *J*=6.2 Hz, 2H), 3.43 (t, *J*=6.7 Hz, 2H), 2.97 (t, *J*=7.0 Hz, 2H), 1.94 (m, 2H), 1.73 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 151.5, 142.9, 131.0, 129.8 (2C), 129.3 (2C), 123.1 (2C), 123.0 (2C), 71.6, 70.2, 36.5, 34.0, 29.9, 28.4; HRMS calcd for $C_{18}H_{21}BrN_2O$ 360.0830, found 360.0837.

4.3.11. (*E*)-4-(4-(Phenyldiazenyl)phenethoxy)butane-1-thiol (6). A solution of the azobenzene bromide **16** (209 mg, 0.578 mmol) and thiourea (220 mg, 2.89 mmol) in ethanol (10 mL) was heated at reflux for 12 h. The solvent was evaporated under reduced pressure. To the concentrated mixture was added KOH (194 mg, 3.47 mmol) in nitrogen purged water (10 mL). The reaction mixture was heated at reflux for 1.5 h and then acidified with 1 N HCl. The aqueous mixture was extracted with CH_2Cl_2 (3×). The extracts were washed with brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel. Elution with 20% EtOAc in hexanes gave the thiol **6** (127 mg, 70%) as an orange solid: mp 31–33 °C; FTIR (KBr) 3425, 3054, 2947, 2851, 2794, 2558, 1955, 1598, 1480, 1434, 1366, 1302 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (m, 2H), 7.85 (d, *J*=8.4 Hz, 2H), 7.52–7.42 (m, 3H), 7.36 (d, *J*=8.4 Hz, 2H), 3.65 (t, *J*=7.0 Hz, 2H), 3.43 (t, *J*=5.7 Hz, 2H), 2.94 (t, *J*=7.0 Hz, 2H), 2.51 (m, 2H), 1.64 (m, 4H), 1.32 (t, *J*=7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 151.7, 143.1, 131.0, 130.1 (2C), 129.5 (2C), 123.6 (2C), 123.3 (2C), 71.8, 70.8, 36.7, 31.0, 28.8, 24.9; HRMS calcd for $C_{18}H_{22}N_2OS$ 314.1449, found 314.1453.

4.3.12. (*E*)-4-((4-(Trifluoromethyl)phenyl)diazenyl)benzeneamine (18). Following the general azobenzene procedure, to a solution of **9b** (1.50 g, 9.31 mmol) in CH_2Cl_2 (20 mL) was added a solution of Oxone® (11.5 g, 18.6 mmol) in H_2O (20 mL). The crude product was dissolved in acetic acid (60 mL) and *p*-phenylenediamine (**17**) (1.00 g, 9.31 mmol) was added. After workup, the crude product was purified on silica gel using CH_2Cl_2 to give **18** (1.05 g, 43%) as an orange solid: mp 134–136 °C; FTIR (KBr) 3503, 3410, 3056, 2920, 1938, 1592, 1499, 1425, 1394, 1316, 1289 cm^{-1}; ^{1}H NMR (500 MHz, CD_3CN) δ 7.92 (d, *J*=9.0 Hz, 2H), 7.81 (d, *J*=9.0 Hz, 2H), 7.77 (d, *J*=8.8 Hz, 2H), 6.76 (d, *J*=8.8 Hz, 2H), 4.96 (br s, 2H);

^{13}C NMR (125 MHz, CD_3CN) δ 156.5, 154.1, 145.6, 131.3 (q, $^2J_{CF}$=32.1 Hz), 127.7 (2C, q, $^3J_{CF}$=3.9 Hz), 127.0 (2C), 125.7 (q, $^1J_{CF}$=271.1 Hz), 123.7 (2C), 115.3 (2C); HRMS calcd for $C_{13}H_{10}F_3N_3$ 265.0827, found 265.0824.

4.3.13. (*E*)-4-((4-(Trifluoromethyl)phenyl)diazenyl)benzendiazonium tetrafluoroborate (7). In a 100 mL round-bottom flask was placed boron trifluoride-diethyl etherate (0.143 mL, 1.13 mmol). The flask was submerged into a dry ice bath to control the temperature at −40 °C. The diazobenzeneamine **18** (100 mg, 0.377 mmol) in THF (4 mL) was added dropwise. The reaction mixture was stirred for 10 min and then *tert*-butylnitrite (0.090 mL, 0.75 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and then anhydrous ether was added to precipitate the diazonium salt. The mixture was filtered and the solid washed with excess ether to afford the diazonium salt **7** (130 mg, 95%): FTIR (KBr) 3363, 3212, 3107, 2291, 1938, 1802, 1577, 1475, 1456, 1332 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d_6) δ 9.13 (d, *J*=9.1 Hz, 2H), 8.51 (d, *J*=9.1 Hz, 2H), 8.26 (d, *J*=8.2 Hz, 2H), 8.06 (d, *J*=8.2 Hz, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 159.2, 155.1, 135.9 (2C), 134.6 (q, $^2J_{CF}$=32.6 Hz), 127.8 (2C, q, $^3J_{CF}$=3.8 Hz), 126.1 (2C), 125.2 (2C), 124.8 (q, $^1J_{CF}$=271.8 Hz), 117.8.

4.3.14. (*E*)-1-(4-Nitrophenyl)-2-(4-tritylphenyl)diazene (22). Following the general azobenzene procedure, to a solution of **19** (1.00 g, 7.24 mmol) in CH_2Cl_2 (20 mL) was added a solution of Oxone® (8.90 g, 14.5 mmol) in H_2O (80 mL). After workup, the crude product was dissolved in acetic acid (50 mL) and 4-tritylaniline **21** (2.43 g, 7.24 mmol) was added to give a product that was recrystallized from hexanes to afford **22** (2.79 g, 82%) as an orange solid: mp 301–302 °C; FTIR (KBr) 3052, 2443, 1957, 1592, 1515, 1492, 1437, 1350, 1312 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J*=9.1 Hz, 2H), 8.01 (d, *J*=9.1 Hz, 2H), 7.87 (d, *J*=8.8 Hz, 2H), 7.46 (d, *J*=8.8 Hz, 2H), 7.30–7.23 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 152.1, 150.6, 148.9, 146.4 (3C), 132.3 (2C), 131.3 (6C), 128.0 (6C), 126.4 (3C), 125.0 (2C), 123.6 (2C), 122.8 (2C), 65.4; HRMS calcd for $C_{31}H_{23}N_3O_2$ 469.1787, found 469.1790.

4.3.15. (*E*)-4-((4-Tritylphenyl)diazenyl)benzeneamine (23). To **22** (1.50 g, 3.19 mmol) in a solution of dioxane (80 mL), ethanol (20 mL), and water (5 mL) was added sodium sulfide (2.30 g, 9.58 mmol). The mixture was heated at 80 °C for 1.5 h. The solvents were partly evaporated and the residue was dissolved in CH_2Cl_2. The solution was washed with 1 N HCl followed by saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel using 60% CH_2Cl_2 in hexanes to give **23** (1.14 g, 81%): mp 230–231 °C; FTIR (KBr) 3476, 3379, 3056, 3025, 1957, 1619, 1503, 1445, 1417, 1289 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, *J*=8.7 Hz, 2H), 7.73 (d, *J*=8.7 Hz, 2H), 7.36 (d, *J*=8.7 Hz, 2H), 7.30–7.20 (m, 15H), 6.75 (d, *J*=8.7 Hz, 2H), 4.11 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.0, 149.8, 148.9, 146.7 (3C), 145.8, 132.0 (2C), 131.3 (6C), 127.8 (6C), 126.2 (3C), 125.3 (2C), 121.5 (2C), 114.9 (2C), 65.2; HRMS calcd for $C_{31}H_{25}N_3$ 439.2048, found 439.2048.

4.3.16. (*E*)-4-((4-Tritylphenyl)diazenyl)benzendiazonium tetrafluoroborate (8). In a 100 mL round-bottom flask was placed boron trifluoride-diethyl etherate (0.432 mL, 3.41 mmol). The flask was submerged into a dry ice bath to control the temperature at −40 °C. The diazobenzeneamine **23** (500 mg, 1.14 mmol) in THF (15 mL) was added dropwise. The reaction mixture was stirred for 10 min and then *tert*-butylnitrite (0.273 mL, 2.28 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and then anhydrous ether was added to precipitate the diazonium salt. The mixture was filtered and the solid was washed with excess ether to afford the pure diazonium salt **8** (520 mg, 85%): FTIR (KBr) 3348, 3111, 3045, 2272, 1957, 1817, 1577, 1487, 1413, 1317 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (d, *J*=8.2 Hz, 2H), 8.29 (d, *J*=8.2 Hz, 2H), 7.99 (d, *J*=7.5 Hz, 2H), 7.65 (d, *J*=7.5 Hz, 2H), 7.42–7.33 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 154.3, 150.9, 146.8 (3C), 135.0 (2C), 132.6 (2C), 131.2 (6C), 128.5 (6C), 126.8 (3C), 125.6 (2C), 123.8 (2C), 114.9, 65.9.

4.3.17. (*E*)-1-(4-Bromophenyl)-2-phenyldiazene (24).[14] Following the general azobenzene procedure, to a solution of 4-bromoaniline (2.50 g, 14.9 mmol) in CH_2Cl_2 (45 mL) was added a solution of Oxone® (17.9 g, 29.8 mmol) in H_2O (180 mL). The crude product (2.78 g) was >90% pure by ^{1}H NMR. A portion (500 mg, 2.70 mmol) was dissolved in acetic acid (30 mL) and was condensed with aniline (210 mg, 2.25 mmol). Purification on silica gel using 5% CH_2Cl_2 in hexanes gave the **24** (520 mg, 74%) as an orange solid: mp 89–90 °C; FTIR (KBr) 3084, 1573, 1480 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J*=8.1 Hz, 2H), 7.80 (d, *J*=8.5 Hz, 2H), 7.65 (d, *J*=8.5 Hz, 2H), 7.55–7.47 (m, 3H).

4.3.18. (*E*)-1-(4-Bromophenyl)-2-(4-iodophenyl)diazene (25). A portion of the crude 4-bromonitrosobenzene (500 mg, 2.70 mmol) from the preparation of **24** was dissolved in acetic acid (30 mL) and **11** (493 mg, 2.25 mmol) was added. Workup provided **25** (810 mg, 93%) as an orange solid: mp 209–212 °C; FTIR (KBr) 3068, 1907, 1561, 1472, 1394, 1336 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J*=8.7 Hz, 2H), 7.80 (d, *J*=8.8 Hz, 2H), 7.66 (d, *J*=8.8 Hz, 2H), 7.65 (d, *J*=8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.0, 151.4, 138.7 (2C), 132.6 (2C), 126.0, 124.74 (2C), 124.67 (2C), 98.3; HRMS calcd for $C_{12}H_8BrIN_2$ 385.8916, found 385.8915.

4.3.19. (*E*)-1-(4-Iodophenyl)-2-(4-((trimethylsilyl)ethynyl)phenyl)diazene (26). Following the general azobenzene procedure, to a solution of 4-((trimethylsilyl)ethynyl)aniline[15] (250 mg, 1.32 mmol) in CH_2Cl_2 (2.5 mL) was added a solution of Oxone® (1.63 g, 2.62 mmol) in H_2O (20 mL). The crude product was dissolved in acetic acid (20 mL) and **11** (289 mg, 1.32 mmol) was added. Workup and chromatography using 5% CH_2Cl_2 in hexane on silica gel gave **26** (185 mg, 35%) as an orange solid: mp 143–144 °C; FTIR (KBr) 2948, 2155, 1926, 1565, 1468, 1398, 1250 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J*=8.7 Hz, 2H), 7.87 (d, *J*=8.7 Hz, 2H), 7.66 (d, *J*=8.7 Hz, 2H), 7.61 (d, *J*=8.7 Hz, 2H), 0.29 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 151.9, 138.6 (2C), 133.1 (2C),

126.4, 124.7 (2C), 123.1 (2C), 104.8, 98.3, 97.6, 0.1 (3C); HRMS calcd for $C_{17}H_{17}IN_2Si$ 404.0213, found 404.0206.

4.3.20. (*E*)-2-(4-((4-Nitrophenyl)diazenyl)phenyl)ethanol (27). Following the general azobenzene procedure, to a solution of 4-nitroaniline (**19**) (1.20 g, 8.69 mmol) in CH_2Cl_2 (20 mL) was added a solution of Oxone® (10.7 g, 17.4 mmol) in H_2O (80 mL). The crude product was dissolved in acetic acid (150 mL) and 4-(hydroxyethyl)aniline (**14**) (1.90 g, 8.69 mmol) was added. After workup the residue was chromatographed on silica gel using 30% EtOAc in hexane to give **27** (1.69 g, 72%) as an orange solid: mp 149–152 °C; FTIR (KBr) 3522, 3402, 3103, 2940, 1915, 1653, 1584, 1522, 1495, 1340, 1207, 1099, 1036 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J=9.1 Hz, 2H), 8.03 (d, J=9.1 Hz, 2H), 7.94 (d, J=8.4 Hz, 2H), 7.44 (d, J=8.4 Hz, 2H), 3.96 (t, J=6.5 Hz, 2H), 2.99 (t, J=6.5 Hz, 2H), 1.53 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 151.4, 148.8, 144.0, 130.2 (2C), 124.9 (2C), 123.9 (2C), 123.6 (2C), 63.5, 39.3; HRMS calcd for $C_{14}H_{13}N_3O_3$ 271.0959, found 271.0957.

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Reaction of ene-bis(phosphinylallenes): [2+2] versus [4+2] cycloaddition

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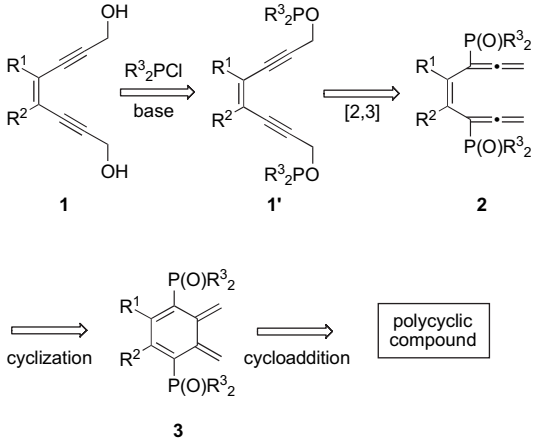
Abstract—Reaction of ene-bis(phosphinylallenes), derived from ene-bis(propargyl alcohols) and chlorodiphenylphosphine, was investigated. Benzene-bridged bis(phosphinylallenes) exclusively gave intramolecular [2+2] cycloadducts in the presence of dimethyl fumarate in sharp contrast to the reaction of benzene-bridged bis(sulfinylallenes), which gave the corresponding [4+2] cycloadducts. On the other hand, substituted ethylene- or five-membered heterocycle-bridged bis(phosphinylallenes) provided [4+2] cycloadducts. Reaction of benzene-bridged diallene bearing both a sulfinyl group and a phosphinyl group on the two allenyl groups was also described.

1. Introduction

Recent efforts from this laboratory[1,2] disclosed that the pericyclic reaction of ene-bis(sulfinylallenes), derived from the reaction of ene-bis(propargyl alcohols), enables the rapid construction of a variety of polycyclic aromatic compounds.[1–3] In our contiguous studies on the utility of the ene-diallene species in organic synthesis, we investigated the pericyclic reaction of ene-bis(phosphinylallene), which is an analogue of ene-bis(sulfinylallene), and should be prepared by the [2,3]-sigmatropic rearrangement[4,5] of a ene-bis(propargyl alcohol) derivative, the same as ene-bis(sulfinylallene)[6] (Scheme 1). This paper describes the unexpected reactivity of the two types of ene-bis(phosphinylallenes); one is the benzene-bridged derivatives, which exclusively underwent [2+2] cycloaddition,[7] while the other is the substituted ethylene derivatives and heterocyclic ones leading to the formation of the [4+2] cycloadducts.



Scheme 1.

2. Results and discussion

According to the previously described procedure for the reaction with PhSCl,[1,6] chlorodiphenylphosphine (Ph_2PCl) was added to the solution of benzene-bridged bis(propargyl alcohol) **4a**, triethylamine, and dimethyl fumarate (**5**) (as a dienophile) in THF at −78 °C, and the resulting mixture was warmed to room temperature to produce the naphtho[*b*]-cyclobutene **6aa**[8] in 85% yield (Scheme 2). The expected cycloadduct **7aa**, predicted on the basis of the reaction of **4a** and **5** in the presence of PhSCl, could not be detected. The reactivity of ene-bis(phosphinylallene) was in sharp contrast to that of bis(sulfinylallene), despite the similarity of the electrical nature between the phosphinyl and sulfinyl groups.[9] On the basis of the experiments in Scheme 2, it was evident that a dienophile did not take part in the formation of **6aa**. Thus, the ring-closing reaction using PhSCl and other chlorophosphines *in the absence of* the dienophile became the next subject of interest (Table 1).

Keywords: Bisallenes; [2+2] Cycloaddition; Naphtho[*b*]cyclobutenes; [4+2] Cycloaddition.
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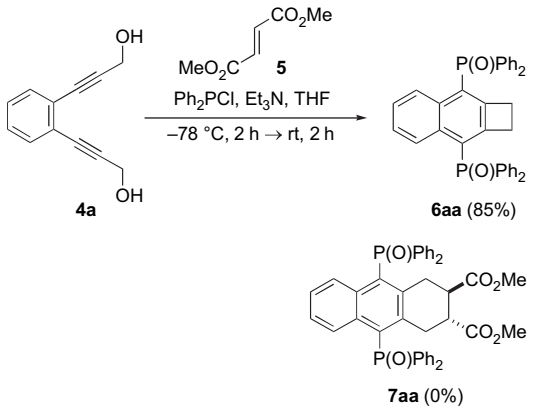
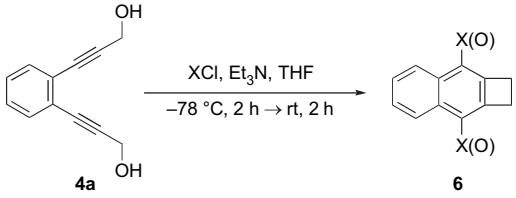
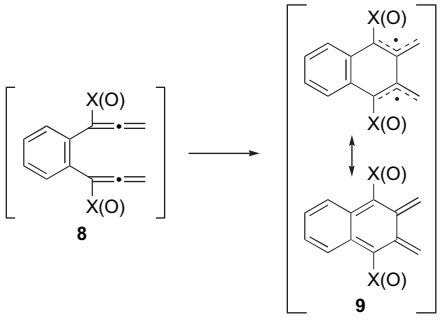


Scheme 2.

Table 1. Synthesis of naphtho[*b*]cyclobutenes **6**[a]



Entry	X	Product	Yield (%)
1	Ph_2P	**6aa**	85
2	Cy_2P	**6ab**	98
3	$^iPr_2P$	**6ac**	81
4	Et_2P	**6ad**	99
5	$(EtO)_2P$	**6ae**	0
6	PhS	**6af**	0



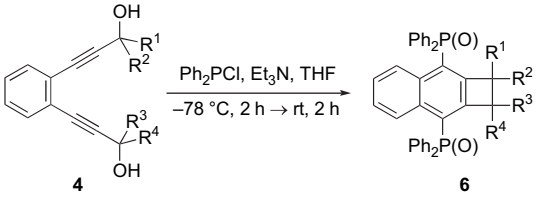
[a] All reactions were performed on a 0.1 mmol scale (0.1 M) with 6 equiv of XCl and 7 equiv of Et_3N.

The reactions with chlorodialkylphosphines instead of Ph_2PCl exclusively afforded the corresponding 3,8-bis(dialkylphosphinyl)naphtho[*b*]cyclobutenes **6ab–6ad** in high yields, regardless of the bulkiness of the alkyl groups on the phosphorus atom (entries 2–4). However, changing chlorodialkylphosphine to PhSCl or diethyl chlorophosphite [$(EtO)_2PCl$] under standard conditions resulted in complex mixtures of products (entries 5 and 6). A comprehensive mechanistic discussion is premature at this point, but it seems reasonable to postulate that the bis(phosphinylallene) **8** [X(O)=phosphinyl], derived from the bis(propargyl phosphinite) by dual [2,3]-sigmatropic rearrangement, would be converted into the biradical (*o*-quinodimethane) species **9**, which spontaneously undergoes intramolecular [2+2] cycloaddition to produce **6**.[10] The production of the naphtho[*b*]-cyclobutene derivative **6aa** as a sole product was observed irrespective of the existence of dimethyl fumarate (**5**) (Scheme 2, Table 1, entry 1). This result may reflect that the intramolecular [2+2] cycloaddition of the plausible biradical intermediate **9** would be much faster than the intermolecular [4+2] cycloaddition with the dienophile. The fact that $(EtO)_2PCl$ could not provide the cyclobutene derivative might be attributable to the comparatively low reactivity in the [2,3]-sigmatropic rearrangement of **1′** to **2** (the second step in Scheme 1).[9]

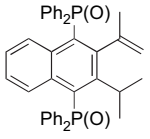
Having identified the effect of the phosphinyl group on the [2+2] cycloaddition, we then synthesized the naphtho[*b*]-cyclobutenes possessing certain substituents on the cyclobutene ring.[11] The results are summarized in Table 2. Treatment of the monomethyl derivative **4b**[1] with Ph_2PCl afforded the naphtho[*b*]cyclobutene **6ba** in 84% yield (entry 1). Similarly, another monosubstituted bis(propargyl alcohol) **4c** furnished **6ca** in a high yield (entry 2). The 1,2-disubstituted naphtho[*b*]cyclobutenes **6da** and **6ea** were obtained as a mixture of two diastereomers from 1,1′-disubstituted bis(propargyl alcohols) **4d** and **4e** in high yields (entries 3 and 4). In addition, the 1,1-disubstituted bis(propargyl alcohol) **4f** provided **6fa** without any difficulties (entry 5). However, fully methyl-substituted bis(propargyl alcohol) **4g** afforded a [1,5] hydrogen-shifted product **10** in 61% yield as the sole isolable product (entry 6).[12]

Stereochemical assignments of **6ea** and **6da** were unambiguously made by a chemical transformation, in particular, using a dephosphinylation reaction,[7] which we have recently developed. Initially, transformation of the major isomer of diphenyl-substituted cycloadduct **6ea** into the known naphtho[*b*]cyclobutene **11**[3c,13] by $LiAlH_4$ in refluxing dioxane was examined; however, an inseparable mixture of the cis- and trans-isomers of **11** and the unknown **12** was obtained (Scheme 3). This result indicated that ring opening of the

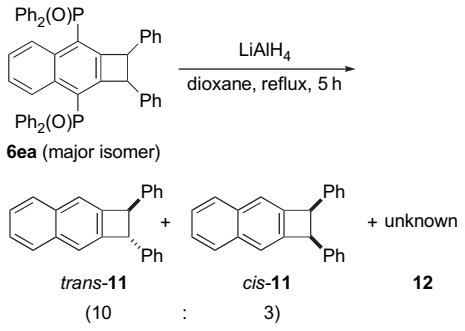
Table 2. Synthesis of substituted naphtho[*b*]cyclobutenes **6**



Entry	Substrate	R^1	R^2	R^3	R^4	Product	Yield (%)
1	**4b**	Me	H	H	H	**6ba**	84
2	**4c**	CH_2OBn	H	H	H	**6ca**	90
3	**4d**	Me	H	Me	H	**6da**	84[a]
4	**4e**	Ph	H	Ph	H	**6ea**	87[b,c]
5	**4f**	Me	Me	H	H	**6fa**	86
6	**4g**	Me	Me	Me	Me	**6ga**	0[b,d]

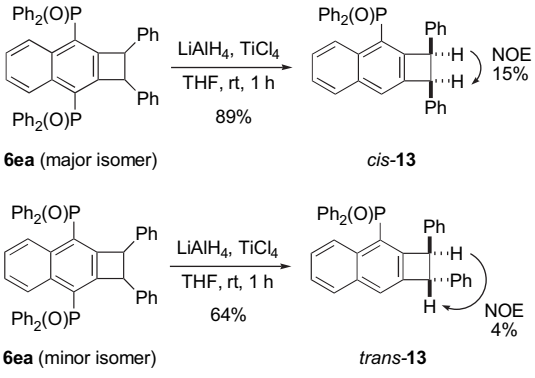


[a] A mixture of trans- and cis-isomers was obtained in a ratio of 3:2.
[b] Reaction mixture was stirred for an additional 15 h at room temperature.
[c] A mixture of trans- and cis-isomers was obtained in a ratio of 1:2.
[d] Compound **10** was obtained in 61% yield.



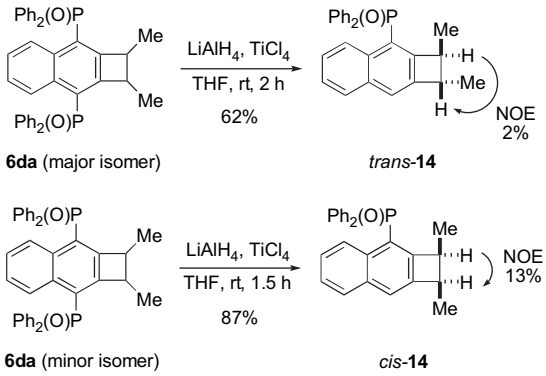
Scheme 3.

cyclobutane framework of **6ea** and/or **11** occurred under the reaction conditions.[14] Thus, a recently developed monodephosphinylation[7] under milder conditions was applied to compound **6ea**. Independent exposure of major and minor isomers of **6ea** to $LiAlH_4$–$TiCl_4$ at room temperature effected the monodephosphinylation without isomerization to provide the *cis*- and *trans*-**13**, respectively (Scheme 4). Their stereochemical assignments were unambiguously established by the NOE experiments as depicted in Scheme 4. As a result, an NOE experiment with *cis*-**13** revealed 15% enhancement between the two benzylic protons, while 4% enhancement was observed by irradiation of one of the two benzylic protons in the NOE experiment with *trans*-**13**. The major and minor isomers of **6da** were transformed to the *trans*- and *cis*-**14**, respectively, under the same reductive conditions (Scheme 5). The stereochemistry of these compounds was also determined on the basis of the NOE experiments. The *cis*-**13** was obtained as a major product in the reaction of the phenyl derivative **4e**, while the major isomer was found to be the *trans*-**14** in the reaction of the methyl congener **4d**. The preferential formation of the *cis*-diphenyl-substituted cycloadduct over the *trans*-one **6ea** in the [2+2] cycloaddition process is uncertain.[15]



Scheme 4.

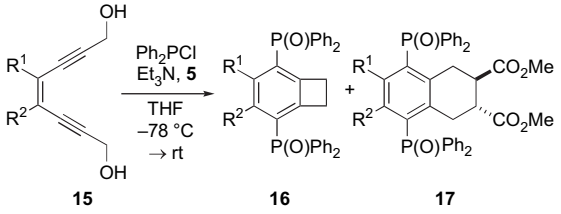
The present [2+2] cycloaddition methodology was found not to be applied to the synthesis of benzocyclobutenes. Indeed, the reaction of ethylene-bridged bis(propargyl alcohol) **15a**[16] with Ph_2PCl in the presence of dimethyl fumarate (**5**) gave neither [2+2] cycloadducts nor [4+2] cycloadducts (Table 3, entry 1). Monosubstituted ethylene **15b**,[1] however, produced the [4+2] cycloadduct **17b** in 88% yield (entry 2). Cycloalkane derivatives **15c**[1] and **15d**[1] (disubstituted ethylene derivatives) also reacted with **5** to give [4+2] cycloadducts **17c** and **17d**, respectively (entries 3 and 4). In

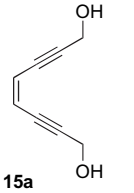
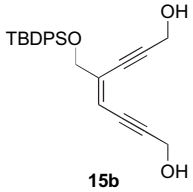
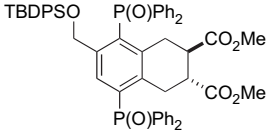
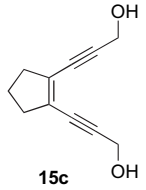
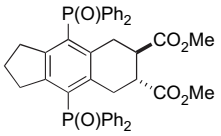
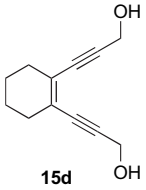
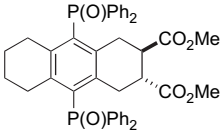
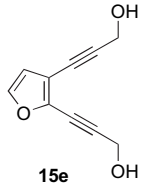
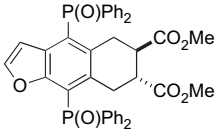
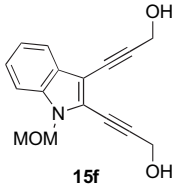
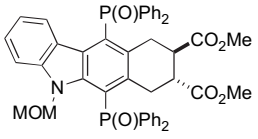
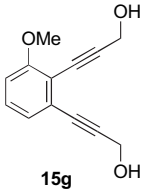
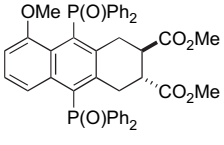


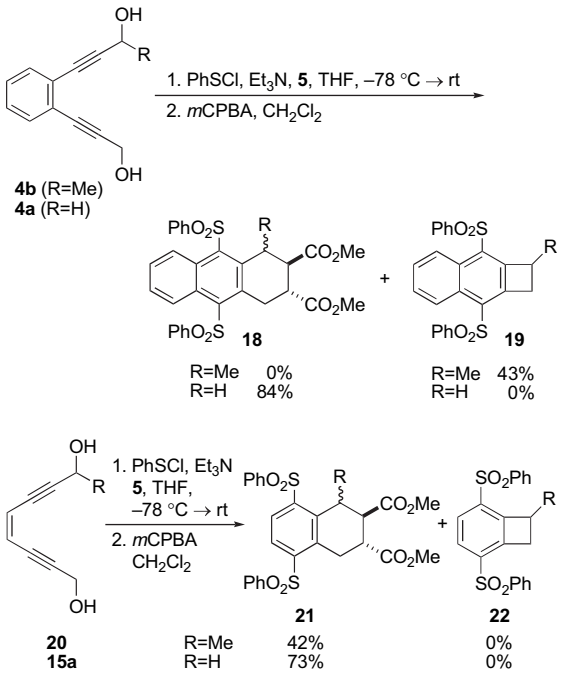
Scheme 5.

addition, the reaction of furan and indole derivatives **15e**[1] and **15f**[1] afforded [4+2] cycloadducts **17e** and **17f** as the sole isolable products in 48% and 77% yields, respectively (entries 5 and 6). The anisole derivative **15g** produced the [4+2] cycloadduct **17g** in 44% yield (entry 7), which was an unexpected result, because the corresponding benzene derivatives **4** consistently afforded naphtho[*b*]cyclobutenes **6** in high yields (see Scheme 2 and Table 2). It should be mentioned that [2+2] cycloadducts could not be detected in the reaction mixture even though the reaction ran in the absence of **5**. Cava and Shirley reported that the 2,3-naphthoquinodimethane (without a sulfinyl or phosphinyl group) is subject to the intramolecular [2+2] cycloaddition in the absence of dienophiles, whereas the *o*-quinodimethane generally tends to undergo dimerization (a kind of [4+2] cycloaddition) under similar conditions. On the basis of these experiments, they claimed that the formation of the naphtho[*b*]cyclobutene framework might be attributed to a higher degree of diradical character of the 2,3-naphthoquinodimethane than that of the *o*-quinodimethane.[17] Thus, the formation of the [4+2] cycloadducts in the reaction of **15b**, **15c**, and **15d** via the corresponding *o*-quinodimethane species (entries 2–4) would be tentatively rationalized by Cava and Shirley's interpretation, although we have no clues yet to understand the result of **15a**. A similar tendency was recorded in the reaction of benzene- and ethylene-bis(propargyl alcohols) **4b** and **20**, having a methyl group at the propargylic position, with PhSCl (Scheme 6).[1] The benzene derivative **4b** gave [2+2] cycloadduct **19** (R=Me) via the 2,3-naphthoquinodimethane intermediate, while ethylene derivative **20** provided a [4+2] cycloadduct **21** (R=Me) via the *o*-quinodimethane intermediate. However, the reaction of both **4a** and **15a** with PhSCl gave the corresponding [4+2] cycloadducts **18** (R=H) and **21** (R=H) in high yields. As aforementioned, the 2,3-naphthoquinodimethane having a bis(diphenylphosphinyl) group, derived from the reaction of **4a** with Ph_2PCl, furnished the [2+2] cycloadduct **6aa** (Scheme 2), whereas the corresponding phenylsulfinyl congener, derived from the reaction of **4a** with PhSCl, provided the [4+2] cycloadduct **18** (R=H). The significant difference in the reactivity observed in these two reactions cannot be rationalized on the basis of Cava and Shirley's results. By taking the similarity of the electrical nature between the phosphinyl and sulfinyl groups into account, we tentatively assumed that the much bulkier diphenylphosphinyl groups might inhibit the approach of dienophiles to the 1,3-diene moiety of the 2,3-naphthoquinodimethane having

Table 3. Reaction of various ene-bis(propargyl alcohols) with Ph_2PCl



Entry	Substrate	Product
1	 **15a**	Complex mixture
2	 **15b**	 **17b** (88%)
3	 **15c**	 **17c** (64%)
4	 **15d**	 **17d** (87%)
5	 **15e**	 **17e** (48%)
6	 **15f**	 **17f** (77%)
7	 **15g**	 **17g** (44%)

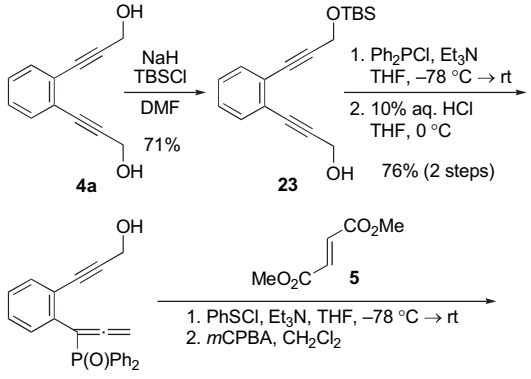


Scheme 6.

a bis(diphenylphosphinyl) group resulting in the exclusive formation of the [2+2] cycloadduct. This would not be the case with the naphthoquinodimethane having a bis(phenylsulfinyl) group where the less sterically hindered circumstances might allow the [4+2] cycloaddition to occur. In the case of other aromatic compounds **15e** and **15f** (Table 3, entries 5 and 6), the plausible furanoquinodimethane and carbazoloquinodimethane intermediates might no longer suffer from the serious non-bonding interaction between the diphenylphosphinyl group and the *peri*-hydrogen atom, which should be associated with the case of the naphthoquinodimethane intermediate derived from **4**. Thus, the rather bulky diphenyl moiety on the phosphorus atom of **15e** and **15f** would be allowed to orient opposite to the quinodimethane moiety. As a result, dimethyl fumarate (**5**) would approach to the 1,3-diene moiety resulting in the [4+2] cycloaddition. This speculation, however, cannot be used to explain the result obtained in the reaction of **15g** (Table 3, entry 7), because the naphthoquinodimethane intermediate from **15g** has a bulkier methoxy group than a hydrogen atom at the *peri*-position.

Independent treatment of the benzene-bis(propargyl alcohol) **4a** with Ph_2PCl (Scheme 2) and PhSCl (Scheme 6) gave the respective formation of the [2+2] cycloadduct **6aa** and the [4+2] cycloadduct **18** (R=H). In other words, the naphthoquinodimethane having a bis(diphenylphosphinyl) group afforded the naphthocyclobutene derivative ([2+2] product), while the bis(phenylsulfinyl) derivative furnished the tetrahydroanthracene derivative ([4+2] product). The outcome of these reactions seems to depend on the property of the substituent on the allenyl moiety. Thus, it would be interesting to examine the reaction of a benzene-bridged diallene bearing both a sulfinyl group and a phosphinyl group on the two allenyl groups. Monosilylation of bis(propargyl alcohol) **4a** under the conventional conditions gave **23**, which was subsequently treated with Ph_2PCl and 10%

aqueous HCl to give phosphinylallene derivative **24** (Scheme 7). The sequential reaction of **24** with PhSCl was carried out in the absence of **5** to give, after *m*CPBA oxidation, the naphtho[*b*]cyclobutene **25** in 35% yield. When **4a** was directly exposed to PhSCl, no characteristic products could be detected, whereas upon treatment with Ph_2PCl, **4a** provided **6aa** in 85% yield as mentioned in Table 1. On the other hand, similar treatment of **24** in the presence of **5** furnished the [4+2] cycloadduct **26** in 53% yield along with the [2+2] cycloadduct **25** (17%) as a by-product. These results may reflect both the natures of the sulfinyl group being susceptible to [4+2] cycloaddition and the phosphinyl group, which is subject to [2+2] cycloaddition.



5	yield of **25** (%)	yield of **26** (%)
x	35	–
○	17	53

Scheme 7.

3. Conclusions

We have shown that benzene-bis(phosphinylallenes), derived from benzene-bis(propargyl alcohols) and Ph_2PCl, underwent intramolecular [2+2] cycloaddition leading to the naphtho[*b*]cyclobutene derivatives in sharp contrast to the reaction of benzene-bis(sulfinylallenes), which gave the corresponding [4+2] cycloadducts. On the other hand, ethylene-bis(phosphinylallenes) afforded the [4+2] cycloadducts instead of the [2+2] cycloadducts. Thus, the reaction pathway could be controlled by proper choice of the reagent (Ph_2PCl and PhSCl) for the [2,3]-sigmatropic rearrangement of the propargyl alcohol moiety. Further studies on the scope and limitations of this method are currently in progress.

4. Experimental

4.1. General

Melting points are uncorrected. IR spectra were measured in $CHCl_3$. ^1H NMR spectra were taken in $CDCl_3$. $CHCl_3$ (7.26 ppm) for silyl compounds and tetramethylsilane (0.00 ppm) for compounds without a silyl group were used as internal standards. ^{13}C NMR spectra were recorded in $CDCl_3$ with $CDCl_3$ (77.00 ppm) as an internal standard. All reactions were carried out under a nitrogen atmosphere. Silica gel (silica gel 60, 230–400 mesh) was used for chromatography. Organic extracts were dried over anhydrous Na_2SO_4.

4.2. General procedure for reaction of ene-bis(propargyl alcohols) with chlorodialkylphosphine

To a solution of bis(propargyl alcohol) (0.100 mmol) in THF (1 mL) were successively added Et_3N (0.10 mL, 0.72 mmol) and chlorodialkylphosphine (0.56 mmol) at −78 °C. After being stirred for 2 h, the reaction mixture was allowed to warm to room temperature. After 2 h, the reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. Chromatography of the residue with AcOEt–MeOH gave the cycloadduct. To the reaction in the presence of dienophile, to a solution of bis(propargyl alcohol), dienophile (4 equiv), and Et_3N were added chlorodialkylphosphine.

4.2.1. 3,8-Bis(diphenylphosphinyl)-1,2-dihydrocyclobuta[*b*]naphthalene (6aa). Colorless prisms: mp >300 °C (AcOEt–MeOH); IR 1439, 1180 cm^{-1}; ^1H NMR δ 8.59–8.55 (2H, m), 7.70–7.25 (22H, m), 2.06 (4H, s); ^{13}C NMR δ 150.4 (dd, J_{C-P}=11.7, 11.7 Hz), 134.4 (J_{C-P}=7.8 Hz), 133.0 (J_{C-P}=105 Hz), 132.2–131.8 (m), 128.8–128.6 (m), 128.3 (dd, J_{C-P}=3.4, 3.4 Hz), 126.1 (J_{C-P}=191 Hz), 30.0; MS *m*/*z* 554 (M^+, 32). Anal. Calcd for $C_{36}H_{28}O_2P_2 \cdot 1/2H_2O$: C, 76.72; H, 5.19. Found: C, 76.78; H, 5.30.

4.2.2. 3,8-Bis(dicyclohexylphosphinyl)-1,2-dihydrocyclobuta[*b*]naphthalene (6ab). Colorless powders: mp >300 °C (AcOEt–MeOH); IR 1448, 1150 cm^{-1}; ^1H NMR δ 9.10 (2H, br s), 7.54–7.50 (2H, m), 3.46 (4H, s), 2.20–1.10 (44H, m); ^{13}C NMR δ 145.4 (br), 136.2 (dd, J_{C-P}=8.3, 6.1 Hz), 127.8, 125.9 (J_{C-P}=0.6 Hz), 124.6 (dd, J_{C-P}=77.1, 2.2 Hz), 37.6 (J_{C-P}=69.3 Hz), 32.2 (J_{C-P}=3.1 Hz), 26.5 (dd, J_{C-P}=13.4, 3.1 Hz), 25.8, 25.3; MS *m*/*z* 578 (M^+, 55); HRMS calcd for $C_{36}H_{52}O_2P_2$ 578.3443, found 578.3436.

4.2.3. 3,8-Bis(diisopropylphosphinyl)-1,2-dihydrocyclobuta[*b*]naphthalene (6ac). Colorless prisms: mp 223–225 °C (AcOEt); IR 1464, 1173, 1140 cm^{-1}; ^1H NMR δ 9.12 (2H, br s), 7.54–7.50 (2H, m), 3.50 (4H, s), 2.50–2.37 (4H, m), 1.33 (6H, d, *J*=7.1 Hz), 1.28 (6H, d, *J*=7.1 Hz), 1.15 (6H, d, *J*=7.1 Hz), 1.09 (6H, d, *J*=7.1 Hz); ^{13}C NMR δ 148.0 (br), 135.8 (br), 127.5, 125.8, 124.4 (dd, J_{C-P}=77.0, 2.8 Hz), 32.1, 27.6 (J_{C-P}=66.5 Hz), 16.3, 15.5; MS *m*/*z* 418 (M^+, 89). Anal. Calcd for $C_{24}H_{36}O_2P_2$: C, 68.88; H, 8.67. Found: C, 68.53; H, 8.75.

4.2.4. 3,8-Bis(diethylphosphinyl)-1,2-dihydrocyclobuta[*b*]naphthalene (6ad). A colorless oil: IR 1458, 1155 cm^{-1}; ^1H NMR δ 9.02–8.98 (2H, m), 7.58–7.54 (2H, m), 3.51 (4H, s), 2.19–2.06 (8H, m), 1.21 (6H, t, *J*=7.6 Hz), 1.15 (6H, d, *J*=7.6 Hz); selected characteristic data for ^{13}C NMR δ 31.9 (dd, J_{C-P}=1.7, 1.7 Hz), 23.4

(J_{C-P}=68.7 Hz), 5.9 (J_{C-P}=5.0 Hz); MS *m/z* 362 (M^+, 100); HRMS calcd for $C_{20}H_{28}O_2P_2$ 362.1565, found 362.1567.

4.2.5. 3,8-Bis(diphenylphosphinyl)-1-methyl-1,2-dihydrocyclobuta[*b*]naphthalene (6ba). Colorless prisms: mp >300 °C (AcOEt–MeOH); IR 1437, 1173 cm^{-1}; ^1H NMR δ 8.59 (1H, d, *J*=7.6 Hz), 8.38 (1H, d, *J*=8.3 Hz), 7.81–7.20 (22H, m), 2.90–2.86 (1H, m), 2.46–2.38 (1H, m), 1.71–1.65 (1H, m), 0.75 (3H, d, *J*=6.9 Hz); selected characteristic data for ^{13}C NMR δ 155.6 (dd, J_{C-P}=13.5, 8.6 Hz), 148.9 (dd, J_{C-P}=13.4, 8.5 Hz), 39.4 (d, J_{C-P}=4.3 Hz), 38.3 (J_{C-P}=3.9 Hz), 20.3; MS *m/z* 568 (M^+, 100); HRMS calcd for $C_{37}H_{30}O_2P_2$ 568.1721, found 568.1725.

4.2.6. 1-(Benzyloxymethyl)-3,8-bis(diphenylphosphinyl)-1,2-dihydrocyclobuta[*b*]naphthalene (6ca). Pale yellow solid: mp 288–293 °C (AcOEt–MeOH); IR 1437, 1173 cm^{-1}; ^1H NMR δ 8.66 (1H, d, *J*=8.5 Hz), 8.22 (1H, d, *J*=8.5 Hz), 7.74–7.02 (27H, m), 4.13 (2H, s), 3.21–3.12 (3H, m), 2.29–2.15 (2H, m); selected characteristic data for ^{13}C NMR δ 151.6 (dd, J_{C-P}=7.2, 7.2 Hz), 149.6 (dd, J_{C-P}=9.3, 4.1 Hz), 72.6, 71.4, 44.4 (d, J_{C-P}=3.1 Hz), 34.7 (J_{C-P}=4.1 Hz); MS *m/z* 674 (M^+, 5.4); HRMS calcd for $C_{44}H_{36}O_3P_2$ 674.2140, found 674.2132. Anal. Calcd for $C_{44}H_{36}O_3P_2 \cdot 1/2H_2O$: C, 77.29; H, 5.45. Found: C, 77.64; H, 5.16.

4.2.7. 3,8-Bis(diphenylphosphinyl)-1,2-dimethyl-1,2-dihydrocyclobuta[*b*]naphthalene (6da): *trans*-6da. Colorless prisms: mp >300 °C (AcOEt–MeOH); IR 1437, 1175 cm^{-1}; ^1H NMR δ 8.46–8.42 (2H, m), 7.82–7.23 (22H, m), 2.39–2.37 (2H, m), 0.82 (6H, d, *J*=6.9 Hz); selected characteristic data for ^{13}C NMR δ 154.7 (dd, J_{C-P}=11.2, 11.2 Hz), 48.1 (dd, J_{C-P}=2.2, 2.2 Hz), 20.1; MS *m/z* 582 (M^+, 100); HRMS calcd for $C_{38}H_{32}O_2P_2$ 582.1878, found 582.1891.

cis-**6da**. Colorless prisms: mp >300 °C (AcOEt–MeOH); IR 1437, 1173 cm^{-1}; ^1H NMR δ 8.32–8.28 (2H, m), 7.85–7.17 (22H, m), 3.26–3.20 (2H, m), 0.71 (6H, d, *J*=6.6 Hz); selected characteristic data for ^{13}C NMR δ 154.8 (dd, J_{C-P}=11.0, 11.0 Hz), 48.2 (J_{C-P}=2.5 Hz), 20.0; MS *m/z* 582 (M^+, 100); HRMS calcd for $C_{38}H_{32}O_2P_2$ 582.1878, found 582.1875.

4.2.8. 3,8-Bis(diphenylphosphinyl)-1,2-diphenyl-1,2-dihydrocyclobuta[*b*]naphthalene (6ea): *trans*-6ea. Pale yellow solid: mp >300 °C (AcOEt–MeOH); IR 1437, 1171 cm^{-1}; ^1H NMR δ 8.68–8.66 (2H, m), 7.71–7.06 (28H, m), 6.50 (4H, d, *J*=6.9 Hz), 3.64 (2H, s); selected characteristic data for ^{13}C NMR δ 150.0 (dd, J_{C-P}=11.2, 11.2 Hz), 135.5 (dd, J_{C-P}=7.3, 7.3 Hz), 60.8 (dd, J_{C-P}=2.2, 2.2 Hz); MS *m/z* 706 (M^+, 72); HRMS calcd for $C_{48}H_{36}O_2P_2$ 706.2191, found 706.2191.

cis-**6ea**. Colorless solid: mp >300 °C (AcOEt–MeOH); IR 1437, 1171 cm^{-1}; ^1H NMR δ 8.61–8.58 (2H, m), 7.56–7.18 (22H, m), 6.71–6.61 (6H, m), 6.22–6.20 (4H, m), 4.39 (2H, s); selected characteristic data for ^{13}C NMR δ 150.1 (dd, J_{C-P}=11.2, 11.2 Hz), 138.0, 135.2 (dd, J_{C-P}=7.3, 7.3 Hz), 55.8; MS *m/z* 706 (M^+, 83); HRMS calcd for $C_{48}H_{36}O_2P_2$ 706.2191, found 706.2185.

4.2.9. 3,8-Bis(diphenylphosphinyl)-1,1-dimethyl-1,2-dihydrocyclobuta[*b*]naphthalene (6fa). Colorless prisms: mp >300 °C (AcOEt–MeOH); IR 1437, 1167 cm^{-1}; ^1H NMR δ 8.62–8.59 (1H, m), 7.73–7.47 (22H, m), 7.27–7.21 (1H, m), 7.09–7.03 (1H, m), 2.09 (2H, s), 1.49 (6H, s); selected characteristic data for ^{13}C NMR δ 47.3 (J_{C-P}=4.7 Hz), 45.8, 26.8; MS *m/z* 582 (M^+, 100); HRMS calcd for $C_{38}H_{32}O_2P_2$ 582.1878, found 582.1893. Anal. Calcd for $C_{38}H_{32}O_2P_2$: C, 78.34; H, 5.54. Found: C, 78.17; H, 5.57.

4.2.10. 1,4-Bis(diphenylphosphinyl)-2-(1-methylethenyl)-3-(1-methylethyl)naphthalene (10). A colorless oil: IR 1437, 1169 cm^{-1}; ^1H NMR δ 8.26 (1H, d, *J*=8.8 Hz), 8.00 (1H, d, *J*=8.8 Hz), 7.79–7.31 (20H, m), 7.02 (1H, t, *J*=7.4 Hz), 6.94 (1H, t, *J*=7.4 Hz), 4.94 (1H, s), 4.81 (1H, s), 3.57 (1H, sep, *J*=6.8 Hz), 1.90 (3H, s), 0.95 (3H, d, *J*=6.9 Hz), 0.67 (3H, d, *J*=6.9 Hz); selected characteristic data for ^{13}C NMR δ 38.3 (J_{C-P}=8.4 Hz), 29.1, 23.7, 20.4; MS *m/z* 610 (M^+, 45); HRMS calcd for $C_{40}H_{36}O_2P_2$ 610.2191, found 610.2192.

4.2.11. Dimethyl *trans*-6-(*tert*-butyldiphenylsiloxy)-methyl-5,8-bis(diphenylphosphinyl)-1,2,3,4-tetrahydronaphthalene-2,3-dicarboxylate (17b). A pale yellow oil: IR 1734, 1437, 1175 cm^{-1}; ^1H NMR δ 7.76–7.19 (31H, m), 4.22 (2H, s), 3.52–3.46 (1H, m), 3.49 (3H, s), 3.45 (3H, s), 3.23–3.06 (3H, m), 2.90–2.82 (2H, m), 0.70 (9H, s); selected characteristic data for ^{13}C NMR δ 174.1, 174.0, 64.7 (J_{C-P}=4.1 Hz), 51.9, 51.8, 40.9, 40.3, 31.0 (J_{C-P}=5.2 Hz), 29.8 (J_{C-P}=5.2 Hz), 26.7, 18.9; FABMS *m/z* 917 (M^++1, 40); FABHRMS calcd for $C_{55}H_{55}O_7P_2Si$ 917.3192, found 917.3164.

4.2.12. Dimethyl *trans*-4,9-bis(diphenylphosphinyl)-2,3,5,6,7,8-hexahydro-1*H*-cyclopenta[*b*]naphthalene-6,7-dicarboxylate (17c). A pale yellow oil: IR 1732, 1437, 1171 cm^{-1}; ^1H NMR δ 7.70–7.47 (20H, m), 3.42 (6H, s), 3.17–3.07 (4H, m), 2.81–2.79 (2H, m), 2.24–2.19 (2H, m), 1.48 (2H, quin, *J*=7.1 Hz); selected characteristic data for ^{13}C NMR δ 174.2, 51.8, 40.6, 34.2 (dd, J_{C-P}=1.7, 1.7 Hz), 30.5 (dd, J_{C-P}=2.8, 2.8 Hz), 25.8; MS *m/z* 688 (M^+, 100); HRMS calcd for $C_{41}H_{38}O_6P_2$ 688.2144, found 688.2140.

4.2.13. Dimethyl *trans*-9,10-bis(diphenylphosphinyl)-1,2,3,4,5,6,7,8-octahydroanthracene-2,3-dicarboxylate (17d). A pale yellow oil: IR 1732, 1437, 1165 cm^{-1}; ^1H NMR δ 7.70–7.47 (20H, m), 3.40 (6H, s), 2.99–2.97 (4H, m), 2.81–2.78 (2H, m), 2.44–2.41 (4H, m), 1.13–1.05 (4H, m); selected characteristic data for ^{13}C NMR δ 174.3, 51.8, 40.6, 30.2 (dd, J_{C-P}=2.8, 2.8 Hz), 28.9 (dd, J_{C-P}=2.8, 2.8 Hz), 19.6; MS *m/z* 702 (M^+, 100); HRMS calcd for $C_{42}H_{40}O_6P_2$ 702.2300, found 702.2304.

4.2.14. Dimethyl *trans*-4,9-bis(diphenylphosphinyl)-5,6,7,8-tetrahydronaphtho[2,3-*b*]furan-6,7-dicarboxylate (17e). A pale yellow oil: IR 1734, 1437, 1172 cm^{-1}; ^1H NMR δ 7.76–7.43 (20H, m), 6.94 (1H, d, *J*=2.3 Hz), 5.72 (1H, dd, *J*=2.3, 2.1 Hz), 4.00 (1H, dd, *J*=16.0, 6.4 Hz), 3.49 (3H, s), 3.45–3.32 (3H, m), 3.43 (3H, s), 3.00–2.91 (1H, m), 2.83–2.75 (1H, m); selected characteristic data for ^{13}C NMR δ 174.4, 174.2, 51.9, 40.5, 30.4 (J_{C-P}=5.6 Hz), 29.0 (J_{C-P}=5.0 Hz); MS *m/z* 688 (M^+, 100); HRMS calcd for $C_{40}H_{34}O_7P_2$ 688.1780, found 688.1776.

4.2.15. Dimethyl *trans*-6,11-bis(diphenylphosphinyl)-5-(methoxymethyl)-7,8,9,10-tetrahydro-5*H*-benzo[*b*]carbazole-8,9-dicarboxylate (17f). A pale yellow oil: IR 1734, 1437, 1172 cm^{-1}; ^1H NMR δ 8.38 (1H, d, J=7.9 Hz), 7.81–7.13 (22H, m), 6.92 (1H, t, J=6.3 Hz), 5.50 (1H, d, J=10.9 Hz), 5.23 (1H, d, J=10.9 Hz), 3.59–3.41 (1H, m), 3.59 (3H, s), 3.41 (3H, s), 2.95–2.56 (5H, m), 2.56 (3H, s); selected characteristic data for ^{13}C NMR δ 173.9, 79.3, 55.8, 52.1, 51.9, 40.3 (J_{C-P}=2.8 Hz), 31.9 (J_{C-P}=6.7 Hz), 30.1; FABMS m/z 782 (M^++1, 2.4); FABHRMS calcd for $C_{46}H_{42}NO_7P_2$ 782.2437, found 782.2440.

4.2.16. Dimethyl *trans*-9,10-bis(diphenylphosphinyl)-5-methoxy-1,2,3,4-tetrahydroanthracene-2,3-dicarboxylate (17g). A pale yellow oil: IR 1732, 1437, 1175 cm^{-1}; ^1H NMR δ 8.00–7.03 (22H, m), 6.33 (1H, d, J=7.7 Hz), 3.89–2.92 (6H, m), 3.47 (3H, s), 3.41 (3H, s), 2.99 (3H, s); selected characteristic data for ^{13}C NMR δ 174.2, 173.9, 53.3, 52.2, 52.0, 40.0, 39.9; MS m/z 728 (M^+, 16); HRMS calcd for $C_{43}H_{38}O_7P_2$ 728.2093, found 728.2089.

4.3. Dephosphinylation of *cis*-6ea with $LiAlH_4$

To a suspension of $LiAlH_4$ (30.4 mg, 0.800 mmol) in 1,4-dioxane (2 mL) was added *cis*-**6ea** (141 mg, 0.200 mmol), and the mixture was refluxed for 5 h. The mixture was cooled to room temperature and quenched by addition of water. Aqueous HCl 10% was added, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated. Chromatography of the residue with hexane afforded an inseparable mixture of the cis- and trans-isomers of **11**[3c,13] and the unknown **12** (38.0 mg, *cis*-**11**: *trans*-**11**=10:3) as colorless solid: ^1H NMR δ 7.88–6.96 (>16H, m), 5.37 (0.46H, s, for *cis*-**11**), 4.67 (1.54H, s, for *trans*-**11**), 4.05 (2.5H, s, for **12**); MS m/z 306 (M^+, 70); HRMS calcd for $C_{24}H_{18}$ 306.1409, found 306.1410.

4.4. Typical procedure for dephosphinylation with $LiAlH_4$–$TiCl_4$

To a suspension of $LiAlH_4$ (20.6 mg, 0.544 mmol) in THF (1 mL) were successively added $TiCl_4$ (0.03 mL, 0.3 mmol) and *cis*-**6ea** (48.1 mg, 6.80×10^{-2} mmol), and the mixture was stirred for 1 h at room temperature. The reaction was quenched by addition of water, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated. Chromatography of the residue with hexane–AcOEt (1:3) afforded *cis*-**13** (30.8 mg, 89%) as a colorless oil.

4.4.1. *cis*-3-(Diphenylphosphinyl)-1,2-diphenyl-1,2-dihydrocyclobuta[*b*]naphthalene (*cis*-13). A colorless oil: IR 1437, 1169 cm^{-1}; ^1H NMR δ 8.56 (1H, d, J=8.5 Hz), 7.91 (2H, s), 7.57–6.70 (20H, m), 6.29 (2H, d, J=7.3 Hz), 5.17 (1H, d, J=6.6 Hz), 4.58 (1H, d, J=6.6 Hz); selected characteristic data for ^{13}C NMR δ 151.2 (J_{C-P}=8.9 Hz), 143.7 (J_{C-P}=14.0 Hz), 60.9 (J_{C-P}=3.9 Hz), 58.2; MS m/z 506 (M^+, 2.5); HRMS calcd for $C_{36}H_{27}OP$ 506.1800, found 506.1802.

4.4.2. *trans*-3-(Diphenylphosphinyl)-1,2-diphenyl-1,2-dihydrocyclobuta[*b*]naphthalene (*trans*-13). A colorless oil: IR 1437, 1169 cm^{-1}; ^1H NMR δ 8.57 (1H, d, J=8.8 Hz), 7.89 (1H, d, J=8.3 Hz), 7.85 (1H, s), 7.55–7.04 (20H, m), 6.63 (2H, d, J=7.1 Hz), 4.34 (1H, d, J=2.4 Hz), 4.02 (1H, d, J=2.4 Hz); selected characteristic data for ^{13}C NMR δ 151.2 (J_{C-P}=9.3 Hz), 143.7 (J_{C-P}=13.4 Hz), 57.3 (J_{C-P}=4.1 Hz), 53.2; MS m/z 506 (M^+, 1.1); HRMS calcd for $C_{36}H_{27}OP$ 506.1800, found 506.1792.

4.4.3. *trans*-3-(Diphenylphosphinyl)-1,2-dimethyl-1,2-dihydrocyclobuta[*b*]naphthalene (*trans*-14). A colorless oil: IR 1437, 1167 cm^{-1}; ^1H NMR δ 8.38 (1H, d, J=8.4 Hz), 7.85–7.26 (14H, m), 3.02–3.00 (1H, m), 2.71–2.68 (1H, m), 1.39 (3H, d, J=6.9 Hz), 0.85 (3H, d, J=6.9 Hz); selected characteristic data for ^{13}C NMR δ 155.5 (J_{C-P}=8.9 Hz), 146.6 (J_{C-P}=14.0 Hz), 49.2 (J_{C-P}=3.9 Hz), 45.2, 19.3, 18.7; MS m/z 382 (M^+, 100); HRMS calcd for $C_{26}H_{23}OP$ 382.1487, found 382.1486.

4.4.4. *cis*-3-(Diphenylphosphinyl)-1,2-dimethyl-1,2-dihydrocyclobuta[*b*]naphthalene (*cis*-14). A colorless oil: IR 1437, 1169 cm^{-1}; ^1H NMR δ 8.35 (1H, d, J=8.6 Hz), 7.86–7.24 (14H, m), 3.66 (1H, quin, J=7.3 Hz), 3.26 (1H, quin, J=7.3 Hz), 1.23 (3H, d, J=7.3 Hz), 0.75 (3H, d, J=7.3 Hz); selected characteristic data for ^{13}C NMR δ 156.7 (J_{C-P}=8.3 Hz), 147.7 (J_{C-P}=14.5 Hz), 43.6 (J_{C-P}=7.2 Hz), 39.2, 14.5, 13.7; MS m/z 382 (M^+, 100); HRMS calcd for $C_{26}H_{23}OP$ 382.1487, found 382.1485.

4.5. Reaction of 24 with PhSCl in the presence of 5

To a solution of **24** (91.9 mg, 0.248 mmol) in THF (2.5 mL) were successively added **5** (71.5 mg, 0.497 mmol), Et_3N (0.21 mL, 1.5 mmol), and a solution of PhSCl (108 mg, 0.747 mmol) in THF (0.5 mL) at −78 °C. After being stirred for 2 h, the reaction mixture was allowed to warm to room temperature. After 13 h, the reaction was quenched by addition of water, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was passed through a short pad of silica gel with hexane–AcOEt (1:3) to afford the crude sulfoxides. To a solution of the crude sulfoxides in CH_2Cl_2 (2 mL) was added *m*CPBA (66.7 mg, 0.386 mmol) at 0 °C, and the reaction mixture was allowed to warm to room temperature. After 12 h, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ and aqueous $Na_2S_2O_3$, and the mixture was extracted with CH_2Cl_2. The extract was washed with water and brine, dried, and concentrated to dryness. Chromatography of the residue with hexane–AcOEt (1:2) afforded **26** (83.7 mg, 53%) and **25** (21.3 mg, 17%) as a colorless oils.

4.5.1. 3-(Diphenylphosphinyl)-8-(phenylsulfonyl)-1,2-dihydrocyclobuta[*b*]naphthalene (25). IR 1437, 1148 cm^{-1}; ^1H NMR δ 8.66–8.61 (2H, m), 7.96 (2H, d, J=6.9 Hz), 7.72–7.34 (15H, m), 3.50–3.46 (2H, m), 2.39–2.36 (2H, m); selected characteristic data for ^{13}C NMR δ 150.7 (J_{C-P}=9.5 Hz), 147.7 (J_{C-P}=14.5 Hz), 141.7, 31.1, 31.0; MS m/z 494 (M^+, 70); HRMS calcd for $C_{30}H_{23}O_3PS$ 494.1106, found 494.1101.

4.5.2. Dimethyl *trans*-9-(diphenylphosphinyl)-10-(phenylsulfonyl)-1,2,3,4-tetrahydroanthracene-2,3-dicarboxylate (26). IR 1732, 1437, 1175 cm^{-1}; ^1H NMR δ 8.94

(1H, d, J=8.7 Hz), 8.26 (1H, d, J=8.7 Hz), 7.94 (2H, d, J=6.9 Hz), 7.71–7.12 (15H, m), 4.05 (1H, dd, J=15.3, 6.3 Hz), 3.61 (3H, s), 3.56–3.34 (2H, m), 3.45 (3H, s), 3.14–3.07 (2H, m), 2.91–2.84 (1H, m); selected characteristic data for ^{13}C NMR δ 173.9, 173.7, 144.3 (J_{C-P}=7.8 Hz), 143.3, 140.0 (J_{C-P}=11.2 Hz), 52.2, 52.0, 39.9, 39.6, 30.4 (J_{C-P}=6.1 Hz), 27.9; MS m/z 638 (M^+, 46); HRMS calcd for $C_{36}H_{31}O_7PS$ 638.1528, found 638.1526.

4.6. Preparation of propargyl alcohols

4.6.1. 1-(4-Benzyloxy-3-hydroxy-1-butynyl)-2-(3-hydroxy-1-propynyl)benzene (4c). To a solution of 1-bromo-2-iodobenzene (1.06 g, 3.75 mmol) and 3-(tetrahydropyran-2-yl)-oxy-1-propyne (1.05 g, 7.50 mmol) in THF (15 mL) were successively added $Pd(PPh_3)_2Cl_2$ (52.6 mg, 7.50×10^{-2} mmol), CuI (71.4 mg, 0.375 mmol), and Et_3N (5.2 mL, 37 mmol) at room temperature. The mixture was stirred for 8 h, and the resulting precipitates were filtered off. The filtrate was concentrated to leave the residue, which was chromatographed with hexane–AcOEt (20:1) to afford 1-bromo-2-[3-(tetrahydropyran-2-yl)oxy-1-propynyl]benzene (988 mg, 89%) as a pale yellow oil: IR 3012, 2237 cm^{-1}; ^{1}H NMR δ 7.59–7.46 (2H, m), 7.28–7.13 (2H, m), 4.99 (1H, t, J=3.2 Hz), 4.55 (2H, s), 3.95–3.86 (1H, m), 3.62–3.54 (1H, m), 1.91–1.54 (6H, m); ^{13}C NMR δ 133.6, 132.4, 129.5, 126.9, 125.5, 96.7, 89.9, 84.3, 62.1, 54.6, 30.3, 25.4, 19.1; MS m/z 294 (M^+, 6.9); HRMS calcd for $C_{14}H_{15}O_2Br$ 294.0255, found 294.0252.

To a solution of the above bromobenzene (295 mg, 1.00 mmol) and (trimethylsilyl)acetylene (0.28 mL, 2.0 mmol) in Et_3N (5 mL) were successively added $Pd(PPh_3)_2Cl_2$ (35.0 mg, 5.00×10^{-2} mmol), CuI (19.0 mg, 0.100 mmol), and PPh_3 (26.3 mg, 0.100 mmol) at room temperature. The mixture was heated under reflux for 15 h, and the resulting precipitates were filtered off. The filtrate was concentrated to leave the residue, which was passed through a short pad of silica gel with hexane–AcOEt (20:1) to afford the crude diyne (311 mg) as a pale yellow oil. To a solution of the above diyne (311 mg) in MeOH (10 mL) was added K_2CO_3 (152 mg, 1.10 mmol) at room temperature. After 30 min, the reaction mixture was diluted with water and extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. Chromatography of the residue with hexane–AcOEt (20:1) gave 1-ethynyl-2-[3-(tetrahydropyran-2-yl)oxy-1-propynyl]benzene (192 mg, 80% for two steps) as a colorless oil: IR 3308, 2230 cm^{-1}; ^{1}H NMR δ 7.50–7.45 (2H, m), 7.30–7.25 (2H, m), 5.00 (1H, t, J=3.4 Hz), 4.55 (2H, s), 3.93–3.88 (1H, m), 3.59–3.55 (1H, m), 3.28 (1H, s), 1.87–1.55 (6H, m); ^{13}C NMR δ 132.5, 132.1, 128.4, 128.0, 125.7, 124.6, 96.5, 89.3, 84.1, 82.0, 80.9, 62.0, 54.6, 30.2, 25.4, 19.1; MS m/z 240 (M^+, 12); HRMS calcd for $C_{16}H_{16}O_2$ 240.1150, found 240.1153.

To a solution of the above ethynylbenzene (243 mg, 1.01 mmol) in THF (8 mL) was added EtMgBr in THF (0.50 M, 2.2 mL, 1.1 mmol) at 0 °C. After 10 min, benzyloxyacetaldehyde (0.16 mL, 1.1 mmol) was added, and the mixture was stirred for 2 h at room temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was passed through a short pad of silica gel with hexane–AcOEt (7:3) to afford the crude alcohol (193 mg, 49%) and to recover the ethynylbenzene (125 mg, 51%). To a solution of the above alcohol (193 mg) in MeOH (10 mL) was added $TsOH\cdot H_2O$ (9.5 mg, 5.0×10^{-2} mmol) at room temperature. After 30 min, the reaction mixture was diluted with water and extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. Chromatography of the residue with hexane–AcOEt (1:1) gave **4c** (125 mg, 82%) as a yellow oil: IR 3421, 2231 cm^{-1}; ^{1}H NMR δ 7.37–7.21 (9H, m), 4.82 (1H, br s), 4.62 (2H, s), 4.39 (2H, s), 3.85–3.62 (4H, m); ^{13}C NMR δ 137.4, 131.2, 130.9, 128.4, 128.1, 127.9, 125.7, 125.1, 92.2, 91.2, 84.3, 84.1, 73.5, 73.4, 62.2, 51.2; FABMS m/z 307 (M^++1, 3.2); FABHRMS calcd for $C_{20}H_{19}O_3$ 307.1334, found 307.1347.

4.6.2. 1,2-Bis(3-hydroxy-3-phenyl-1-propynyl)benzene (4e). To a solution of *o*-diiodobenzene (387 mg, 1.17 mmol) and 1-phenyl-2-propyn-1-ol (0.90 mL, 7.0 mmol) in THF (8.5 mL) were successively added $Pd(PPh_3)_2Cl_2$ (16.0 mg, 2.34×10^{-2} mmol), CuI (22.0 mg, 0.117 mmol), and $^iPr_2NH$ (1.3 mL, 12 mmol) at room temperature. The mixture was stirred for 16 h, and the resulting precipitates were filtered off. The filtrate was concentrated to leave the residue, which was chromatographed with hexane–AcOEt (8:1→1:3) to afford **4e** (380 mg, 96%) as yellow solid: IR 3587, 3384, 2199 cm^{-1}; ^{1}H NMR δ 7.54–7.22 (14H, m), 5.59 (2H, s), 3.58 (2H, br s); ^{13}C NMR δ 140.4, 140.3, 131.3, 131.2, 128.7, 128.6, 128.5, 128.5, 128.3, 128.2, 128.1, 126.9, 126.8, 126.6, 125.4, 125.3, 93.2, 85.2, 65.0, 64.8; FABMS m/z 339 (M^++1, 0.1); FABHRMS calcd for $C_{24}H_{19}O_2$ 339.1385, found 339.1368.

4.6.3. 1-(3-Hydroxy-3-methyl-1-propynyl)-2-(3-hydroxy-1-propynyl)benzene (4f). To a solution of 1-(3-hydroxy-1-propynyl)-2-iodobenzene[18] (50.0 mg, 0.194 mmol) and 2-methyl-3-butyn-2-ol (0.06 mL, 0.6 mmol) in THF (2 mL) were successively added $Pd(PPh_3)_2Cl_2$ (2.7 mg, 3.9×10^{-3} mmol), CuI (3.7 mg, 1.9×10^{-2} mmol), and Et_3N (0.3 mL, 2 mmol) at room temperature. The mixture was stirred for 3 d, and the resulting precipitates were filtered off. The filtrate was concentrated to leave the residue, which was chromatographed with hexane–AcOEt (3:1) to afford **4f** (35.3 mg, 85%) as yellow solid: IR 3597, 3383, 2230 cm^{-1}; ^{1}H NMR δ 7.39–7.20 (4H, m), 4.53 (2H, s), 3.91 (2H, br s), 1.63 (6H, s); ^{13}C NMR δ 131.1, 131.0, 127.9, 127.8, 125.5, 125.4, 98.2, 91.7, 84.2, 80.8, 65.7, 51.2, 31.2; MS m/z 214 (M^+, 25); HRMS calcd for $C_{14}H_{14}O_2$ 214.0994, found 214.0998.

4.6.4. 2,3-Bis(3-hydroxy-1-propynyl)anisole (15g). To a biphasic mixture of 3-methoxycatechol (700 mg, 5.00 mmol) in toluene (10 mL) and 30% aqueous K_3PO_4 (20 mL) was added Tf_2O (2.02 mL, 12.0 mmol) at 0 °C. After the mixture was stirred for 3 h at room temperature, the layer was separated. The toluene layer was washed with water and brine, dried, and concentrated to dryness. The residue was chromatographed with hexane–AcOEt (8:1) to afford 2,3-bis(trifluoromethanesulfonyloxy)anisole (2.05 g, quant.) as pale yellow solid: ^{1}H NMR δ 7.41–7.38 (1H, m), 7.10–7.04 (2H, m), 3.97 (3H, s).

To a solution of the above bis(triflate) (992 mg, 2.45 mmol) in Et_3N–DMF (24 mL, 1:5) were successively added tetrabutylammonium iodide (2.71 g, 7.35 mmol), (trimethylsilyl)-acetylene (1.38 mL, 9.80 mmol), $Pd(PPh_3)_2Cl_2$ (172 mg, 0.245 mmol) and CuI (140 mg, 0.735 mmol) at room temperature. After being stirred for 8 h at 70 °C, the mixture was cooled, diluted with saturated aqueous NH_4Cl, and extracted with Et_2O. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was passed through a short pad of silica gel with hexane–AcOEt (20:1) to afford the crude bis[(trimethylsilyl)acetylene] (720 mg) as a brown oil. To a solution of the above bis[(trimethylsilyl)acetylene] (720 mg) in MeOH (15 mL) was added K_2CO_3 (728 mg, 5.28 mmol) at room temperature. After 3 h, MeOH was evaporated off, and the residue was dissolved in water and extracted with Et_2O. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was passed through a short pad of silica gel with hexane–AcOEt (20:1) to afford the crude diyne (97.1 mg). To a solution of the above diyne (97.1 mg) in THF (5 mL) was added ^{n}BuLi in hexane (1.35 M, 1.01 mL, 1.36 mmol) at −40 °C. After 30 min, paraformaldehyde (188 mg, 12.8 mmol) was added to the reaction mixture, which was stirred for an additional 3 h at room temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was chromatographed with hexane–AcOEt (3:1) to afford **15g** (38.5 mg, 7% for three steps) as pale yellow solid: IR 3367, 3022 cm^{-1}; ^1H NMR δ 7.22 (1H, t, J=7.9 Hz), 7.03 (1H, d, J=7.9 Hz), 6.85 (1H, d, J=7.9 Hz), 4.60 (2H, s), 4.54 (2H, s), 3.89 (3H, s), 2.37 (2H, br s); ^{13}C NMR δ 129.1, 123.4, 110.7, 96.1, 91.8, 56.0, 52.1, 51.8; MS *m/z* 216 (M^+, 100); HRMS calcd for $C_{13}H_{12}O_3$ 216.0787, found 216.0790.

4.6.5. 1-[1-(Diphenylphosphinyl)-1,2-propadienyl]-2-(3-hydroxy-1-propynyl)benzene (24). To a suspension of NaH (60% in oil, 44.0 mg, 1.1 mmol) in THF (5 mL) was added a solution of **4a** (186 mg, 1.00 mmol) in THF (5 mL) at 0 °C. After 20 min, TBSCl (166 mg, 1.10 mmol) was added to the mixture, which was stirred for an additional 1 h at room temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was chromatographed with hexane–AcOEt (5:1) to afford 1-[3-(*tert*-butyldimethylsiloxy)-1-propynyl]-2-(3-hydroxy-1-propynyl)benzene (**23**) (213 mg, 71%) as a pale yellow oil: IR 3607, 3421, 2233, 2189 cm^{-1}; ^1H NMR δ 7.43–7.41 (2H, m), 7.27–7.24 (2H, m), 4.59 (2H, s), 4.52 (2H, br s), 2.37 (1H, br s), 0.94 (9H, s), 0.18 (6H, s); ^{13}C NMR δ 131.8, 131.7, 128.0, 127.9, 125.4, 125.1, 91.7, 91.5, 84.2, 83.4, 52.4, 51.5, 25.8, 14.1, −5.1; MS *m/z* 243 ($M^+-{}^t$Bu, 99); HRMS calcd for $C_{14}H_{15}O_2Si$ 243.0841, found 243.0846.

To a solution of the above propargyl alcohol **23** (30.0 mg, 0.100 mmol) and Et_3N (0.05 mL, 0.4 mmol) in THF (1 mL) was added Ph_2PCl (0.05 mL, 0.3 mmol) at −78 °C. After 45 min, the reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was passed through a short pad of silica gel with hexane–AcOEt (1:1) to afford the crude phosphinylallene (41.3 mg) as a yellow oil. To a solution of the above crude phosphinylallene (21.6 mg) in THF (1 mL) was added 10% aqueous HCl (0.1 mL) at 0 °C. The reaction mixture was stirred for 2 h at that temperature and then diluted with water. The mixture was extracted with Et_2O, and the extract was washed with water and brine, dried, and concentrated to dryness. The residue was chromatographed with hexane–AcOEt (1:3) to afford **24** (14.9 mg, 76% for two steps) as a yellow oil: IR 3306, 1958, 1927, 1439, 1173 cm^{-1}; ^1H NMR δ 7.83–7.13 (14H, m), 4.88 (2H, d, J_{P-H}=10.6 Hz), 4.50 (2H, s), 1.26 (1H, s); characteristic data for ^{13}C NMR δ 213.9 (J_{C-P}=5.6 Hz), 99.4 (J_{C-P}=100.6 Hz), 93.1, 84.4, 78.0 (J_{C-P}=12.3 Hz), 51.3; MS *m/z* 370 (M^+, 82); HRMS calcd for $C_{24}H_{19}O_2P$ 370.1123, found 370.1129.

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Suzuki–Miyaura coupling on the three upper rims of hexahomotrioxacalix[3]arenes

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Abstract—The efficient functionalization of three upper rims based on Suzuki–Miyaura coupling to temporarily lower rim-protected hexahomotrioxacalix[3]arenes was developed. After deprotection of the three protecting groups, the three upper rim-functionalized and lower rim-free hexahomotrioxacalix[3]arenes **5a**–**5m** were synthesized.

1. Introduction

Hexahomotrioxacalix[3]arene is a member of the calixarene family and has structural features of both a calixarene and an 18-crown-6 ether, having a cavity composed of a C_3-symmetric 18-membered ring (similar to 18-crown-6 ether), and also a three-dimensional cavity, which can adopt cone and/or partial-cone conformations (by analogy with calixarenes).[1] Hexahomotrioxacalix[3]arene skeleton having such features would be expected to be a useful platform for selective and specific function or host–guest recognition properties. However, functionalization of the upper or lower rim of hexahomotrioxacalix[3]arenes, especially the transformation of functional groups on the upper rim in the presence of a phenolic hydroxy group on the lower rim, is quite difficult due to structural weakness attributed to the combination of three dibenzyletheral linkages and phenolic hydroxyl groups (Fig. 1). To overcome these synthetic difficulties, we developed a stepwise construction of hexahomotrioxacalix[3]-arenes based on cyclization of the corresponding linear trimers[2] as well as several functional group transformations at one of the three upper rim aryl groups of hexahomotrioxacalix[3]arenes using the so-called 'Mannich route' in calixarene chemistry.[3] However, the Mannich route is not suitable for transformation of the three upper rims in one operation because the yield of each reaction is around 50%. In this paper, we report functionalization of the three upper rims based on Suzuki–Miyaura coupling[4] to temporarily lower rim-protected hexahomotrioxacalix[3]arenes.

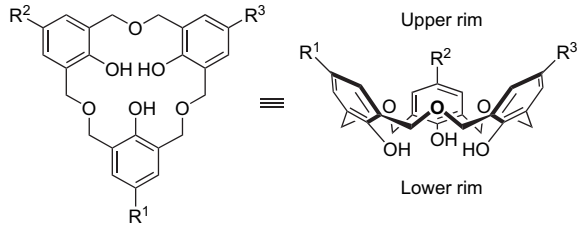
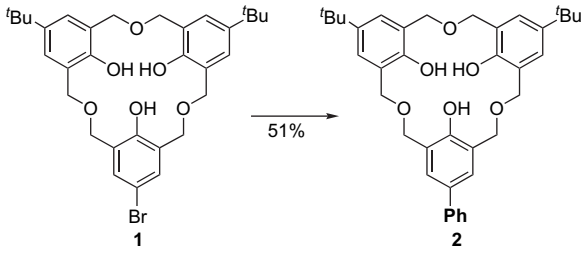


Figure 1. Hexahomotrioxacalix[3]arenes.

2. Results and discussion

Since there have been a few reports on the Suzuki–Miyaura coupling of the three upper rims of lower rim-alkylated hexahomotrioxacalix[3]arenes,[5] we applied this coupling to the mono-brominated hexahomotrioxacalix[3]arene **1**.[2a] First, we tried the modified Suzuki–Miyaura coupling reported by Buchwald et al.[6] on one of the three upper rims of **1** (Scheme 1).



Scheme 1. Conditions: (2-biphenyl) di-*tert*-butylphosphine, $Pd(OAc)_2$, and KF.

Hexahomotrioxacalix[3]arene with one bromine on the upper rim **1** was reacted with 1.5 equiv of boric acid in the presence of (2-biphenyl) di-*tert*-butylphosphine (10 mol %),

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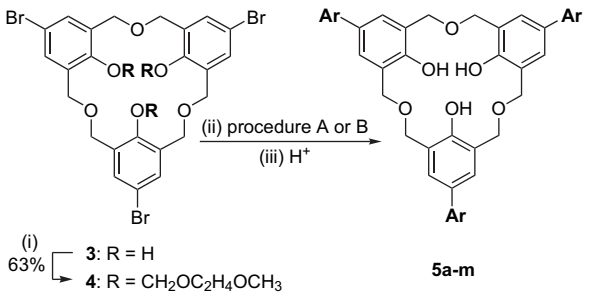
palladium acetate (5 mol %), and potassium fluoride (3 equiv) in THF at 50 °C to give **2** in 51% yield. However, our attempt to introduce three phenyl groups to the three upper rims on **3**[7] was not successful due to the formation of inseparable and complicated product mixtures. Therefore, we changed our synthetic strategy, so that active hydroxy groups at the lower rim were temporarily protected by methoxyethoxymethyl (MEM) groups (Scheme 2). Tribromohexahomotrioxacalix[3]arene **3** was treated with 10 equiv of MEMCl and sodium hydride in THF at room temperature to give the desired **4** in 63% yield. Shinkai and co-workers reported that ring-inversion of hexahomotrioxacalix[3]arenes took place through oxygen-through-the-annulus rotation, and cone/partial-cone ring-inversion was inhibited by introducing butyl and bulkier groups to the lower rim.[8] Based on the results of ^{1}H NMR, MEM-protected **4** is fixed in a partial-cone conformation.



Scheme 2. Conditions: (i) MEMCl and NaH; (ii) procedure A: $ArB(OH)_2$, $Pd(OAc)_2$, (2-biphenyl) di-*tert*-butylphosphine; procedure B: $ArB(OH)_2$, $Pd(PPh_3)_4$; (iii) 0.2 N HCl.

Suzuki–Miyaura coupling of the three upper rims of lower rim-protected **4** was investigated. Among the several procedures reported on modified Suzuki–Miyaura coupling,[5] two procedures (procedures A and B; see Section 3 and Table 1) were suitable for introducing aromatic rings to the three upper rims of MEM-protected **4**. Procedure A was suitable for *para*-substituted boric acids (entries 1–4), and procedure B was suitable for boric acids possessing a strong electron-withdrawing group and/or *ortho*-substitution (entries 5–13). After a coupling reaction through procedure A or B, deprotection of the three MEM groups was successively performed for concise purification of the lower rim-free hexahomotrioxacalix[3]arenes **5**, and the yields in Table 1 were calculated in two steps based on isolated **5**. Since procedure A had milder reaction conditions, the combined yields (74–90%) were generally higher than those using procedure B (36–78%).

Table 1. Suzuki–Miyaura coupling on the three upper rims of hexahomotrioxacalix[3]arenes **4**

Entry	Ar	Product	Procedure[a]	Yield (%)[b]
1	Ph	**5a**[9]	A	77
2	*p*-Me–C_6H_4	**5b**	A	90
3	*p*-MeO–C_6H_4	**5c**	A	89
4	*p*-F–C_6H_4	**5d**	A	74
5	*o*-NC–C_6H_4	**5e**	B	36
6	*p*-CHO–C_6H_4	**5f**	B	59
7	*p*-O_2N–C_6H_4	**5g**	B	62
8	*o*-MeO–C_6H_4	**5h**	B	78
9[c]	*o*-HO–C_6H_4	**5i**	B	67
10	*o*-EtO_2C–C_6H_4	**5j**	B	63
11[c]	*o*-MeCONH–C_6H_4	**5k**	B	43
12	3-Pyridinyl	**5l**	B	50
13	4-Pyridinyl	**5m**	B	60

[a] Procedure A: $ArB(OH)_2$ (10 equiv), $Pd(OAc)_2$ (10 mol %), (2-biphenyl) di-*tert*-butylphosphine (20 mol %), rt, 2 days. Procedure B: $ArB(OH)_2$ (10 equiv), $Pd(PPh_3)_4$ (20 mol %), 80 °C, 6–19 h.
[b] Isolated yield.
[c] Corresponding pinacol ester of boric acid was used.

In summary, efficient functionalization of the three upper rims of (lower rim-free) hexahomotrioxacalix[3]arenes based on Suzuki–Miyaura coupling was developed. We believe that products **5a**–**5m**, which have deep cavities and binding sites on peripheral aromatic rings, will contribute to the field of host–guest chemistry. In particular, further studies on products **5e** and **5j** with regard to fluorescence and complexation with various guest molecules are now in progress.

3. Experimental

3.1. General

Nuclear magnetic resonance (NMR) spectra were taken at 200 or 300 MHz in $CDCl_3$ with chemical shifts being reported as δ ppm from tetramethylsilane as an internal standard and couplings are expressed in hertz. Preparative TLC was carried out with silica gel 60 F_{254} plates (Merck).

3.2. Phenylhexahomotrioxacalix[3]arene 2

A mixture of **1**[2a] (50.0 mg, 0.083 mmol), phenylboric acid (15.3 mg, 0.125 mmol), 2-(di-*tert*-butylphosphino)biphenyl (3.0 mg, 0.008 mmol), and KF (14.6 mg, 0.25 mmol) was heated with a heat gun under vacuum and flushed with argon. A solution of palladium acetate (1.0 mg, 0.004 mmol) in dry THF (1 ml) was added to the mixture and stirred overnight at 50 °C. To the reaction mixture, 0.1 N aqueous hydrochloric acid and EtOAc were added. The organic layer was separated, and washed successively with water, aqueous sodium carbonate solution, and brine. After being dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was purified by PTLC to furnish pure **2** (25.4 mg) in 51% yield. Mp=118–120 °C; IR (KBr) 3348, 2957, 1611, 1486, 1361 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (s, 18H), 4.74 (s, 4H), 4.75 (s, 4H), 4.76 (s, 4H), 7.14 (s, 4H), 7.20–7.50 (m, 5H), 7.35 (s, 2H), 8.58 (s, 2H), 8.87 (s, 1H); HRMS (EI$^+$) calcd for $C_{38}H_{44}O_6$ (M^+): 596.3138. Found: 596.3145. Anal. Calcd for $C_{38}H_{44}O_6 \cdot 0.5H_2O$: C, 75.34; H, 7.49. Found: C, 75.03; H, 7.37.

3.3. MEM-protected hexahomotrioxacalix[3]arene 4

To a solution of **3**[7] (33.9 mg, 0.053 mmol) in THF (3 ml) were added sodium hydride (28.3 mg, 0.71 mmol, 60% oil dispersion in mineral oil) and 2-methoxyethoxymethyl chloride (60 µl, 0.53 mmol), and the mixture was stirred for 5 h at room temperature. Aqueous ammonium chloride solution and EtOAc were added to the reaction mixture. The organic layer was separated, and washed successively with water, sodium hydrogen carbonate solution, and brine. After being dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was purified by PTLC (hexane/EtOAc=2/3) to give **4** as a colorless oil (29.9 mg, 63%

yield). IR (film) 2876, 1579, 1454, 1361, 1200 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.17–3.20 (m, 2H), 3.32–3.36 (m, 2H), 3.36 (s, 9H), 3.46–3.51 (m, 4H), 3.67–3.72 (m, 4H), 4.17 (s, 2H), 4.19 (d, J=10.3 Hz, 2H), 4.20 (d, J=12.8 Hz, 2H), 4.32 (d, J=11.2 Hz, 2H), 4.39 (d, J=11.4 Hz, 2H), 4.62 (d, J=11.2 Hz, 2H), 4.68 (d, J=11.2 Hz, 2H), 4.85 (s, 4H), 7.37 (d, J=2.4 Hz, 2H), 7.41 (d, J=2.4 Hz, 2H), 7.47 (s, 2H); HRMS (FAB^+) calcd for $C_{36}H_{45}O_{12}{}^{79}Br_3Na$ ($M+Na^+$): 929.0359. Found: 929.0380, calcd for $C_{36}H_{45}O_{12}{}^{79}Br_2{}^{81}BrNa$ ($M+Na^+$): 931.1034. Found: 931.0357, calcd for $C_{36}H_{45}O_{12}{}^{79}Br^{81}Br_2Na$ ($M+Na^+$): 933.0318. Found: 933.0338, calcd for $C_{36}H_{45}O_{12}{}^{81}Br_3Na$ ($M+Na^+$): 935.0298. Found: 935.0320. Anal. Calcd for $C_{36}H_{45}O_{12}Br_3\cdot H_2O$: C, 46.62; H, 5.11. Found: C, 46.37; H, 4.83.

3.4. Procedure A for the synthesis of compounds 5a–5d

The synthesis of **5a** is typical. A solution of **4** (35.3 mg, 0.039 mmol), phenylboric acid (47.3 mg, 0.388 mmol), 2-(di-*tert*-butylphosphino)biphenyl (1.7 mg, 0.008 mmol), palladium acetate (0.9 mg, 0.004 mmol) in degassed toluene (2 ml), and 2 M aqueous sodium carbonate (0.23 ml) was stirred for 2 days at room temperature under an Ar atmosphere. Water and EtOAc were added to the reaction mixture. The organic layer was separated, and washed successively with 0.1 N aqueous hydrochloric acid, water (twice), and brine. After being dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was dissolved in EtOAc (10 ml) and 4 N hydrogen chloride in EtOAc (0.5 ml) was added to the solution and stirred overnight. The reaction mixture was poured into a mixture of ethyl acetate and water. The organic layer was separated, washed successively with water (twice) and brine, dried over Na_2SO_4, and evaporated in vacuo to give a pale yellow viscous oil. The residue was purified by PTLC ($CHCl_3$/hexane=3/1) to furnish pure **5a** (19.0 mg) in 77% yield.

3.4.1. Hexahomotrioxacalix[3]arene 5a. Known.[9]

3.4.2. Hexahomotrioxacalix[3]arene 5b. Mp=229–231 °C; IR (KBr) 3361, 1611, 1479, 1361, 1193 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.37 (s, 9H), 4.81 (s, 12H), 7.20 (d, J=8.2 Hz, 6H), 7.35 (s, 6H), 7.39 (d, J=8.2 Hz, 6H), 8.83 (s, 3H); HRMS (FAB^+) calcd for $C_{45}H_{42}O_6$ (M^+): 678.2981. Found: 678.2989. Anal. Calcd for $C_{45}H_{42}O_6\cdot 0.5H_2O$: C, 78.58; H, 6.30. Found: C, 78.37; H, 6.24.

3.4.3. Hexahomotrioxacalix[3]arene 5c. Mp=134–136 °C; IR (KBr) 3347, 1609, 1519, 1360, 1181 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.83 (s, 9H), 4.80 (s, 12H), 6.93 (d, J=8.8 Hz, 6H), 7.32 (s, 6H), 7.42 (d, J=8.4 Hz, 6H), 8.81 (s, 3H); HRMS (FAB^+) calcd for $C_{45}H_{42}O_9$ (M^+): 726.2829. Found: 726.2834. Anal. Calcd for $C_{45}H_{42}O_6\cdot 0.5H_2O$: C, 73.45; H, 5.89. Found: C, 73.09; H, 5.84.

3.4.4. Hexahomotrioxacalix[3]arene 5d. Mp=137–139 °C; IR (KBr) 3342, 1605, 1481, 1361, 1223 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.81 (s, 12H), 7.03–7.12 (m, 6H), 7.31 (s, 6H), 7.39–7.47 (m, 6H), 8.84 (s, 3H); HRMS (FAB^+) calcd for $C_{42}H_{33}F_3O_6$ (M^+): 690.2229. Found: 690.2219. Anal. Calcd for $C_{42}H_{33}F_3O_6\cdot H_2O$: C, 71.18; H, 4.98. Found: C, 71.37; H, 4.82.

3.5. Procedure B for the synthesis of compounds 5e–5m

The synthesis of **5e** is typical. A solution of **4** (57.6 mg, 0.063 mmol), 2-cyanophenylboric acid (93.1 mg, 0.63 mmol), $Pd(PPh_3)_4$ (14.6 mg, 0.013 mmol), and 2 M aqueous sodium carbonate (0.38 ml) in toluene (2 ml) and methanol (2 ml) was stirred for 17 h at 80 °C under an Ar atmosphere. Water and EtOAc were added to the reaction mixture. The organic layer was separated, and washed successively with 0.1 N aqueous hydrochloric acid, water (twice), and brine. After being dried over sodium sulfate, the solvent was evaporated in vacuo. The residue was dissolved in EtOAc (10 ml) and 4 N hydrogen chloride in EtOAc (0.5 ml) was added to the solution and stirred overnight. The reaction mixture was poured into a mixture of ethyl acetate and water. The organic layer was separated, washed successively with water (twice) and brine, dried over Na_2SO_4, and evaporated in vacuo to give a pale yellow viscous oil. The residue was purified by PTLC ($CHCl_3$/EtOAc=20/1) to furnish pure **5e** (16.3 mg).

3.5.1. Hexahomotrioxacalix[3]arene 5e. Mp=145–148 °C; IR (KBr) 3340, 2223, 1612, 1473, 1362 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.83 (s, 12H), 7.36 (s, 6H), 7.36–7.45 (m, 6H), 7.56–7.63 (m, 3H), 7.72 (d, J=7.6 Hz, 3H), 9.01 (s, 3H); HRMS (FAB^+) calcd for $C_{45}H_{34}N_3O_6$ ($M+H^+$): 712.2447. Found: 712.2444. Anal. Calcd for $C_{45}H_{33}N_3O_6\cdot 0.5CHCl_3$: C, 70.84; H, 4.38; N, 5.45. Found: C, 70.84; H, 4.69; N, 5.11.

3.5.2. Hexahomotrioxacalix[3]arene 5f. Mp=157–159 °C; IR (KBr) 3340, 1696, 1602, 1480, 1388 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.85 (s, 12H), 7.45 (s, 6H), 7.87 (d, J=8.0 Hz, 6H), 7.92 (d, J=8.0 Hz, 6H), 8.95 (s, 3H), 10.03 (s, 3H); HRMS (FAB^+) calcd for $C_{45}H_{36}O_9$ (M^+): 720.2359. Found: 720.2347. Anal. Calcd for $C_{45}H_{36}O_9\cdot 1/3CHCl_3$: C, 71.59; H, 4.82. Found: C, 71.91; H, 5.04.

3.5.3. Hexahomotrioxacalix[3]arene 5g. Mp=236–238 °C; IR (KBr) 3340, 1595, 1514, 1476, 1343 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.86 (s, 12H), 7.44 (s, 6H), 7.65 (d, J=8.6 Hz, 6H), 8.27 (d, J=8.6 Hz, 6H), 8.97 (s, 3H); HRMS (FAB^+) calcd for $C_{42}H_{33}N_3O_{12}Na$ ($M+Na^+$): 794.1962. Found: 794.1944. Anal. Calcd for $C_{42}H_{33}N_3O_{12}\cdot CHCl_3$: C, 57.96; H, 3.85; N, 4.72. Found: C, 57.75; H, 3.89; N, 4.54.

3.5.4. Hexahomotrioxacalix[3]arene 5h. Mp=115–117 °C; IR (KBr) 3351, 1601, 1478, 1360, 1243 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.77 (s, 9H), 4.79 (s, 12H), 6.92–7.01 (m, 6H), 7.20–7.31 (m, 6H), 7.30 (s, 6H), 8.90 (s, 3H); HRMS (FAB^+) calcd for $C_{45}H_{42}O_9$ (M^+): 726.2829. Found: 726.2818. Anal. Calcd for $C_{45}H_{42}O_9\cdot 1.5H_2O$: C, 71.70; H, 6.02. Found: C, 72.09; H, 5.74.

3.5.5. Hexahomotrioxacalix[3]arene 5i. Mp=138–141 °C; IR (KBr) 3363, 1608, 1480, 1361, 1204 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.78 (s, 12H), 5.13 (s, 3H), 6.91–6.98 (m, 6H), 7.12–7.25 (m, 6H), 7.25 (s, 6H), 8.92 (s, 3H); HRMS (FAB^+) calcd for $C_{42}H_{36}O_9$ (M^+): 684.2360.

Found: 684.2371. Anal. Calcd for $C_{42}H_{36}O_9 \cdot 0.5CHCl_3$: C, 68.57; H, 4.94. Found: C, 68.86; H, 5.27.

3.5.6. Hexahomotrioxacalix[3]arene 5j. Mp=103–106 °C; IR (KBr) 3343, 1728, 1612, 1473, 1364 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.07 (t, J=7.0 Hz, 9H), 4.11 (q, J=7.0 Hz, 6H), 4.76 (s, 12H), 7.11 (s, 6H), 7.26–7.48 (m, 9H), 7.78 (d, J=7.4 Hz, 3H), 8.86 (s, 3H); HRMS (FAB^+) calcd for $C_{51}H_{48}O_{12}$ (M^+): 852.3146. Found: 852.3158. Anal. Calcd for $C_{51}H_{48}O_{12} \cdot 0.5H_2O$: C, 71.07; H, 5.73. Found: C, 71.15; H, 5.72.

3.5.7. Hexahomotrioxacalix[3]arene 5k. Mp=154–156 °C; IR (KBr) 3345, 1665, 1580, 1521, 1447 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.99 (s, 9H), 4.81 (s, 12H), 7.00 (s, 3H), 7.17 (br s, 12H), 7.30–7.40 (m, 3H), 8.24 (d, J=7.6 Hz, 3H), 8.96 (s, 3H); HRMS (FAB^+) calcd for $C_{48}H_{46}N_3O_9$ ($M+H^+$): 808.3234. Found: 808.3223. Anal. Calcd for $C_{48}H_{45}N_3O_9 \cdot 1/3CHCl_3$: C, 68.48; H, 5.39; N, 4.96. Found: C, 68.84; H, 5.57; N, 5.04.

3.5.8. Hexahomotrioxacalix[3]arene 5l. Mp=135–137 °C; IR (KBr) 3348, 2854, 1612, 1471, 1322 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.85 (s, 12H), 7.29–7.33 (m, 3H), 7.34 (s, 6H), 7.78 (d, J=7.6 Hz, 3H), 8.55 (d, J=3.8 Hz, 3H), 8.76 (s, 3H), 8.91 (s, 3H); HRMS (FAB^+) calcd for $C_{39}H_{34}N_3O_6$ ($M+H^+$): 640.2448. Found: 640.2450. Anal. Calcd for $C_{39}H_{33}N_3O_6 \cdot 0.5CHCl_3$: C, 67.83; H, 4.83; N, 6.01. Found: C, 68.12; H, 5.22; N, 5.86.

3.5.9. Hexahomotrioxacalix[3]arene 5m. Mp>300 °C; IR (KBr) 3332, 1600, 1548, 1477, 1317 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.85 (s, 12H), 7.43 (d, J=6.2 Hz, 6H), 7.46 (s, 6H), 8.61 (d, J=6.2 Hz, 6H), 8.97 (s, 3H); HRMS (FAB^+) calcd for $C_{39}H_{34}N_3O_6$ ($M+H^+$): 640.2448. Found: 640.2451. Anal. Calcd for $C_{39}H_{33}N_3O_6 \cdot 1.5H_2O$: C, 70.26; H, 5.44; N, 6.30. Found: C, 70.27; H, 5.32; N, 6.22.

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Supplementary data

The ^1H NMR spectra of **2**, **4**, and **5a**–**5m**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.08.073.

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Synthesis of aza-analogues of *Ganciclovir*

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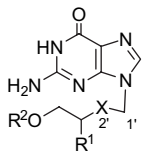
Abstract—Aza-analogues of *ganciclovir* have been prepared via coupling of nucleobases with *N*-[2-pivaloyloxy-1-(pivaloyloxymethyl)-ethyl]methanesulfonamide or 3-mesyl-4-(benzoyloxymethyl)-1,3-oxazolidine.

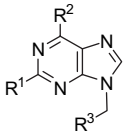
1. Introduction

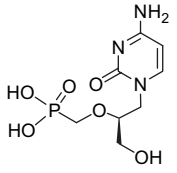
The synthesis of acyclic nucleosides[1,2] has attracted considerable attention due to their antiviral[3] or anticancer activity.[4] Some of them (Fig. 1) have been approved for the clinical treatment of herpes virus infections (e.g. *acyclovir*, *ganciclovir*, *penciclovir*, *cidofovir*, *famciclovir*) or hepatitis B virus infections (e.g. *adefovir*).[3] In order to improve their efficacy, the parent antiviral drugs are also used in the form of prodrugs, usually esters (e.g. *valacyclovir* or *valganciclovir*, Fig. 1).[5] The synthesis of new nucleoside analogues bearing molecular modifications on the sugar mimic is one of the approaches towards promising antiviral agents.[1] Some of these modifications involve replacement of the oxygen or carbon atom at the 2′-position with sulfur or nitrogen.[1a,6] Acyclic analogues with a nitrogen atom at the 2′-position (termed acyclic azanucleosides) are much less known than the corresponding oxa or carbo derivatives.[7]

Most of them are amino acid or peptide derivatives of 5-fluorouracil. They are obtained by the condensation of *N*-(α-acetyloxyalkyl)amides (which, are derivatives of amino acids or peptides) with the nucleobase or its silylated derivative in the presence of triethylamine (or sodium hydride)[6e,f,k] or tin(IV) chloride.[6d] The acyclic aza-analogues have also been synthesized by the coupling of silylated nucleobases, natural or synthetic, with *N*-(acetoxymethyl)amides,[6m] *N*-(chloromethyl)sulfonamides[6g] or *N*-(chloromethyl)amides.[6i] Other methods, such as Curtius rearrangement, have also been reported.[6a] Acyclic azanucleosides containing a nitrogen atom at the 3′-position are also known; they disclose biological activity.[8]

Recently we have shown that acyclic azanucleosides can be readily obtained by the coupling of *N*-(pivaloyloxymethyl)-amides or sulfonamides with silylated nucleobases in the presence of Lewis acids. Using this approach we have synthesized the aza-analogues of *ganciclovir*[9] and *acyclovir*[10] protected with mesyl or tosyl group, respectively, at a nitrogen atom of the sugar mimic.[11] Herein we describe two methods for the first synthesis of the aza-analogues of *ganciclovir*: (i) from *N*-[2-pivaloyloxy-1-







	X	R^1	R^2		R^1	R^2	R^3	
Acyclovir	O	H	H	*Adefovir*	H	NH_2	$(HO)_2P(O)CH_2OCH_2$	*Cidofovir*
Valacyclovir	O	H	L-Val					
Ganciclovir	O	CH_2OH	H	*Famciclovir*	NH_2	H	$(AcOCH_2)_2CHCH_2$	
Valganciclovir	O	CH_2OH	L-Val					
Penciclovir	CH_2	CH_2OH	H					

Figure 1.

Keywords: *N*-Pivaloyloxymethyl sulfonamides; *N*-Mesyl-5-hydroxymethyl-1,3-oxazolidine; Acyclic azanucleosides; *Ganciclovir analogues*.
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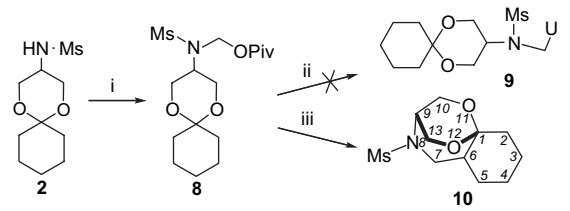
(pivaloyloxymethyl)ethyl]-methanesulfonamide or (ii) from 3-mesyl-4-(benzoyloxymethyl)-1,3-oxazolidine. These both substrates are easily accessible from 3-nitro-1,5-dioxaspiro[5.5]undecane **1**.[12] Antiviral activities of aza-analogues of *ganciclovir* are also reported.

2. Results and discussion

Nitrodioxane **1** was converted into the serinol derivative **4** in four steps (Scheme 1): (i) palladium catalyzed hydrogenation, (ii) reaction of the resulting amine with methanesulfonyl chloride (MsCl) in the presence of pyridine, (iii) acidic hydrolysis of **2**, (iv) O-pivaloylation of **3** with pivaloyl chloride in the presence of pyridine. The alkylation of **4** with chloromethyl pivaloate in the presence of sodium hydride in dry DMF gave *N*-(pivaloyloxymethyl)sulfonamide **5** required for the nucleoside coupling.

A one-pot base silylation/nucleoside coupling procedure was employed for the syntheses of protected azanucleosides **6C^{Bz}** and **6A^{Cbz}** (Scheme 1). Compound **5** was coupled with silylated N^4-Bz-cytosine (**C^{Bz}**) or N^6-Cbz-adenine (**A^{Cbz}**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) or tin(IV) chloride, respectively.[13] Thus, these nucleobases were silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in acetonitrile and subsequently **5** and a catalyst (Lewis acid) was added to afford **6C^{Bz}** or **6A^{Cbz}** in 72% or 44% yield, respectively. Heating of **6C^{Bz}** with ammonium hydroxide in methanol furnished deprotected azanucleoside **7C** in 83% yield. Deprotection of **6A^{Cbz}** was completed in two steps: (i) Cbz protecting group was removed by palladium catalyzed hydrogenolysis and (ii) hydroxy groups were deprotected by heating of the corresponding *O*-pivaloyl derivative with ammonium hydroxide to afford **7A** in 73% overall yield.

We also tried to obtain the uracil azanucleoside **9** from *N*-(pivaloyloxymethyl)-*N*-dioxanesulfonamide **8** (resulting from alkylation of **2** with chloromethyl pivaloate) (Scheme 2). However, attempts to couple **8** with silylated uracil (**U**) in the presence of TMSOTf failed. Instead of the expected derivative **9** the tricyclic derivative **10** was isolated in 39% yield from the complex reaction mixture.



Scheme 2. Reagents and conditions: (i) NaH, DMF, $ClCH_2OPiv$, rt, 72 h, 72%; (ii) uracil (**U**), BSA, TMSOTf, MeCN, rt, 24 h and (iii) Lewis acid (TMSOTf or $AlCl_3$ or $BF_3{\cdot}Et_2O$), MeCN, rt, 24 h, 39%.

A plausible pathway for formation of **10** from **8** in the presence of TMSOTf is shown in Scheme 3. According to the literature data,[14] we assume that **8** reacts with TMSOTf to give the intermediate enol ether **A**.

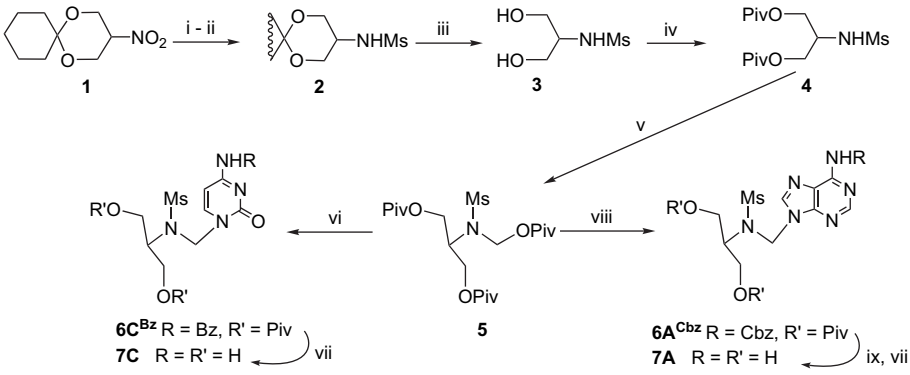
N-(Pivaloyloxymethyl)sulfonamide group is then converted into iminium cation **B** (Scheme 3), which undergoes intramolecular Mannich type reaction with the enol ether moiety. The process is completed with closure of the acetal ring to yield tricyclic derivative **10**.

The compound **10** was also obtained in the same yield in the presence of aluminum(III) chloride or boron trifluoride etherate.

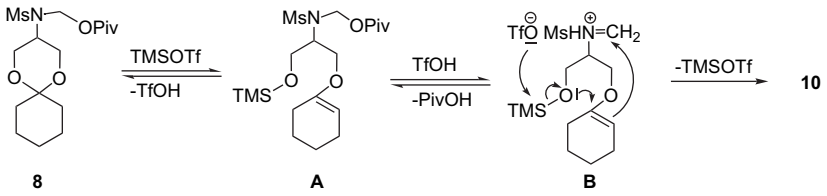
Unexpectedly, *N*-(pivaloyloxymethyl)-*N*-dioxanesulfonamide **8** underwent transformation into 3-mesyl-4-(hydroxymethyl)-1,3-oxazolidine **11** in 90% yield in the presence of a Brønsted acid [*p*-toluenesulfonic acid (PTSA) or Dowex-50(H^+)] in methanol (Scheme 4). Compound **11** was fully analyzed as *O*-benzoyl derivative **12** (Scheme 5).

Considering the literature data concerning the hydrolysis of *N*-(pivaloyloxymethyl)sulfonamides,[15] we assume that protonation of the carbonyl oxygen of the pivaloyl group with a Brønsted acid takes place. Then, loss of pivalic acid gives iminium cation **C**, which undergoes intramolecular rearrangement to give 1,3-oxazolidine intermediate **D**. The methanolysis of **D** gives **11**.

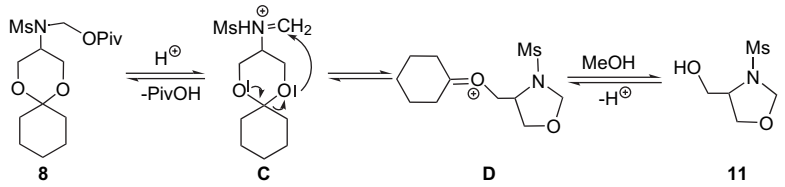
Some acyclic azanucleosides were prepared from 3-tosyloxazolidine-5-ones,[61] obtained from *N*-tosyl derivatives of



Scheme 1. Reagents and conditions: (i) H_2, 10% Pd/C, EtOH, rt, 60 bar, 24 h, quantitative; (ii) $MeSO_2Cl$ (MsCl), pyridine, CH_2Cl_2, rt, 67%; (iii) MeOH, Dowex-50 (H^+), rt, 48 h, 67%; (iv) PivCl, pyridine, rt, 24 h, 42%; (v) NaH, DMF, $ClCH_2OPiv$, rt, 72 h, 72%; (vi) N^4-Bz-cytosine (**C^{Bz}**), BSA, TMSOTf, MeCN rt, 24 h, 71%; (vii) NH_4OH_{concd}, MeOH, sealed tube, 70 °C, 1 d; (viii) N^6-Cbz-adenine (**A^{Cbz}**), BSA, $SnCl_4$, MeCN, 24 h, 44% and (ix) H_2 (balloon), 10% Pd/C, MeOH, rt, 1 d.



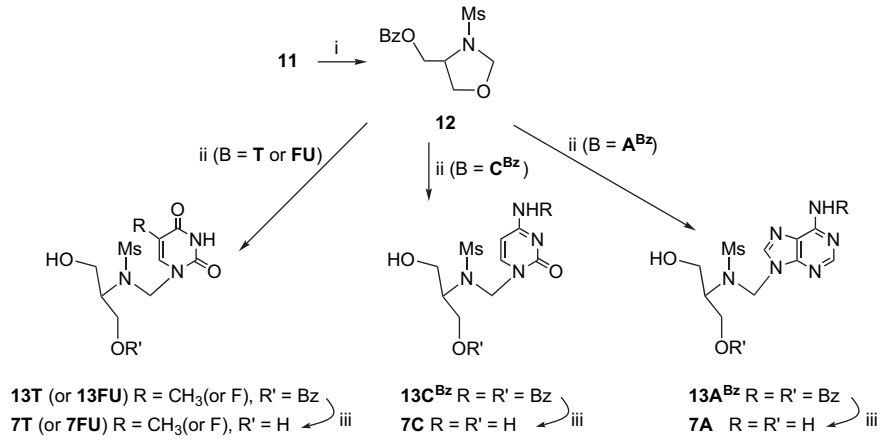
Scheme 3.



Scheme 4. Dowex-50(H^+) MeOH, rt, 1 d; 90% (crude).

amino acids. Consequently we envisaged that oxazolidine **11** could be used for the synthesis of aza-analogues of *ganciclovir* as well (Scheme 5, Table 1).

Crude **11** was treated with benzoyl chloride in the presence of pyridine in dry dichloromethane to afford *O*-benzoyl derivative **12** in 72% overall yield. The aforementioned one-pot base silylation/nucleoside coupling procedure was employed for the coupling of oxazolidine **12** with thymine (**B**=**T**), 5-fluorouracil (**B**=**FU**), N^4-Bz-cytosine (**B**=$\mathbf{C^{Bz}}$), N^6-Bz-adenine (**B**=$\mathbf{A^{Bz}}$) or N^2-acetyl-O^6-(diphenylcarbamoyl)guanine (**B**=$\mathbf{G^{PAC}}$). Accordingly, **12** and TMSOTf were added to the acetonitrile solution of the corresponding silylated nucleobase to yield compounds **13** (Table 1). Pyrimidine nucleosides **13** (**T**, **FU** or $\mathbf{C^{Bz}}$) were obtained in satisfactory yields, which were higher than that of the adenine derivative $\mathbf{13A^{Bz}}$. Attempts to obtain the guanine derivative using this procedure as well the original Robins' procedure[16] (nucleoside coupling was conducted in hot toluene in the presence of TMSOTf) were unsuccessful.[17] In the case of 5-fluorouracil and adenine derivatives (**13FU** and $\mathbf{13A^{Bz}}$, respectively) the yield was improved when TMSOTf was replaced by tin(IV) chloride (Table 1, entries 2–3 and 5–6). The *N*-1 or *N*-9 regioselectivity of the coupling was proved



Scheme 5. Reagents and conditions: (i) BzCl, pyridine, CH_2Cl_2, rt, 1 d, 72% (from **8**); (ii) **B**, BSA, catalyst (TMSOTf or $SnCl_4$), MeCN, rt, 2 d (for details see Table 1) and (iii) NH_4OH_{concd}, MeOH, rt, 1 d.

Table 1. Syntheses of aza-analogues of *Ganciclovir* **13** and **7**

Aza-analogues of *Ganciclovir* **13**					Aza-analogues *of Ganciclovir* **7**			
Entry	Base (**B**)	Catalyst	Nucleoside	Yield [%]	Entry	Base	Nucleoside	Yield [%]
1	Thymine (**T**)	TMSOTf	**13T**	67	7	Thymine (**T**)	**7T**	99
2	5-Fluorouracil (**FU**)	TMSOTf	**13FU**	44	8	5-Fluorouracil (**FU**)	**7FU**	80
3		$SnCl_4$		56				
4	N^4-Bz-cytosine ($\mathbf{C^{Bz}}$)	TMSOTf	$\mathbf{13C^{Bz}}$	66	9	Cytosine (**C**)	**7C**	74
5	N^6-Bz-Adenine ($\mathbf{A^{Bz}}$)	TMSOTf	$\mathbf{13A^{Bz}}$	14	10	Adenine (**A**)	**7A**	67
6		$SnCl_4$		26				

by ^{1}H–^{13}C HMBC correlations observed at thymine derivative **13T** or adenine derivative **13A^{Bz}**, respectively.

Treatment of compounds **13** with concd ammonium hydroxide in methanol at room temperature for 1 d afforded the azanucleosides **7** in high yields (Table 1, entries 7–10).

2.1. Antiviral activity

The antiviral activities of compounds **7** (**T**, **FU**, **C** or **A**), **13T** and **13FU** were evaluated in vitro against a variety of viruses. The following viruses and host cells were used for the evaluation:

(a) Vero cell cultures: parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie B4 virus and Punta Toro virus;
(b) E_6SM cell cultures: herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), herpes simplex virus-1 (TK^- KOS ACV^r), vaccinia virus and vesicular stomatitis virus;
(c) HeLa cell cultures: vesicular stomatitis virus, Coxsackie B4 virus and respiratory syncytial virus.

Brivudin, (*S*)-9-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA), *ribavirin*, *acyclovir* and *ganciclovir* were used as the reference compounds. Among them, only thymine derivative **13T** showed very low activity in test against respiratory syncytial virus in HeLa cell cultures (IC_{50}=49 μg/ml). In the same test *ribavirin* displayed IC_{50} of 9.6 μg/ml. The following minimum cytotoxic concentration values were estimated for tested azanucleosides:[18] (i) Vero cell cultures: 200 μM; (ii) E_6SM cell cultures: >200 μM and (iii) HeLa cell cultures: >200 μM.[19]

3. Conclusions

We have shown that the aza-analogues of *ganciclovir* can be readily obtained from *N*-[2-pivaloyloxy-1-(pivaloyloxymethyl)ethyl]methanesulfonamide **5** or 3-mesyl-4-(benzoyloxymethyl)-1,3-oxazolidine **11** by employing the one-pot base silylation/nucleoside coupling procedure. The oxazolidine **11** is useful for the synthesis of mono *O*-substituted aza-analogues of *ganciclovir*. Further studies on the improvement and extension of the methodologies described for the syntheses of various acyclic azanucleosides are in progress.

4. Experimental

4.1. General

High Resolution Mass Spectra (Electrospray Ionization, ESI) were performed on a Mariner™ spectrometer in positive ionization mode unless otherwise indicated. IR spectra were recorded on a Specord M80 (Carl-Zeiss Jena) spectrometer in KBr disc unless otherwise indicated; absorption maxima (ν_{max}) are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer at 200 MHz and 50 MHz, respectively. ^{1}H and ^{13}C chemical shifts are reported in parts per million relative to the solvent signals: $CDCl_3$, δ_H (residual $CHCl_3$) 7.26 ppm, δ_C 77.16 ppm or DMSO-d_6, δ_H (residual DMSO) 2.50 ppm, δ_C 39.52 ppm; signals are quoted as 's' (singlet), 'd' (doublet), 't' (triplet), 'dt' (doublet of triplets), 'm' (multiplet) and 'br s' (broad singlet). Coupling constants (*J*) are reported in Hertz. ^{13}C-NMR APT (Attached Proton Test) spectra were recorded on a Varian Gemini 200 spectrometer at 50 MHz; signals quoted as (–) indicate signals of carbon atoms from methyl (CH_3) or methylidene group (CH). Precoated Merck silica gel 60 F_{254} (0.2 mm) plates were used for thin-layer chromatography (TLC), and the spots were detected under UV light (254 nm). Column chromatography was performed using silica gel (200–400 mesh, Merck). Solvents were purified by routine methods. The anhydrous $MgSO_4$ was employed as a drying agent. Solvents were distilled off under reduced pressure on a rotating evaporator.

4.1.1. 3-(Mesylamino)-1,5-dioxaspiro[5.5]undecane (2). 3-Nitro-1,5-dioxaspiro[5.5]undecane (**1**, 20.1 g, 0.1 mol)[12] in ethanol (100 cm^3) was hydrogenated under 60 bar pressure in the presence of 10% Pd/C (0.5 g) at room temperature for 24 h. The mixture was filtered through a Celite pad and the solvent was distilled off from the filtrate. The residue was dried in vacuum desiccator (over P_2O_5) for 24 h. The solution of the crude amine and pyridine (20.24 g, 0.2 mol, 27.9 cm^3) in DCM (160 cm^3) was cooled in ice water and the solution of methanesulfonyl chloride (17.2 g, 0.15 mol, 11.7 cm^3) in DCM (40 cm^3) was added dropwise. When the addition was completed, stirring was continued for 10 min. The mixture was washed subsequently with water (3×100 cm^3), diluted (5%) hydrochloric acid, water and dried. Solvent was distilled off and the residue was crystallized (ethyl acetate–hexane, 9/1, v/v) to yield **1** as white crystals (16.9 g, 67%), mp 101–103 °C. δ_H ($CDCl_3$) 1.52 (m, 8H), 1.84 (m, 2H), 3.01 (s, 3H), 3.41 (m, 1H), 3.75 (m, 2H), 4.14 (m, 2H), 5.30 (d, 1H, ^3J 9.0). δ_C ($CDCl_3$) 22.47, 22.56, 25.63, 28.15, 36.81, 42.51, 48.22, 63.57, 99.00. IR: 3324, 2944, 1312, 1164, 1136, 1104. HRMS (EI, 70 eV) *m/z* calcd for $C_{10}H_{19}NO_4S$ (M^+) 249.1032, found 249.1035.

4.1.2. *N*-[2-Pivaloyloxy-1-(pivaloyloxymethyl)ethyl]-methanesulfonamide (4). A mixture of **2** (1.0 g, 4 mmol) and Dowex-50(H^+) (1 g) in methanol (20 cm^3) was shaken at room temperature for 24 h. The ion exchange resin was filtered off and the solvent was distilled off. The residue was dried in vacuum desiccator (over P_2O_5) for 24 h, and then this was dissolved in dry pyridine (6 cm^3) and cooled in water bath. Pivaloyl chloride (1.16 g, 9.6 mmol, 1.2 cm^3) was added to this solution in one portion. The mixture was kept at room temperature for 24 h. The reaction was quenched by addition of water (16 cm^3) and DCM (4 cm^3). The organic phase was separated, washed with water, brine and dried. The solvent was distilled off and the residue was purified by flash chromatography (chloroform) to yield **4** (oil, 0.57 g, 42%). δ_H ($CDCl_3$) 1.27 (s, 18H), 3.02 (s, 3H), 3.98 (m, 1H), 4.17 (m, 4H), 4.81 (br s, 1H). δ_C ($CDCl_3$) 22.92, 39.06, 42.33, 52.11, 63.43 (×2), 97.14, 178.34. IR: 3288, 2976, 1728, 1320, 1156. HRMS *m/z* calcd for $C_{14}H_{27}NO_6NaS$ $(M+Na)^+$ 360.1451, found 360.1463.

4.1.3. *N*-(Pivaloyloxymethyl)-*N*-[2-pivaloyloxy-1-(pivaloyloxymethyl)ethyl]methanesulfonamide (5). A mixture of sodium hydride (63% suspension in mineral oil, 2.8 g,

70 mmol) and **4** (15.8 g, 35 mmol) in dry DMF (20 cm^3) was stirred at room temperature for 1 h. Then chloromethyl pivaloate (15.8 g, 105 mmol, 11.2 cm^3) was added in one portion. After 3 d of stirring at room temperature the mixture was poured into cold water (40 cm^3) followed by extraction with ethyl acetate (3×20 cm^3). The combined extracts were washed with water and dried. The low boiling solvents were distilled off under reduced pressure and residual DMF was removed in vacuum at 80 °C (0.05 mmHg). The residue was purified by flash chromatography (hexane–ethyl acetate, 3/1, v/v) to yield **5** as an oil (88%, 13.9 g). δ_H ($CDCl_3$) 1.21 (s, 27H), 3.09 (s, 3H), 4.27 (m, 5H), 5.53 (s, 2H). δ_C ($CDCl_3$) 27.15, 27.27, 38.95, 42.41, 54.26, 62.32, 70.58, 177.55, 178.04. IR: 2976, 1728, 1340, 1148. HRMS *m/z* calcd for $C_{20}H_{37}NO_8NaS$ $(M+Na)^+$ 474.2132, found 474.2126.

4.1.4. 4-(Benzoylamino)-1-{*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-1*H*-pyrimidin-2-one (6C^{Bz}). A mixture of N^4-benzoylcytosine (**B**=**C**^{Bz}, 430 mg, 2.0 mmol) and BSA (814 mg, 4.0 mmol, 1.0 cm^3) in dry acetonitrile (10 cm^3) was stirred at room temperature under argon for 1 h. Then a solution of **5** (363 mg, 1.0 mmol) in acetonitrile (1 cm^3) and TMSOTf (0.3 cm^3, 1.66 mmol) were added, successively. After 24 h the reaction mixture was quenched by the addition of $CHCl_3$ (50 cm^3) and a saturated solution of sodium bicarbonate (1 cm^3) and the resulting mixture was stirred for 1 h. Insoluble material was removed by filtration through a Celite pad. The organic layer was separated, washed with water, brine and dried. The solvent was distilled off and the residue was purified by flash chromatography (CH_2Cl_2/MeOH, 98/2, v/v) to give **6C**^{Bz} as amorphous foam, yield 71% (400 mg). δ_H ($CDCl_3$) 1.18 (s, 18H), 3.06 (s, 3H), 4.32 (m, 5H), 5.49 (s, 2H), 7.55 (m, 4H), 7.90 (m, 2H), 8.17 (d, ^3J 7.6, 1H), 8.78 (br s, 1H). δ_C ($CDCl_3$) 27.26, 38.89, 41.85, 56.67, 58.42, 62.04, 97.77, 127.74, 129.22, 132.94, 133.51, 148.41, 155.75, 163.00, 178.05. IR: 3308, 2976, 2932, 1732, 1696, 1676, 1556, 1484, 1340, 1312, 1276, 1252, 1148. HRMS *m/z* calcd for $C_{26}H_{37}N_4O_8S$ $(M+H)^+$ 565.2327, found 565.2346.

4.1.5. 4-Amino-1-{*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-1*H*-pyrimidin-2-one (7C). A mixture of **6C**^{Bz} (305 mg, 0.54 mmol), concd NH_4OH (1 cm^3) and MeOH (2 cm^3) was heated in a sealed tube at 70 °C for 24 h. The solvent was distilled off and the residue was purified by flash chromatography (acetone–methanol–NH_3 aq, 6/1/0.4, v/v/v) to give **7C** as white crystals, yield 74% (117 mg), mp 212–222 °C (dec). δ_H (DMSO-d_6) 3.06 (s, 3H), 3.44 (m, 4H), 3.79 (m, 1H), 4.90 (m, 1H), 5.19 (s, 2H), 5.76 (d, ^3J 7.4, 1H), 7.18 (s, 1H), 7.24 (s, 1H), 7.63 (d, ^3J 7.4 1H). δ_C (DMSO-d_6) 39.94, 55.49, 59.57, 62.04, 94.60, 143.98, 155.63, 165.81. IR: 3504, 3428, 3348, 3140, 1680, 1616, 1512, 1320, 1148. HRMS *m/z* calcd for $C_9H_{17}N_4O_5S$ $(M+H)^+$ 293.0914, found 293.0925.

4.1.6. 6-(Benzyloxycarbonylamino)-9-{*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-9*H*-purine (6A^{Cbz}). A mixture of the N^6-(benzyloxycarbonyl)adenine (**B**=**A**^{Cbz} 270 mg, 1.0 mmol) and BSA (814 mg, 4.0 mmol, 1.0 cm^3) in dry acetonitrile (10 cm^3) was stirred at room temperature under argon for 1 h, and then a solution of **5** (260 mg, 0.58 mmol) in acetonitrile (1 cm^3) and $SnCl_4$ (0.18 cm^3, 1.5 mmol) were added, successively. The resulted mixture was worked-up as described for **6C**^{Bz}. The residue was purified by flash chromatography ($CHCl_3$–acetone, 98/2, v/v) to give **6A**^{Cbz} as amorphous foam, yield 44% (158 mg). δ_H ($CDCl_3$) 1.08 (m, 18H), 2.96 (s, 3H), 4.27 (m, 5H), 5.28 (s, 2H), 5.75 (s, 2H), 7.45 (m, 5H), 8.43 (s, 1H), 8.74 (s, 1H), 9.26 (br s, 1H). δ_C ($CDCl_3$) 27.07, 38.72, 41.98, 52.05, 56.08, 61.93, 67.83, 121.36, 128.51, 128.62, 135.46, 143.54, 149.87, 151.02, 151.16, 153.17, 177.84. IR: 2976, 1732, 1616, 1588, 1472, 1340, 1284, 1212, 1148. HRMS *m/z* calcd for $C_{28}H_{38}N_6O_8NaS$ $(M+Na)^+$ 641.2364, found 641.2380.

4.1.7. 6-Amino-9-{*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-9*H*-purine (7A). The mixture of **6A**^{Cbz} (141 mg, 22.8 mmol) and 10% Pd/C (20 mg) in MeOH (3 cm^3) was hydrogenated under atmospheric pressure (balloon) at room temperature for 2 d. The catalyst was filtered off through a short pad of Celite. Concd NH_4OH (1 cm^3) was added to the filtrate and the mixture was heated in a sealed tube at 70 °C for 24 h. The solvent was distilled off and the residue was purified by flash chromatography (chloroform–methanol, 9/1, v/v) to give **7A** as white crystals, yield 75% (54 mg), mp 240–242 °C. δ_H (DMSO-d_6) 3.10 (s, 3H), 3.47 (m, 4H), 3.82 (m, 1H), 5.00 (m, 2H), 5.65 (s, 2H), 7.36 (br s, 2H), 8.13 (s, 1H), 8.19 (s, 1H). δ_C (DMSO-d_6) 39.97, 51.47, 59.50, 61.93, 118.34, 140.29, 149.05, 152.67, 156.08. IR: 3324, 3160, 1664, 1600, 1332, 1152. HRMS *m/z* calcd for $C_{10}H_{17}N_6O_4S$ $(M+H)^+$ 317.1027, found 317.1042.

4.1.8. 3-[*N*-Mesyl-*N*-(pivaloyloxymethyl)amino]-1,5-dioxaspiro[5.5]undecane (8). A mixture of sodium hydride (63% suspension in mineral oil, 2.8 g, 70 mmol) and **2** (8.7 g, 35 mmol) in dry DMF (20 cm^3) was stirred at room temperature for 1 h. Then, chloromethyl pivaloate (15.8 g, 105 mmol, 11.2 cm^3) was added to the resulted suspension in one portion. After 3 d stirring at room temperature the reaction mixture was poured into ice water (40 cm^3). The mixture was extracted with ethyl acetate (3×20 cm^3) and the combined extracts were washed with water and dried. The solvents were distilled off and residual DMF was removed in vacuum (80 °C, 0.05 mmHg), successively. The residue was purified by flash chromatography (hexane–ethyl acetate, 3/1, v/v) to yield **8** as white crystals (9.2 g, 72%), mp 91–93 °C. δ_H ($CDCl_3$) 1.21 (s, 9H), 1.60 (m, 10H), 3.00 (s, 3H), 3.89 (m, 3H), 4.14 (m, 2H), 5.75 (s, 2H). δ_C ($CDCl_3$) 22.44, 22.65, 25.62, 27.22, 30.65, 34.08, 38.82, 42.27, 50.31, 62.33, 72.62, 98.77, 177.46. IR: 2936, 1712, 1332, 1164, 1156. HRMS *m/z* calcd for $C_{16}H_{29}NO_6NaS$ $(M+Na)^+$ 386.1608, found 386.1627.

4.1.9. 8-Mesyl-11,12-dioxa-8-aza-tricyclo[7.2.2.0^{1,6}]tridecane (10). A mixture of **8** (100 mg, 0.28 mmol) and a Lewis acid (TMSOTf, $AlCl_3$ or $BF_3 \cdot Et_2O$) (0.3 mmol) in acetonitrile (4 ml) was kept at room temperature for 24 h and then poured into a saturated solution of sodium bicarbonate. The product was extracted with ethyl acetate (2×2 cm^3). The combined extracts were washed with brine and dried. The solvent was distilled off and the residue was purified by flash chromatography (DCM) to give **10** as oil. δ_H ($CDCl_3$) 1.32–1.90 (m, 8H), 2.16 (m, 1H), 2.98 (s, 3H), 3.13 (dd, 1H, ^3J 9.5, ^2J 14.2), 3.75 (dd, 1H, ^3J 7.4,

^2J 14.2), 3.94 (dd, 1H, ^3J 2.0, ^3J 10.4), 4.01–4.09 (m, 2H), 4.19–4.24 (m, 2H). δ_C ($CDCl_3$, APT) 22.37, 24.61, 29.55, 38.97(–), 39.25, 46.50, 46.74(–), 51.67(–), 64.33, 65.02, 99.20. IR: 2932, 2860, 1320, 1256, 1196, 1144, 1112, 1052, 956. HRMS *m/z* calcd for $C_{11}H_{19}NO_4NaS$ $(M+Na)^+$ 284.0927, found 284.0929.

4.1.10. 3-Mesyl-4-(benzoyloxymethyl)-1,3-oxazolidine (12). A mixture of **8** (2.08 g, 5.5 mmol) and Dowex-50(H^+) (2.4 g) in methanol (50 cm^3) was shaken at room temperature for 24 h. The ion exchange resin was filtered off and the filtrate was concentrated to dryness. An analytical sample of **11** was purified by flash chromatography (methylene chloride). δ_H ($CDCl_3$) 2.69 (m, 1H), 2.89 (s, 3H), 3.68 (m, 3H), 3.88 (m, 1H), 4.26 (m, 1H), 4.52 (d, ^3J 7.2, 1H), 5.11 (d, ^3J 7.2, 1H). δ_C ($CDCl_3$) 35.90, 60.13, 63.10, 68.56, 81.13. IR: (neat) 3504, 3380, 3016, 2936, 2888, 1332, 1164, 1060.

The crude **11** was dissolved in dry DCM (4 cm^3). Dry pyridine (0.9 cm^3, 0.87 g, 11 mmol) and benzoyl chloride (0.7 cm^3, 0.84 g, 6 mmol) were added to that solution, subsequently. The mixture was left at room temperature for 24 h. Water (30 cm^3) was added and the mixture was stirred for 1 h. The organic layer was separated and the aqueous phase was extracted with DCM (2×10 cm^3). The organic phases were combined and washed with water and dried. The solvent was distilled off and the residue was crystallized from hexane–ethyl acetate mixture (1/1, v/v) to give **12** as white crystals, yield 72% (1.13 g), mp 102–103 °C. δ_H ($CDCl_3$) 2.91 (s, 3H), 3.78 (m, 1H), 4.29 (m, 2H), 4.44 (m, 2H), 4.62 (d, ^3J 7.1, 1H), 5.20 (d, ^3J 7.1, 1H), 7.45 (m, 2H), 7.59 (m, 1H), 8.04 (m, 2H). δ_C ($CDCl_3$) 37.04, 57.06, 64.70, 69.00, 80.97, 128.68, 129.52, 129.84, 133.55, 166.26. IR: (neat) 3012, 2932, 2892, 1708, 1456, 1340, 1284, 1160, 1128. HRMS *m/z* calcd for $C_{12}H_{15}NO_5NaS$ $(M+Na)^+$ 308.0563, found 308.0571.

4.2. Synthesis of azanucleosides 13

Azanucleosides **13** were obtained according to the procedure described for the syntheses of **6C^{Bz}** and **6A^{Cbz}** (for details see Table 1). For the syntheses of **13T** and **13C^{Bz}** a molar ratio of nucleobase (**B**)–BSA–Lewis acids–**12** was 2.0/4.0/1.66/1.0, respectively. In the case of the preparation of **13FU** and **13A^{Bz}** the above ratio was 2.0/4.0/3.0/1.0. The reaction mixtures were purified by flash chromatography to give the corresponding azanucleosides **13**; eluting solvents are given in parentheses below.

4.2.1. 1-{*N*-[2-Benzoyloxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-5-methyl-1*H*,3*H*-pyrimidin-2,4-dione (13T). Chromatographic purification (chloroform–methanol, 95/5, v/v) afforded **13T**, yield 67% (273 mg), mp 182–186 °C. δ_H (DMSO-d_6) 1.42 (s, 3H), 3.14 (s, 3H), 3.70 (m, 2H), 4.20 (m, 3H), 5.14 (d, ^2J 15.1, 1H), 5.27 (m, 1H), 5.41 (d, ^2J 15.1, 1H), 7.49 (m, 2H), 7.54 (s, 1H), 7.63 (m, 1H), 7.91 (m, 2H), 11.35 (br s, 1H). δ_C (DMSO-d_6) 11.80, 40.37, 54.82, 57.94, 58.43, 61.92, 109.77, 128.70, 129.14, 129.26, 133.39, 138.41, 151.14, 163.62, 165.26. IR: 3408, 3036, 1724, 1692, 1668, 1336, 1280, 1148. HRMS *m/z* calcd for $C_{17}H_{21}N_3O_7NaS$ $(M+Na)^+$ 434.0992, found 434.1000.

4.2.2. 1-{*N*-[2-Benzoyloxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-5-fluoro-1*H*,3*H*-pyrimidin-2,4-dione (13FU). Chromatographic purification (chloroform–methanol, 93/7, v/v) afforded **13FU**, yield 44% (177 mg, TMSOTf) or 56% (230 mg, $SnCl_4$), mp 183–187 °C. δ_H (DMSO-d_6) 3.15 (s, 3H), 3.71 (m, 2H), 4.27 (m, 3H), 5.28 (m, 3H), 7.72 (m, 6H), 11.90 (br s, 1H). δ_C (DMSO-d_6) 40.20, 55.84, 58.19, 58.86, 62.06, 127.08 (d, $^2J_{C-F}$ 33.8), 128.72, 129.13, 133.48, 139.77 (d, $^1J_{C-F}$ 230.3), 149.84, 157.02 (d, $^2J_{C-F}$ 26.2), 165.35. IR: 3504, 3012, 2856, 1712, 1692, 1660, 1328, 1272, 1148, 1100. HRMS *m/z* calcd for $C_{16}H_{18}N_3O_7FNaS$ $(M+Na)^+$ 438.0742, found 438.0738.

4.2.3. 4-(Benzoylamino)-1-{*N*-[2-benzoyloxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-1*H*-pyrimidin-2-one (13C^{Bz}). Chromatographic purification (chloroform–methanol, 95/5, v/v) gave **13C^{Bz}**, yield 66% (324 mg), foam. δ_H (DMSO-d_6) 3.19 (m, 3H), 3.73 (m, 2H), 4.35 (m, 3H), 5.43 (m, 3H), 7.20–8.24 (m, 12H), 11.20 (br s, 1H). δ_C (DMSO-d_6) 40.17, 57.43, 58.62, 59.21, 62.64, 96.74, 128.47, 128.64, 129.19, 129.30, 129.38, 129.70, 132.78, 133.12, 133.35, 148.17, 155.33, 163.38, 167.23. IR: 3410, 3030, 1721, 1690, 1660, 1338, 1147. HRMS *m/z* calcd for $C_{23}H_{25}N_4O_7S$ $(M+H)^+$ 501.1438, found 501.1451.

4.2.4. 6-(Benzoylamino)-9-{*N*-[2-benzoyloxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-9*H*-purine (13A^{Bz}). Chromatographic purification (chloroform–acetone, 98/2, v/v) afforded **13A^{Bz}**, yield 14% (74 mg, TMSOTf) or 26% (138 mg, $SnCl_4$), foam. δ_H ($CDCl_3$) 2.74 (s, 3H), 4.06 (m, 2H), 4.39 (m, 1H), 4.55 (m, 2H), 4.94 (m, 1H), 5.88 (s, 2H), 7.52 (m, 6H), 7.98 (m, 4H), 8.36 (s, 1H), 8.77 (s, 1H), 9.17 (br s, 1H). δ_C ($CDCl_3$) 42.90, 53.96, 60.32, 61.30, 63.38, 123.33, 128.67, 129.34, 129.72, 129.94, 130.39, 133.81, 134.11, 134.24, 145.29, 150.82, 151.80, 153.54, 165.33, 166.86. IR: 3410, 1716, 1623, 1527, 1325, 1143. HRMS *m/z* calcd for $C_{24}H_{24}N_6O_6NaS$ $(M+Na)^+$ 547.1370, found 547.1383.

4.3. Deprotection of 13

Nucleosides **13** were treated with concd ammonium hydroxide in MeOH at room temperature for 24 h. The ratio of **13**–NH_4OH_{concd}–MeOH was 0.25 mmol/4 cm^3/4 cm^3, respectively. The reaction mixtures were evaporated to dryness under reduced pressure and the residues were purified by flash chromatography to give the corresponding azanucleosides: **7T**, **7FU**, **7C** or **7A** from **13T**, **13FU**, **13C^{Bz}** or **13A^{Bz}**, respectively (Table 1). The nucleosides **7C** and **7A** obtained from corresponding **13C^{Bz}** and **13A^{Bz}**, respectively, are identical with those obtained from **5**.

4.3.1. 1-{*N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-5-methyl-1*H*,3*H*-pyrimidin-2,4-dione (7T). Yield 99% (74 mg), (chloroform–methanol, 9/1, v/v, mp 160–187 °C). δ_H (DMSO-d_6) 1.77 (d, ^4J 1.2, 3H), 3.08 (s, 3H), 3.47 (m, 4H), 3.78 (m, 1H), 4.92 (m, 2H), 5.18 (s, 2H), 7.50 (q, ^4J 1.2, 2H). δ_C (DMSO-d_6) 12.30, 40.20, 54.87, 59.59, 62.04, 109.55, 138.69, 150.98, 163.96. IR: 3424, 3364, 1704, 1656, 1340, 1280, 1164, 1128. HRMS *m/z* calcd for $C_{10}H_{17}N_3O_6NaS$ $(M+Na)^+$ 330.0730, found 330.0744.

4.3.2. 1-{*N*-[2-Hydroxy-1-(hydroxymethyl)ethyl]mesylaminomethyl}-5-fluoro-1*H*,3*H*-pyrimidin-2,4-dione (7FU). Yield 80% (137 mg), chloroform–methanol–NH_3 aq, 7/3/0.5 and then 6/4/0.8, v/v/v, mp 172–190 °C (dec). δ_H (200 MHz, DMSO-d_6) 3.10 (s, 3H), 3.49 (m, 4H), 3.78 (m, 1H), 4.96 (m, 2H), 5.18 (s, 3H), 7.87 (d, $^3J_{F-H}$ 6.6, 1H). δ_C (50 MHz, DMSO-d_6) 39.67, 55.52, 59.48, 62.22, 127.17 (d, $^2J_{C-F}$ 33.8), 139.70 (d, $^1J_{C-F}$ 229.5), 149.61, 157.12 (d, $^2J_{C-F}$ 25.8). IR: 3512, 3436, 3064, 2836, 1700, 1348, 1316, 1260, 1144. HRMS *m/z* calcd for $C_9H_{14}N_3O_6FNaS$ $(M+Na)^+$ 334.0480, found 334.0496.

Acknowledgements

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17. In the light of successful synthesis of *N*-acetyl[9] and *N*-tosyl[10] acyclic aza-analogues of guanosine as well as adenosine (independently of kind of *N*-substituent), the fiasco of preparation of the *N*-mesyl analogue guanosine by various methods (Ref. 9 and this paper) is somewhat puzzling, indeed. Studies on solution of this problem are in progress.
18. Required to cause a microscopically detectable alteration of normal cell morphology.
19. The minimum cytotoxic concentrations of reference compounds were as follows: *brivudin* and *ribavirin* (Vero, E_6SM or HeLa cells, >400 μM); (*S*)-DHPA (Vero or HeLa cells, >400 μM); *acyclovir* (E_6SM cells, >400 μM); and *ganciclovir* (E_6SM cells, >100 μM).

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X=Y–ZH compounds as potential 1,3-dipoles. Part 63: Silver catalysed azomethine ylide cycloaddition—the synthesis of spiro homoserine lactone analogues

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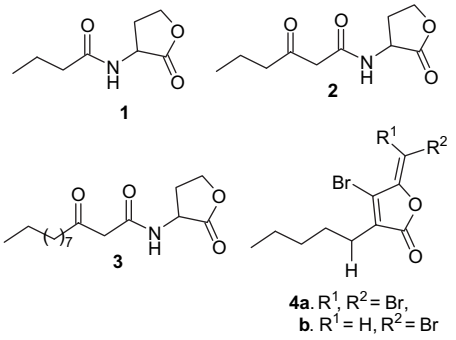
Abstract—A range of room temperature 1,3-dipolar cycloaddition reactions of imines of 2-amino-γ-lactone and thiolactone, catalysed by a combination of AgOAc or Ag_2O with NEt_3 or DBU, are described. The spiro lactones/thiolactones are formed regio- and stereoselectively as single cycloadducts in good yield via the *syn* dipoles and an *endo*-transition states.

1. Introduction

Homoserine lactone derivatives have attract much attention due to their biological activity profile.[1] Acyl homoserine lactones (AHLs) **1**–**3** are important intercellular signalling molecules in many Gram-negative bacteria and are responsible for bacterial quorum sensing.[2] Both antagonist and agonist AHLs have been reported for Gram-negative bacteria.[3] The seaweed *Delisea pulchra* produces a number of halogenated furanones **4a**,**b**, structurally similar to the bacterial AHLs, which exhibit antifouling and antimicrobial properties.[4] Synthetic analogues of AHLs possess inhibitory and immune modulatory activity in both eukaryotic and prokaryotic cells.[5]

Spiropyrrolidines have attracted attention because of their antiviral[6] and local anaesthetic[7] activity and as potential anitleukemic and anticonvulsant agents.[8] Recently, several reports[9–12] have appeared of the synthesis of substituted spiropyrrolidines using azomethine ylide cycloaddition reactions. Raghunathan et al. reported the synthesis and biological activity of new classes of spiro- and bis-spiropyrrolidines.[11] The spiropyrrolidine ring system also occurs in alkaloids,[12] for example, (−)-horsfiline[12a] and spirotryprostatin A,[12b] which have been synthesised using azomethine ylide cycloaddition reactions. A few reports describing the use of methylene lactones as dipolarophiles in azomethine ylide cycloaddition reactions generating spiropyrrolidines have appeared.[13]



Our group has developed a wide variety of protocols for the synthesis of polysubstituted pyrrolidines involving imine substrates.[14–16] Appropriate imines generate azomethine ylides in situ via thermal 1,2-prototropy or Bronsted acid catalysis[14] or by decarboxylative processes.[15] Alternatively metallo-azomethine ylides can be generated catalytically at room temperature by combination of a metal salt and a tertiary amine.[16] In this context, imines of homoserine lactone **5** and homocysteine thiolactone **6** offer access to spiro analogues. This paper describes application of the catalytic metallo-azomethine ylide process to the synthesis of diverse analogues of **5** and **6** via silver salt catalysis.

A variety of aldehydes (aryl, heteroaryl or aliphatic) were employed to illustrate the diversity of the metallo-azomethine ylide cycloaddition (Scheme 1). In some cases long-chain aliphatic aldehydes were used to increase the lipophilicity of the cycloadducts. A series of aryl/heteroaryl

Keywords: Metallo-azomethine ylides; Cycloaddition; Silver oxide; Homoserine lactones.
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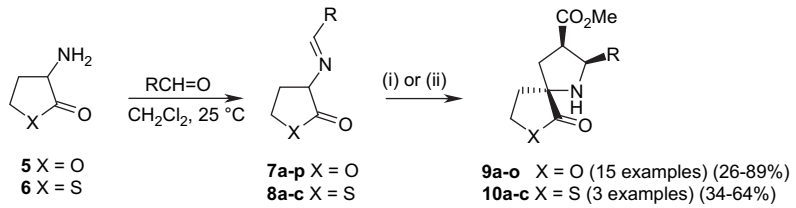
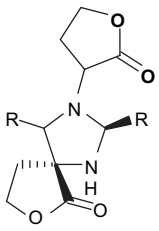


Scheme 1. Reagents and conditions: (i) NEt_3, AgOAc in MeCN, 25 °C; (ii) NEt_3 or DBU, AgO in toluene, 25 °C.

7b–d and aliphatic **7e–o** imines of α-amino-γ-butyrolactone **5** were prepared in good yield (85–99%) by condensation of **5** with the appropriate aldehydes in the presence of a dehydrating agent ($MgSO_4$) in a suitable solvent at room temperature (Table 1, entries 1–15). Imine **7a** (R=Ph) was obtained using a literature method.[17] Attempts to prepare imines of **5** with 2- and 4-pyridine carboxaldehyde and *N*-methyl imidazole-2-carboxaldehyde resulted in complex reaction mixtures. Imines **8a–c** were obtained at room temperature in 86–95% yield by the analogous condensation of **6** with the appropriate aldehyde (Table 1, entries 16–18).

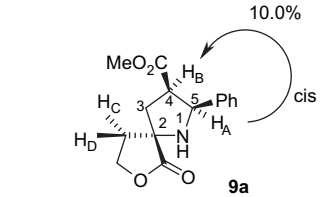
The aryl/heteroaryl imines **7a–d** reacted regio- and stereospecifically with methyl acrylate (1.2 equiv) in acetonitrile at room temperature in the presence of AgOAc (1.5 equiv) and NEt_3 (1.1 equiv) to give single cycloadducts **9a–d** in 48–86% yield (Table 1, entries 1–4). The low yield of cycloadduct **9b** probably arises from the formation of some imine dimer product **11** due to the high reactivity of the 3-pyridyl imine.[18] The reactions were completed in 3–4 h in the case of imines **7a**,**b**,**d** whilst **7c** required 24 h. This longer reaction time appears to be due to the poor solubility of the imine in acetonitrile.



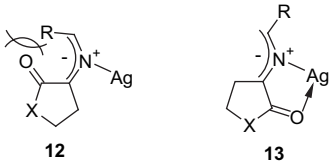
11

In contrast to aryl imines, cycloaddition of aliphatic aldimines **7e–o** was carried out in toluene. Previous experiments[19] suggested that the Ag(I) catalysed cycloaddition reactions of alanine ester imines of aliphatic aldehydes in acetonitrile resulted in the formation of an additional (minor) cycloadduct arising from the *E*,*Z*-metallo-dipole **12**. However, it has also been reported that a mixture of *syn*-endo and *anti*-endo cycloadducts were obtained in both acetonitrile and toluene.[20]

The recent introduction[21] of a catalytic method using Ag_2O (10 mol %) for the generation of metallo-azomethine ylide is very effective in the case of imines **7e–o.** The latter were subjected to 1,3-dipolar cycloaddition reactions with methyl acrylate in toluene in the presence of NEt_3 and Ag_2O (10 mol %) at room temperature (Table 1, entries 5–15). The cycloaddition of imines **7e**,**m** afforded single cycloadducts **9e**,**m** (Table 1, entries 5 and 13) in good yield (86–89%), whereas racemic aldimines **7f**,**h–l**,**o** afforded diastereomeric mixtures (dr; 1:1 to 1:3) of cycloadducts **9f**,**h–l**,**o** (Table 1, entries 6, 8–12 and 15) (due to the chiral centre present in the aldimine substituent). In the case of **9l**,**o** it was possible to separate both diastereomers using silica gel chromatography but it did not prove possible to achieve a similar separation for **9h–k** (see Section 2). The chiral aldimines **7g**,**n** afforded 1:1 mixtures of chiral diastereomers **9g**,**n** (Table 1, entries 7 and 14). The diastereomers of **9n** were separated by silica gel chromatography, whereas those of **9g** were inseparable. The low yield of **9g** appears to be due to competitive metal catalysed hydrolysis of the imine as evidenced by the isolation of the hydrolysis products and the ^1H NMR spectrum of the crude reaction mixture which showed it to comprise a 1:1 mixture of cycloadducts and hydrolysis products.



9a

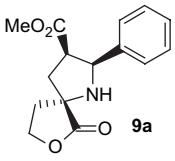
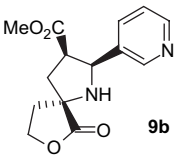
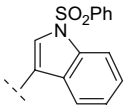
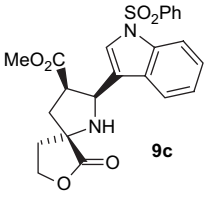
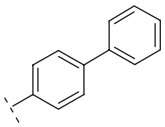
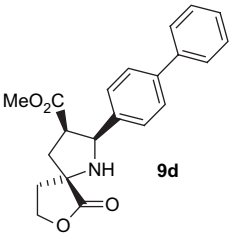
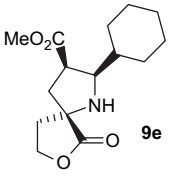
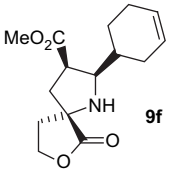
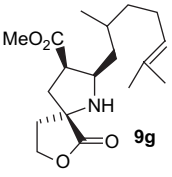
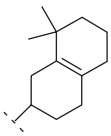
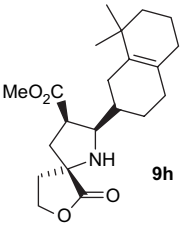


12 **13**

Racemic imines **8a–c** underwent cycloaddition with methyl acrylate in the presence of DBU as base to afford mixture of diastereomeric cycloadducts **10a–c** in 34–64% yield (Table 1, entries 16–18). Previously, we reported[19] related cycloadditions of aliphatic aldimines using NEt_3 as base. However, in the case of **8a–c** NEt_3 was ineffective. Imines **8a**,**b** gave a 1:1 mixture of diastereomers **10a**,**b** (inseparable), whereas **8c** gave a 3:1 mixture of separable diastereomers of **10c**. The cycloadditions of **8a–c** were regio- and *endo*-selective.

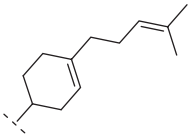
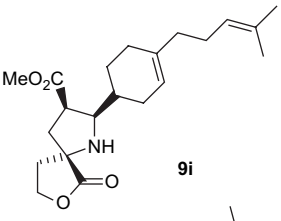
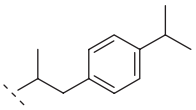
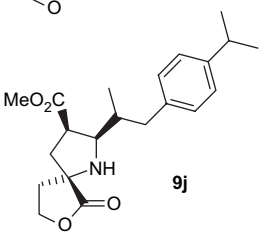
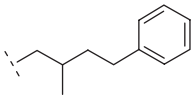
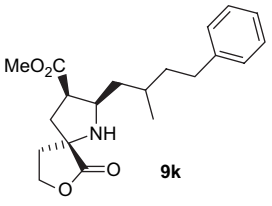
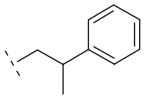
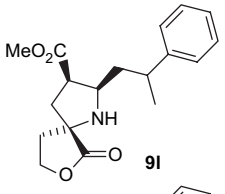
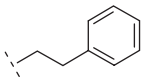
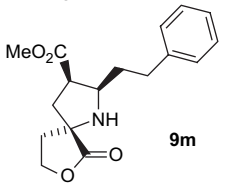
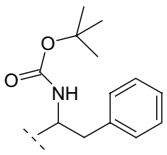
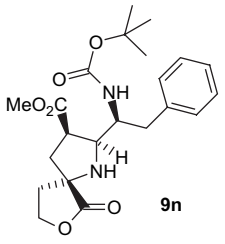
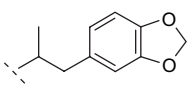
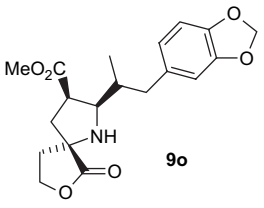
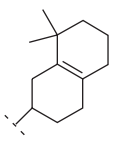
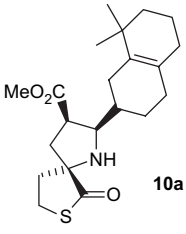
In all cases, the imines **7a–o** and **8a–c** generate the corresponding metallo-1,3-dipoles **13** stereoselectively, based on cycloadduct stereochemistry, and the dipole configuration is analogous to that obtained from imines of α-amino esters.[22] In these cases, the dipoles are formed under kinetic control and the co-ordination of the metal depicted in **13** together with steric effects arising from interaction of R and the carbonyl group in **12** are believed to be responsible for this kinetic preference. The cycloadducts arise from **13** via *endo*-transition states. The relative stereochemistry of the cycloadducts was determined by NOE studies. For example, the irradiation of H_A in **9a** effects a 10% enhancement of the signal for H_B indicating a cis relationship between them.

Table 1. Silver salt/base catalysed cycloaddition of **7a–o** and **8a–c** with methyl acrylate[a–c]

Entry	Imine	R	Cycloadduct	Yield (%)[d]
1[a]	**7a**		 **9a**	86
2[a]	**7b**		 **9b**	48
3[a]	**7c**		 **9c**	54
4[a]	**7d**		 **9d**	57
5[b]	**7e**		 **9e**	89
6[b]	**7f**		 **9f**	57[e]
7[b]	**7g**		 **9g**	26[e]
8[b]	**7h**		 **9h**	52[e]

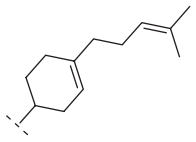
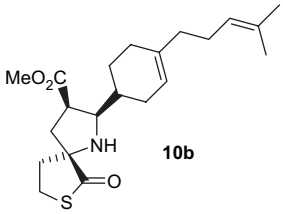
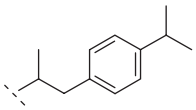
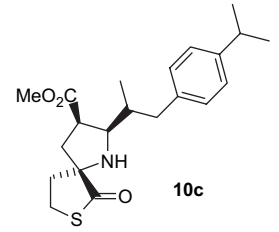
(*continued*)

Table 1. (*continued*)

Entry	Imine	R	Cycloadduct	Yield (%)[d]
9[b]	**7i**		 **9i**	81[e]
10[b]	**7j**		 **9j**	72[f]
11[b]	**7k**		 **9k**	68[e]
12[b]	**7l**		 **9l**	71[e]
13[b]	**7m**		 **9m**	86
14[b]	**7n**		 **9n**	46[e]
15[b]	**7o**		 **9o**	80[f]
16[c]	**8a**		 **10a**	34[e]

(*continued*)

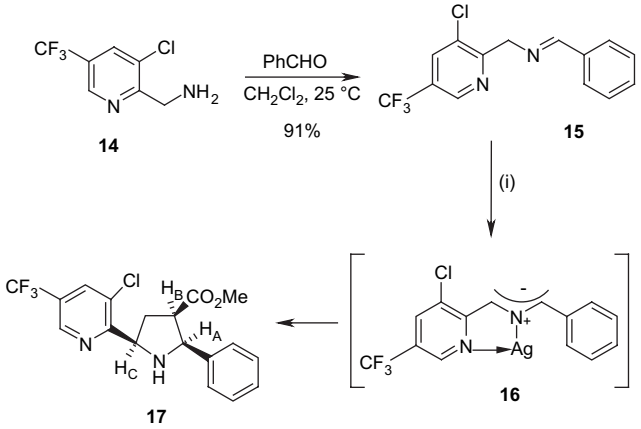
Table 1. (*continued*)

Entry	Imine	R	Cycloadduct	Yield (%)[d]
17[c]	**8b**		 **10b**	64[e]
18[c]	**8c**		 **10c**	64[f]

[a] Entries 1–4: acetonitrile, NEt_3 (1.1 equiv), AgOAc (1.5 equiv), 25 °C.
[b] Entries 5–15: toluene, NEt_3 (1.1 equiv), Ag_2O (10 mol %), 25 °C.
[c] Entries 16–18: toluene, DBU (1 equiv), Ag_2O (10 mol %), 25 °C.
[d] Isolated yield.
[e] 1:1 Mixture of diastereomers.
[f] 1:3 Mixture of diastereomers.

The regiochemistry of the cycloadducts was assigned by comparing the coupling pattern of the relevant protons in the ^{1}H NMR spectra. In the spectra of the cycloadducts, H_A appears as a dd (due to coupling with H_B and NH protons) whilst H_B appears either as a ddd or dt due to coupling with H_A and the C(3)–CH_2 group.

Trifluoromethylated pyridines are widely applied in the field of medicinal and agricultural chemistry.[23] Imine **15**, derived from trifluoromethyl pyridine **14**, underwent regio- and stereo-specific 1,3-dipolar cycloaddition with methyl acrylate in toluene in the presence of Ag_2O (10 mol %) and NEt_3 to give a single cycloadduct **17** in 44% yield (Scheme 2). Cycloadduct **17** arises from dipole **16** via an *endo*-transition state. Thermal 1,2-prototropy-cycloaddition gave a mixture of *endo* -and *exo*-cycloadduct for similar reactions.[24] The dipole **16** is formed under kinetic control and the coordination of the metal depicted in **16** is believed to be responsible for this kinetic preference.



Scheme 2. Reagents and conditions: (i) Methyl acrylate, NEt_3, Ag_2O, toluene, 5 h at 25 °C.

The relative stereochemistry of **17** was assigned by NOE studies (see Section 2). The irradiation of H_A effects a 9% enhancement of the signal for H_B and a 2% enhancement for H_C, whilst irradiation of H_B effects an 8% enhancement of the signal for H_A and a 1% enhancement of H_C. These results suggested that all three protons are cis related. The regiochemistry of the cycloadduct was assigned by considering the coupling pattern of the associated protons. In the ^{1}H NMR spectrum, H_A proton is a doublet whilst H_C proton appeared as an apparent triplet (coupling with CH_2 proton).

2. Experimental

2.1. General

Melting points were determined on a Reichert hot-stage apparatus and are uncorrected. Microanalysis was performed using a Carlo Erba MOD 1108 instrument. Mass spectrometric data were recorded on a V.G.-AutoSpec instrument operating at 70 eV in EI and used Cs ions for FAB spectra. Accurate molecular weights were recorded on a Micromass LCT KAIII electrospray (ES) machine. Infrared spectra were recorded on a Nicolet Magna FT-IR Spectrometer. Optical rotations were measured at ambient temperature using an AA1000 polarimeter. Nuclear magnetic resonance spectra were recorded at 250 MHz on a Bruker AC250 instrument or at 500 MHz on a Bruker DRX500 instrument. Chemical shifts (δ) are given in parts per million (ppm). Deuteriochloroform was used as the solvent unless otherwise stated. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, dd=double doublet, dt=double triplet, ddd=double double doublet, m=multiplet, b=broad. Flash chromatography employed silica gel 60 (230–400 mesh). All solvents were purified according to standard procedures. The term ether refers to diethyl ether. Analytical grade anhydrous silver salts were used as purchased. In all reactions involving silver(I) salts the reaction flask was covered with aluminium foil.

2.2. General procedure for aldimines

A mixture of α-amino-γ-butyrolactone hydrobromide or α-amino-γ-butyrothiolactone hydrochloride (1.1 equiv), triethylamine (1.2 equiv) and anhydrous magnesium sulfate (5 g for each 1 g of hydrobromide salt) was stirred in dry dichloromethane for 1 h before the addition of the appropriate aldehyde (1 equiv). After stirring at room temperature for an appropriate time, the suspension was filtered and the filtrate washed with water (2×) to remove the triethylammonium bromide salt. The organic layer was separated, dried ($MgSO_4$), evaporated under reduced pressure and the residue triturated with ether to afford the solid imine. In the case of aliphatic aldimines the water wash was omitted and the residue triturated with ether to precipitate salts and then filtered. The filtrate was evaporated under reduced pressure to give the product as an oil. All the imines were used for the cycloaddition reactions without further purification due to their thermal and chromatographic instability.

2.2.1. (3-Pyridylmethylidene)-amino-dihydro-furan-2-one (7b). Compound **7b** was prepared from α-amino-γ-butyrolactone hydrobromide (2.00 g, 11.0 mmol), 3-pyridine

carboxaldehyde (1.03 mL, 11 mmol) and triethylamine (1.68 mL, 12.1 mmol) in DCM (35 mL) over 4 h. The crude imine (1.70 g, 81%) was a pale yellow viscous oil, which was used without further purification. δ (^{1}H, 250 MHz): 9.0 and 8.68 (2×m, 2×1H, pyridyl-H), 8.59 (s, 1H, CH═N) 8.12 and 7.36 (2×m, 2×1H, pyridyl-H), 4.68 and 4.48 (2×m, 2×1H, CH_2O), 4.35 (t, 1H, *J* 7.8 Hz, CHN) and 2.85–2.58 (m, 2H, CH_2CH_2O); *m/z* (%): 190 (M^+, 36), 145 (35), 144 (83), 131 (26), 118 (75), 107 (48), 105 (56), 92 (100), 86 (71) and 51 (55).

2.2.2. 3′-(*N*-Phenylsulfonyl-indolylmethylidene)-amino-dihydro-furan-2-one (7c). α-Amino-γ-butyrolactone (1.00 g, 5.49 mmol), *N*-sulfonyl-3-indolylcarboxaldehyde (1.56 g, 5.49 mmol) and triethylamine (0.8 mL, 6.0 mmol) in DCM (25 mL) were reacted over 4 h to afford the imine (1.50 g, 72.6%), which crystallised from ether as colourless needles, mp 130–132 °C. Found (HRMS, M^++H): 369.0910. $C_{19}H_{16}O_4N_2S$ requires: 369.0909; δ (^{1}H, 250 MHz): 8.56 (s, 1H, CH═N), 8.30 (m, 1H, indolyl-H), 7.98–7.89 (m, 4H, indolyl-H), 7.60–7.29 (m, 5H, phenyl-H), 4.63 (ddd, 1H, *J* 8.9, 7.6 and 5.2 Hz, CH_2O), 4.41 (m, 1H, CH_2O), 4.18 (t, 1H, *J* 7.7 Hz, CHN) and 2.69–2.59 (m, 2H, CH_2CH_2O); ν_{max} (film): 1773, 1638, 1447, 1369, 1269, 1219 and 1175 cm^{-1}; *m/z* (%): 368 (M^+, 29), 285 (64), 227 (8), 183 (38), 169 (17), 156 (34), 141 (53) and 77 (100).

2.2.3. (4-Biphenylmethylidene)-amino-dihydro-furan-2-one (7d). Compound **7d** was prepared from α-amino-γ-butyrolactone hydrobromide (1.00 g, 5.0 mmol), 4-biphenyl carboxaldehyde (0.91 g, 5.0 mmol) and triethylamine (0.85 mL, 6.04 mmol) in DCM (25 mL) over 6 h to afford the imine (0.74 g, 56%) as a colourless solid, mp 110–112 °C. Found (HRMS, M^++H): 266.1183. $C_{17}H_{15}O_2N$ requires: 266.1181; δ (^{1}H, 250 MHz): 8.46 (s, 1H, CH═N), 7.86–7.82 (m, 2H, phenyl-H), 7.67–7.61 (m, 4H, phenyl-H), 7.46–7.40 (m, 3H, phenyl-H), 4.62 (ddd, 1H, *J* 9.0, 7.4 and 5.2 Hz, CH_2O), 4.40 (m, 1H, CH_2O), 4.25 (dd, 1H, *J* 7.3 and 7.9 Hz, CHN) and 2.68–2.62 (m, 2H, CH_2CH_2O); ν_{max} (film): 1773, 1637, 1607, 1487, 1371, 1218, 1164, 1109 and 972 cm^{-1}; *m/z* (%): 265 (M^+, 9), 220 (25), 193 (36), 180 (39), 165 (44) and 86 (100).

2.2.4. 3-(Cyclohexylmethylidene)amino-dihydro-furan-2-one (7e). α-Amino-γ-butyrolactone hydrobromide (5.00 g, 27.5 mmol), triethylamine (4.2 mL, 30.2 mmol), cyclohexane carboxaldehyde (3.08 g, 27.5 mmol) and magnesium sulfate in DCM (100 mL) after 2 h gave the product (5.26 g, 98%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. Found (HRMS, M^++H): 196.1337. $C_{11}H_{17}O_2N$ requires: 196.1337; δ (^{1}H, 250 MHz): 7.69 (d, 1H, *J* 5.1 Hz, CH═N), 4.52 and 4.32 (2×m, 2×1H, CH_2O), 3.95 (t, 1H, *J* 8.1 Hz, CHN), 2.61–2.38 (m, 2H, CH_2CH_2O), 2.25 (m, 1H, cyclohexyl-H), and 1.93–1.65 and 1.38–1.16 (2×m, 2×5H, cyclohexyl-H); ν_{max} (film): 2926, 2852, 1776, 1695, 1662, 1449, 1371, 1218 and 1165 cm^{-1}; *m/z* (%): 195 (M^+, 3), 180 (1), 166 (5), 153 (5), 140 (71), 127 (100) and 86 (66).

2.2.5. 3-(3′-Cyclohexenylmethylidene)amino-dihydro furan-2-one (7f). Compound **7f** was prepared from α-amino-γ-butyrolactone (2.00 g, 11 mmol), racemic 1,2,3,6-tetrahydrobenzaldehyde (1.29 mL, 11.0 mmol) and triethylamine (1.7 mL, 12.0 mmol) in DCM (40 mL) stirring for 8 h. The crude imine (2.11 g, 99%) was obtained as a pale yellow oil, which was used without further purification. Found (HRMS, M^++H): 194.1184. $C_{11}H_{15}O_2N$ requires: 194.1181; δ (^{1}H, 250 MHz): 7.79 (d, 1H, *J* 4.9 Hz, CH═N), 5.71 (br s, 2H, olefinic-H), 4.52 and 4.34 (2×m, 2×1H, CH_2O), 3.99 (t, 1H, *J* 8.1 Hz, CHN), 2.63–2.39 (m, 3H, CH_2CH_2O and cyclohexenyl-H), 2.26–1.87 (m, 5H, cyclohexenyl-H) and 1.58 (m, 1H, cyclohexenyl-H); ν_{max} (film): 2917, 2839, 1775, 1696, 1663, 1437, 1371, 1276 and 1163 cm^{-1}; *m/z* (%): 193 (M^+, 11), 164 (8), 148 (5), 134 (14), 127 (23), 120 (20), 108 (100), 102 (13), 91 (46) and 77 (26).

2.2.6. 3-(*N*-Citronellylidene)amino-dihydro-furan-2-one (7g). α-Amino-γ-butyrolactone hydrobromide (3.00 g, 16.5 mmol), triethylamine (2.5 mL, 18.1 mmol) and (*R*)-(+)-citronellal (3.0 mL, 16.5 mmol) were reacted for 4 h. Work-up gave the product (3.10 g, 80%) as a pale yellow oil, which was used without further purification for the subsequent cycloaddition reactions. Found (HRMS, M^++H): 238.1808. $C_{14}H_{23}O_2N$ requires: 238.1807; δ (^{1}H, 250 MHz): 7.83 (d, 1H, *J* 4.9 Hz, CH═N), 5.08 (m, 1H, olefinic-H), 4.52 and 4.32 (2×m, 2×1H, CH_2O), 4.0 (t, 1H, *J* 8.0 Hz, CHN), 2.58–2.49 (m, 2H, CH_2CH_2O), 2.04–1.70 (m, 4H, aliphatic-H), 1.68 and 1.60 (2×s, 2×3H, Me_2C═C), 1.55–1.16 (m, 3H, aliphatic-H) and 0.97 and 0.95 (d, 3H, *J* 4.0 Hz, CH_3CH); ν_{max} (film): 2960, 2917, 1778, 1696, 1448, 1376, 1217, 1167 and 1055 cm^{-1}; *m/z* (%): 237 (M^+, 2), 222 (4), 194 (6), 154 (100), 121 (47) and 69 (33).

2.2.7. 3-(8,8-Dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-2-yl)methylidene amino-dihydrofuran-2-one (7h). α-Amino-γ-butyrolactone hydrobromide (2.00 g, 11.0 mmol), cyclemone (2.13 g, 11.0 mmol), triethylamine (1.7 mL, 12.1 mmol) and magnesium sulfate in DCM (50 mL) after 7 h gave the product (2.9 g, 95%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. Found (HRMS, M^++H): 276.1965. $C_{17}H_{25}O_2N$ requires: 276.1963; δ (^{1}H, 250 MHz): 7.76 (m, 1H, CH═N), 4.52 and 4.34 (2×m, 2×1H, CH_2O), 3.98 (t, 1H, *J* 7.9 Hz, CHN), 2.57–2.39 (m, 2H, CH_2CH_2O), 2.13–1.37 (m, 10H, aliphatic-H) and 1.18–0.82 (m, 8H, aliphatic-H and 2×CH_3); ν_{max} (film): 2925, 2866, 1777, 1695, 1663, 1456, 1360, 1217 and 1165 cm^{-1}; *m/z* (%): 275 (M^+, 36), 260 (37), 206 (11), 190 (100), 174 (44) 159 (65) and 104 (86).

2.2.8. 3-[4-(4-Methylpent-3-enyl)-cyclohex-3-en-1-yl]-methylidene-amino-dihydrofuran-2-one (7i). Compound **7i** was prepared from α-amino-γ-butyrolactone (3.00 g, 16.5 mmol), racemic emfetal (3.20 g, 16.5 mmol), triethylamine (2.5 mL, 18.1 mmol) and magnesium sulfate in DCM (70 mL) over 8 h. The crude imine (4.25 g, 93%) was obtained as a pale yellow oil, which was used without further purification. Found (HRMS, M^++H): 276.1960. $C_{17}H_{25}O_2N$ requires: 276.1963; δ (^{1}H, 250 MHz): 7.77 (d, 1H, *J* 4.9 Hz, CH═N), 5.41 (br, 1H, olefinic-H), 5.08 (m, 1H, olefinic-H), 4.52 and 4.34 (2×m, 2×1H, CH_2O), 3.98 (t, 1H, *J* 8.0 Hz, CHN), 2.56–2.40 (m, 2H, CH_2CH_2O), 2.20–1.94 (m, 10H, aliphatic-H), 1.65 and 1.50 (2×s, 2×3H, 2×CH_3) and 1.45 (m, 1H, aliphatic-H); ν_{max} (film): 2916, 1778, 1662, 1438, 1373, 1217, 1164 and 1104 cm^{-1}; *m/z* (%): 275 (M^+, 15), 206 (40), 190 (31), 121 (29), 105 (100) and 69 (61).

2.2.9. 3-[3-(4-Isopropylphenyl)-2-methylpropylidene]-amino-dihydrofuran-2-one (7j). α-Amino-γ-butyrolactone hydrobromide (3.00 g, 16.5 mmol), cyclamen aldehyde (3.13 g, 16.5 mmol), triethylamine (2.5 mL, 18.1 mmol) and magnesium sulfate in DCM (50 mL) after 8 h gave the product (4.4 g, 95%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. Found (HRMS, M^++H): 274.1806. $C_{17}H_{23}O_2N$ requires: 274.1807; δ (^{1}H, 250 MHz): 7.79 and 7.73 (d, 1H, *J* 4.9 Hz, CH═N), 7.16–7.07 (m, 4H, phenyl-H), 4.48 and 4.30 (2×m, 2×1H, CH_2O), 3.94 (t, 1H, *J* 8.2 Hz, CHN), 2.94–2.84 (m, 2H, C*H*$_2$CH$_2$O), 2.69–2.35 (m, 4H, aliphatic-H), 1.23 (d, 2×3H, *J* 7.0 Hz, 2×CH_3), and 1.10 and 1.17 (d, 3H, *J* 6.0 Hz, CH_3); ν_{max} (film): 2960, 2928, 2871, 1778, 1695, 1663, 1513, 1457 and 1366 cm^{-1}; *m*/*z* (%): 273 (M^+, 51), 258 (61), 214 (7), 200 (21), 188 (100), 172 (24), 157 (34) and 133 (78).

2.2.10. 3-(3-Methyl-5-phenylpentylidene)amino-dihydrofuran-2-one (7k). Compound **7k** was prepared from α-amino-γ-butyrolactone (3.00 g, 16.5 mmol), racemic mefanal (2.90 g, 16.5 mmol), triethylamine (2.5 mL, 18.1 mmol) and magnesium sulfate in DCM (70 mL) over 8 h. The crude imine (3.95 g, 92%) was obtained as a pale yellow oil, which was used without further purification. δ (^{1}H, 250 MHz): 7.82 (t, 1H, *J* 5.2 Hz, CH═N), 7.31–7.16 (m, 5H, phenyl-H), 4.51 and 4.30 (2×m, 2×1H, CH_2O), 3.99 (t, 1H, *J* 8.1 Hz, CHN), 2.89–2.15 (m, 6H, C*H*$_2$CH$_2$O and aliphatic-H), 1.94–1.43 (m, 3H, aliphatic-H), and 1.04 and 1.01 (d, 3H, *J* 4.5 Hz, CH_3); *m*/*z* (%): 259 (M^+, 1.5), 244 (4), 216 (3), 174 (20), 154 (91), 143 (12), 127 (63) and 91 (100).

2.2.11. 3-(3-Phenylbutylidene)amino-dihydrofuran-2-one (7l). α-Amino-γ-butyrolactone hydrobromide (2.00 g, 11.0 mmol), 3-phenylbutyraldehyde (1.5 mL, 10.0 mmol), triethylamine (1.7 mL, 12.1 mmol) and magnesium sulfate in DCM (50 mL) after 2 h gave the product (2.20 g, 95%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. Found (HRMS, M^++H): 232.1333. $C_{14}H_{17}O_2N$ requires: 232.1337; δ (^{1}H, 250 MHz): 7.75–7.69 (m, 1H, CH═N), 7.36–7.16 (m, 5H, phenyl-H), 4.47 and 4.26 (2×m, 2×1H, CH_2O), 3.97 (m, 1H, CHN), 3.12 (m, 1H, aliphatic-H), 2.72–2.28 (m, 4H, C*H*$_2$CH$_2$O and olefinic-H), 1.34 and 1.32 (d, 3H, *J* 2.8 Hz, CH_3) and 1.21 (m, 1H, aliphatic-H); ν_{max} (film): 2961, 1774, 1696, 1663, 1602, 1494, 1452, 1372 and 1277 cm^{-1}; *m*/*z* (%): 231 (M^+, 4.5), 216 (44), 172 (6), 146 (36) 130 (51), 105 (100), 86 (25) and 41 (29).

2.2.12. 3-(3-Phenylpropylidene)amino-dihydrofuran-2-one (7m). Compound **7m** was prepared from α-amino-γ-butyrolactone (1.00 g, 5.5 mmol), 3-phenyl propionaldehyde (0.66 mL, 5.0 mmol), triethylamine (0.85 mL, 6.0 mmol) and magnesium sulfate in DCM (25 mL) over 2 h. The crude imine (1.00 g, 92%) was a pale yellow oil, which was used without further purification. Found (HRMS, M^++H): 218.1180. $C_{13}H_{15}O_2N$ requires: 218.1181; δ (^{1}H, 250 MHz): 7.88 (m, 1H, CH═N), 7.30–7.19 (m, 5H, phenyl-H), 4.51 and 4.30 (2×m, 2×1H, CH_2O), 3.99 (t, 1H, *J* 8.2 Hz, CHN) and 2.91–2.36 (m, 6H, C*H*$_2$CH$_2$O and aliphatic-H); ν_{max} (film): 2918, 1774, 1696, 1663, 1602, 1496, 1453, 1371 and 1276 cm^{-1}; *m*/*z* (%): 217 (M^+, 9), 158 (10), 132 (77), 117 (57) and 91 (100).

2.2.13. *tert*-Butyl (1*S*)-1-benzyl-2-(2-oxotetrahydrofuran-3-yl)imino-ethylcarbamate (7n). α-Amino-γ-butyrolactone hydrobromide (0.80 g, 4.4 mmol), (*S*)-Boc-L-phenylalaninal (1.00 g, 4.0 mmol), triethylamine (0.67 mL, 4.8 mmol) and magnesium sulfate in DCM (20 mL) after 2 h gave the product (1.31 g, 98%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. δ (^{1}H, 250 MHz): 7.80 (m, 1H, CH═N), 7.31–7.18 (m, 5H, phenyl-H), 5.25 (b, 1H, NH), 4.63–4.19 (m, 3H, NHC*H* and CH_2O), 4.04 (m, 1H, CHN), 3.10–2.95 (m, 2H, aliphatic-H), 2.58–2.41 (m, 2H, C*H*$_2$CH$_2$O) and 1.45 (s, 3×3H, 3×CH_3); *m*/*z* (%): 332 (M^+, <1), 276 (11), 258 (9), 141 (47), 120 (47), 91 (58) and 57 (100).

2.2.14. 3-[3-(1,3-Benzodioxol-5-yl)-2-methylpropylidine]-amino-dihydrofuran-2-one (7o). α-Amino-γ-butyrolactone (1.00 g, 5.49 mmol), racemic 2-methyl-3-(3,4-methylenedioxy phenyl)propanal (0.91 mL, 5.5 mmol), triethylamine (0.85 mL, 6.0 mmol) and magnesium sulfate in DCM (30 mL) after 4 h gave the product (1.41 g, 94%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. Found (HRMS, M^++H): 276.1238. $C_{15}H_{17}O_4N$ requires: 276.1236; δ (^{1}H, 250 MHz): 7.77 and 7.73 (d, 1H, *J* 4.8 Hz, CH═N), 6.74–6.59 (m, 4H, phenyl-H), 5.92 (s, 2H, OCH_2O), 4.50 and 4.30 (2×m, 2×1H, CH_2O), 3.98 (m, 1H, CHN), 2.98–2.35 (m, 5H, C*H*$_2$CH$_2$O and aliphatic-H), and 1.09 and 1.06 (d, 3H, *J* 5.0 Hz, CH_3); ν_{max} (film): 2915, 1774, 1695, 1662, 1502, 1489, 1441, 1369 and 1246 cm^{-1}; *m*/*z* (%): 275 (M^+, 14), 260 (13), 202 (9), 190 (51), 135 (100), 105 (13) and 77 (35).

2.2.15. 3-(2-Naphthylidine)-amino-dihydrofuran-2-one (7p). α-Amino-γ-butyrolactone (3.00 g, 16.4 mmol), naphthalene-2-carboxaldehyde (1.70 mL, 11.0 mmol), triethylamine (2.50 mL, 18.1 mmol) and magnesium sulfate in DCM (50 mL) after 4 h gave the product (1.95 g, 74%) as colourless plates, mp 135–137 °C. Found: C, 75.00; H, 5.25; N, 5.65. $C_{15}H_{13}O_2N$ requires: C, 75.30; H, 5.50; N, 5.85%; δ (^{1}H, 250 MHz): 8.58 (s, 1H, CH═N), 8.09 (s, 1H, ArH), 8.01–7.84 (m, 4H, ArH), 7.56–7.51 (m, 2H, ArH), 4.64 (ddd, 1H, *J* 5.3 and 9.0 Hz, OCH_2), 4.44 (m, 1H, OCH_2), 4.28 (dd, 1H, *J* 0.5 and 7.8 Hz, CHN) and 2.71–2.60 (m, 5H, C*H*$_2$CH$_2$O); ν_{max} (film): 2876, 1770, 1636, 1370, 1218, 1168 and 1020 cm^{-1}; *m*/*z* (ES$^+$, %): 240 (M^++H, 100), 212 (32).

2.2.16. 3-(8,8-Dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-2-yl)methylidene amino-dihydrothiophen-2-one (8a). α-Amino-γ-butyrothiolactone hydrochloride (2.00 g, 13.0 mmol), cyclemone aldehyde (2.50 g, 13.0 mmol), triethylamine (2 mL, 14.3 mmol) and magnesium sulfate in DCM (60 mL) after 4 h gave the product (3.60 g, 95%) as a pale yellow gum, which was used without further purification for the subsequent cycloaddition reactions. δ (^{1}H, 250 MHz): 7.66 (d, 1H, *J* 5.4 Hz, CH═N), 3.84 (m, 1H, CHN), 3.48 and 3.34 (2×m, 2×1H, CH_2S), 2.60–2.44 (m, 2H, C*H*$_2$CH$_2$S), 2.17–1.21 (m, 13H, aliphatic-H) and 0.96 (s, 2×3H, 2×CH_3); *m*/*z* (%): 291 (M^+, 7), 263 (23), 248 (21), 190 (49), 175 (73), 91 (85) and 41 (100).

2.2.17. 3-[4-(4-Methylpent-3-enyl)-cyclohex-3-en-1-yl]-methylidene-amino-dihydrothiophen-2-one (8b). α-Amino-γ-butyrothiolactone hydrochloride (1.00 g, 6.51 mmol),

emfetal (1.25 g, 6.5 mmol), triethylamine (1.0 mL, 7.2 mmol) and magnesium sulfate in DCM (25 mL) after 4 h gave the product (1.71 g, 90%) as a pale yellow oil, which was used without further purification for the subsequent cycloaddition reactions. δ (^{1}H, 250 MHz): 7.67 (d, 1H, *J* 5.1 Hz, CH=N), 5.40 and 5.11 (2×m, 2×1H, olefinic-H), 3.84 (dd, 1H, *J* 8.6 and 6.8 Hz, CHN), 3.50 and 3.35 (2×m, 2×1H, CH_2S), 2.55–2.39 (m, 2H, CH_2CH_2S), 2.08–1.87 (m, 11H, aliphatic-H) and 1.68 and 1.60 (2×s, 2×3H, 2×CH_3); *m/z* (%): 291 (M^+, 4), 263 (11), 222 (39), 194 (36), 105 (66), 69 (91) and 41 (100).

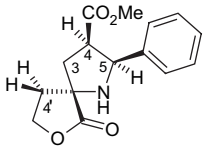
2.2.18. 3-[3-(4-Isopropylphenyl)-2-methylpropylidene]-amino-dihydrothiophen-2-one (8c). α-Amino-γ-butyrothiolactone hydrochloride (1.00 g, 6.5 mmol), cyclamen aldehyde (1.24 g, 6.5 mmol), triethylamine (1.0 mL, 7.2 mmol) and magnesium sulfate in DCM (25 mL) after 4 h gave the product (1.63 g, 86%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. δ (^{1}H, 250 MHz): 7.70 and 7.67 (d, 1H, *J* 5.1 Hz, CH=N), 7.16–7.06 (m, 4H, phenyl-H), 3.81 (m, 1H, CHN), 3.52–3.24 (m, 2H, CH_2S), 2.95–2.83 (m, 2H, CH_2CH_2S), 2.71–2.27 (m, 4H, aliphatic-H), 1.24 and 1.21 (2×d, 2×3H, *J* 6.9 Hz, 2×CH_3) and 1.08 and 1.05 (d, 3H, *J* 6.6 Hz, CH_3); *m/z* (%): 289 (M^+, 8), 274 (13), 261 (11), 200 (31), 133 (100), 117 (77), 91 (80) and 41 (68).

2.2.19. *N*-3-[Chloro-5-(trifluoromethyl)pyridin-2-yl]-methyl-*N*-phenylmethylidene amine (15). 2-Aminomethyl-3-chloro-5-(trifluoromethyl)pyridine hydrochloride (0.50 g, 2.0 mmol), benzaldehyde (0.20 mL, 2.0 mmol), triethylamine (0.31 mL, 2.2 mmol) and magnesium sulfate in DCM (25 mL) after 1 h gave the product (0.55 g, 91%) as a colourless oil, which was used without further purification for the subsequent cycloaddition reactions. δ (^{1}H, 250 MHz): 8.77 (d, 1H, *J* 1.0 Hz, pyridyl-H), 8.51 (s, 1H, CH=N), 7.95 (d, 1H, 1.8 Hz, pyridyl-H), 7.91–7.26 (m, 5H, phenyl-H) and 5.12 (s, 2H, NCH_2); *m/z* (%, FAB): 301 (M^++1, 31), 299 (M^++1, 100), 284 (8), 282 (20), 196 (6), 194 (22) and 91 (7).

2.3. General procedure for silver(I) catalysed cycloaddition reactions

Aldimine (1.0 equiv), triethylamine (1.1 equiv), dipolarophile (1.1 equiv) and silver acetate (1.5 equiv) were mixed in anhydrous acetonitrile. Silver oxide (10 mol %) and toluene (dried over sodium wire) were used in the case of aliphatic aldimines. The resulting suspension was stirred for an appropriate period (see below) at room temperature (monitored by TLC and ^{1}H NMR). After completion of the reaction the mixture was quenched with saturated aqueous ammonium chloride and extracted with ether or dichloromethane (2×). The dried (magnesium sulfate) organic layer was evaporated under reduced pressure. The ratio of any isomers present in the residue was calculated from the integrals of appropriate peaks in the ^{1}H NMR spectra. Flash chromatography of the residue afforded the product.

2.3.1. Methyl 6-oxo-2-phenyl-7-oxa-1-azaspiro[4.4]-nonane-3-carboxylate (9a). A mixture of imine **7a** (2.00 g, 10.6 mmol), triethylamine (1.60 mL, 11.6 mmol), methyl acrylate (1.14 mL, 12.7 mmol) and silver acetate (2.64 g, 15.8 mmol) were stirred in acetonitrile (70 mL) over 4 h. Flash chromatography eluting with ether afforded the product (2.50 g, 86%) as a colourless solid, which crystallised from dichloromethane/hexane as colourless plates, mp 103–105 °C. Found: C, 65.45; H, 6.15; N, 5.00. $C_{15}H_{17}O_4N$ requires: C, 65.45; H, 6.20; N, 5.10%; δ (^{1}H, 250 MHz): 7.43–7.23 (m, 5H, ArH), 4.64 (t, 1H, *J* 7.1 Hz, NHC*H*), 4.46 (ddd, 1H, *J* 3.8, 7.6 and 9.2 Hz, CH_2O), 4.25 (dt, 1H, *J* 6.8 and 9.0 Hz, CH_2O), 3.52 (dt, 1H, *J* 7.4 and 9.2 Hz, C*H*CO_2Me), 3.25 (s, 3H, OMe), 2.76 (d, 1H, *J* 5.0 Hz, NH), 2.55 (dd, 1H, *J* 9.7 and 12.8 Hz, CH_2CHCO_2Me), 2.43–2.26 (m, 2H, CH_2CH_2O) and 2.14 (dd, 1H, *J* 7.4 and 12.8 Hz, CH_2CHCO_2Me); ν_{max} (film): 2949, 1770, 1733, 1495, 1455, 1436, 1373, 1281 and 1180 cm^{-1}; *m/z* (%): 275 (M^+, 0.6), 244 (18), 231 (100), 217 (40), 177 (42), 172 (76), 158 (79), 143 (25) and 91 (31).



NOE data for **9a**:

Signal irradiated	Enhancement (%)					
	5-H	4-H	NH	3-H	Ar-H	3′-H
5-H		9.5	2.0	2.9	10.0	2.9
4-H	10.0		—	3.9	—	2.3

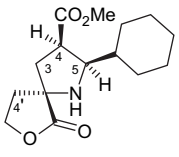
2.3.2. Methyl 6-oxo-2-phenyl-7-oxa-1-azaspiro[4.4]-nonane-3-carboxylate (9b). Methyl acrylate (114 μl, 1.26 mmol) was added to a mixture of imine **7b** (200 mg, 1.05 mmol), triethylamine (0.16 mL, 1.15 mmol) and silver acetate (0.26 g, 1.57 mmol) in acetonitrile (10 mL) and the resulting mixture was stirred at room temperature for 4 h. After work-up, the residue was subjected to flash chromatography eluting with ether to afford the product (0.14 g, 48%) as pale yellow plates, mp 147–149 °C. Found: C, 60.70; H, 6.00; N, 10.10. $C_{14}H_{16}O_4N_2$ requires: C, 60.85; H, 5.85; N, 10.15%; δ (^{1}H, 250 MHz): 8.53–8.50 (m, 2H, pyridyl-H), 7.99 and 7.31 (2×m, 2×1H, pyridyl-H), 4.72 (d, 1H, *J* 8.6 Hz, NHC*H*), 4.46 (m, 1H, CH_2O), 4.27 (dt, 1H, *J* 7.9 and 9.0 Hz, CH_2O), 3.58 (ddd, 1H, *J* 7.4, 8.6 and 10.1 Hz, C*H*CO_2Me), 3.32 (s, 3H, OMe), 2.58 (dd, 1H, *J* 10.3 and 12.9 Hz, CH_2CHCO_2Me), 2.37–2.31 (m, 2H, CH_2CH_2O), 2.17 (dd, 1H, *J* 7.2 and 12.9 Hz, CH_2CHCO_2Me) and 2.05 (b, 1H, NH); ν_{max} (film): 2950, 1769, 1733, 1684, 1652, 1576, 1558, 1480, 1456, 1373, 1289 and 1153 cm^{-1}; *m/z* (%): 276 (M^+, 1.8), 245 (18), 232 (100), 218 (38), 173 (59), 159 (66), 145 (16), 118 (26) and 91 (18).

2.3.3. Methyl 6-oxo-2-(3-*N*-sulfonyl-indolyl)-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9c). A mixture of imine **7c** (200 mg, 0.54 mmol), triethylamine (0.08 mL, 0.6 mmol), methyl acrylate (0.06 mL, 0.65 mmol) and silver acetate (0.13 g, 0.8 mmol) in acetonitrile (15 mL) was stirred over 24 h. Work-up followed by flash chromatography eluting with ether afforded the product (132 mg, 54%) as a colourless solid, which crystallised from dichloromethane/hexane as colourless plates, mp 75–77 °C. Found: C, 60.80; H, 4.85; N, 6.00; S, 7.15. $C_{23}H_{22}O_6N_2S$ requires: C, 60.75; H, 4.90; N, 6.20; S, 7.05%; δ (^{1}H, 250 MHz): 8.00–7.19 (m, 10H, ArH and indolyl-H), 4.87 (d, 1H,

J 7.8 Hz, NHC*H*), 4.47 (ddd, 1H, *J* 4.0, 7.2 and 9.3 Hz, CH_2O), 4.28 (dt, 1H, *J* 6.9 and 8.9 Hz, CH_2O), 3.56 (dt, 1H, *J* 7.4 and 9.3 Hz, C*H*CO_2Me), 2.90 (s, 3H, OMe), 2.70 (b, 1H, NH), 2.62 (dd, 1H, *J* 9.4 and 13.0 Hz, CH_2CHCO_2Me), 2.38–2.31 (m, 2H, CH_2CH_2O) and 2.20 (dd, 1H, *J* 7.2 and 13.0 Hz, CH_2CHCO_2Me); ν_{max} (film): 1771, 1733, 1653, 1559, 1447, 1437, 1367, 1214 and 1123 cm^{-1}; *m*/*z* (%): 454 (M^+, 11), 410 (11), 368 (80), 356 (35), 313 (13), 269 (35), 255 (21), 227 (100) and 183 (34).

2.3.4. Methyl 2-(1.1′-biphenyl-4-yl)-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9d). A mixture of imine **7d** (0.25 g, 0.94 mmol), triethylamine (0.15 mL, 1.03 mmol), methyl acrylate (0.10 mL, 1.13 mmol) and silver acetate (0.23 g, 1.41 mmol) in acetonitrile (15 mL) was stirred for 18 h. Work-up followed by flash chromatography eluting with ether afforded the product (0.19 g, 57%) as a colourless solid, which crystallised from dichloromethane/hexane as colourless plates, mp 125–127 °C. Found: C, 72.00; H, 6.15; N, 3.85. $C_{21}H_{21}O_4N$ requires: C, 71.80; H, 6.00; N, 4.00%; δ (^{1}H, 250 MHz): 7.60–7.26 (m, 9H, phenyl-H), 4.68 (d, 1H, *J* 8.3 Hz, NHC*H*), 4.45 and 4.28 (2×m, 2×1H, CH_2O), 3.56 (m, 1H, CHCO_2Me), 3.28 (s, 3H, OMe), 2.77 (b, 1H, NH), 2.63 (dd, 1H, *J* 9.8 and 12.8 Hz, CH_2CHCO_2Me), 2.38–2.30 (m, 2H, CH_2CH_2O) and 2.16 (dd, 1H, *J* 7.3 and 12.8 Hz, CH_2CHCO_2Me); ν_{max} (film): 1771, 1734, 1487, 1436, 1373, 1216, 1194 and 1086 cm^{-1}; *m*/*z* (%): 351 (M^+, 1.5), 320 (17), 307 (100), 293 (39), 248 (89), 219 (43) and 165 (66).

2.3.5. Methyl 2-cyclohexyl-6-oxo-7-oxa-1-azaspiro[4.4]-nonane-3-carboxylate (9e). A mixture of imine **7e** (1.00 g, 5.11 mmol), triethylamine (0.78 mL, 5.62 mmol), silver oxide (118 mg, 0.51 mmol) and methyl acrylate (0.55 mL, 6.13 mmol) in toluene (50 mL) was stirred at room temperature for 4 h. Work-up without the need for chromatography gave the product (1.28 g, 89%), which crystallised from dichloromethane/hexane as colourless plates, mp 88–90 °C. Found: C, 64.30; H, 8.15; N, 4.75. $C_{15}H_{23}O_4N$ requires: C, 64.05; H, 8.25; N, 5.00%; δ (^{1}H, 250 MHz): 4.40 (dt, 1H, *J* 4.2 and 8.4 Hz, CH_2O), 4.23 (dt, 1H, *J* 6.7 and 8.6 Hz, CH_2O), 3.71 (s, 3H, OMe), 3.14 (ddd, 1H, *J* 4.0, 6.5 and 8.4 Hz, CHCO_2Me), 2.86 (dd, 1H, *J* 6.7 and 9.5 Hz, NHC*H*), 2.40–2.07 (m, 4H, CH_2CHCO_2Me and CH_2CH_2O) and 1.73–1.15 (m, 12H, NH and cyclohexyl-H); ν_{max} (film): 2923, 2851, 1773, 1728, 1436, 1370, 1211, 1170 and 1126 cm^{-1}; *m*/*z* (%): 282 (M^++1, 8), 250 (5), 237 (34), 223 (13), 198 (86), 154 (100), 140 (52) and 94 (29). In NOE 4′-H proton was overlapped with cyclohexyl protons.



NOE data for **9e**:

Signal irradiated	Enhancement (%)			
	5-H	4-H	3-H	Aliphatic-H
5-H		6.8	2.9	13.0
4-H	4.1		5.9	2.3

2.3.6. Methyl 2-(3-cyclohexen-1-yl)-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9f). A mixture of imine **7f** (1.00 g, 5.17 mmol), triethylamine (0.80 mL, 5.68 mmol), silver oxide (0.12 g, 0.517 mmol) and methyl acrylate (0.56 mL, 6.2 mmol) in toluene (50 mL) was stirred for 2 h. Work-up followed by flash chromatography eluting with ether afforded the product (0.83 g, 57%) as a 1:1 mixture of diastereomers, which crystallised from dichloromethane/hexane as colourless plates, mp 84–90 °C. Found: C, 64.50; H, 7.55; N, 4.85. $C_{15}H_{21}O_4N$ requires: C, 64.50; H, 7.60; N, 5.00%; δ (^{1}H, 250 MHz): 5.70–5.57 (m, 2H, olefinic-H), 4.42 (dt, 1H, *J* 4.0 and 8.3 Hz, CH_2O), 4.23 (ddd, 1H, *J* 1.2, 7.3 and 9.0 Hz, CH_2O), 3.71 (s, 3H, OMe), 3.25–3.09 (m, 1H, CHCO_2Me), 2.97 (b, 1H, NHC*H*) and 2.43–1.22 (m, 12H, NH, CH_2CH_2O, CH_2CHCO_2Me and cyclohexenyl-H); ν_{max} (film): 2912, 1773, 1727, 1652, 1456, 1436, 1290, 1103 and 1079 cm^{-1}; *m*/*z* (%): 279 (M^+, 7), 235 (23), 221 (10), 198 (100), 176 (32), 166 (45), 154 (97), 140 (60), 94 (50), 80 (41), 67 (27) and 53 (19).

2.3.7. Methyl 2-(2,6-dimethyl-5-heptenyl)-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9g). A mixture of imine **7g** (700 mg, 2.95 mmol), triethylamine (0.45 mL, 3.24 mmol), silver oxide (0.068 g, 0.29 mmol) and methyl acrylate (0.30 mL, 3.54 mmol) in toluene (30 mL) was stirred for 4 h. Work-up followed by flash chromatography eluting with ether afforded the product (0.25 g, 26%) as a colourless oil, which comprised a 1:1 mixture of diastereomers. Found: C, 67.00; H, 8.85; N, 4.60. $C_{15}H_{21}O_4N$ requires: C, 66.85; H, 9.05; N, 4.60%; δ (^{1}H, 250 MHz): 5.08 (m, 1H, olefinic-H), 4.40 (ddd, 1H, *J* 4.0, 8.1 and 8.9 Hz, CH_2O), 4.22 (dt, 1H, *J* 6.9 and 8.7 Hz, CH_2O), 3.70 (s, 3H, OMe), 3.42 (m, 1H, CHCO_2Me), 3.12 (m, 1H, NHC*H*), 2.45–1.86 (m, 7H, NH, CH_2CH_2O, CH_2CHCO_2Me and citronellyl-H), 1.68 and 1.60 (2×s, 2×3H, Me_2C=C), 1.55–1.13 (m, 3H, citronellyl-H) and 0.95 and 0.90 (d, 3H, *J* 6.5 Hz, citronellyl-CH_3); ν_{max} (film): 2954, 2916, 1773, 1733, 1436, 1375, 1213, 1171, 1117, 1069 and 1019 cm^{-1}; *m*/*z* (%): 323 (H^+, 9), 279 (12), 264 (13), 238 (54), 220 (69), 206 (21), 194 (55), 180 (31), 168 (76), 154 (100), 140 (39) and 94 (60).

2.3.8. Methyl 2-(8,8-dimethyl-1,2,3,4,5,6,7,8-octahydro-2-naphthalenyl)-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9h). A mixture of imine **7h** (1.00 g, 3.6 mmol), triethylamine (0.55 mL, 3.9 mmol), silver oxide (0.08 g, 0.36 mmol) and methyl acrylate (0.40 mL, 4.3 mmol) in toluene (40 mL) was stirred for 3 h. Work-up followed by flash chromatography eluting with 2:1 v/v ether/hexane afforded the product (0.68 g, 52%) as colourless gum, which comprised a 1:1 mixture of diastereomers. Found: C, 70.05; H, 8.90; N, 3.70. $C_{21}H_{31}O_4N$ requires: C, 69.80; H, 8.65; N, 3.90%; δ (^{1}H, 250 MHz): 4.41 and 4.22 (2×m, 2×1H, CH_2O), 3.70 and 3.69 (s, 3H, OMe), 3.18 (m, 1H, CHCO_2Me), 2.94 (m, 1H, NHC*H*), 2.44–2.08 (m, 5H, NH, CH_2CH_2O and CH_2CHCO_2Me), 2.0–1.25 (m, 13H, aliphatic-H) and 0.95 (s, 2×3H, 2×CH_3); ν_{max} (film): 2924, 1774, 1728, 1436, 1371, 1211 and 1169 cm^{-1}; *m*/*z* (%): 361 (M^+, 14), 317 (6), 258 (5), 224 (12), 198 (100) and 154 (36).

2.3.9. Methyl 2-[4-(4-methyl-3-pentenyl)-3-cyclohexen-1-yl]-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9i). A mixture of imine **7i** (1.50 g, 5.4 mmol), triethylamine

(0.83 mL, 5.9 mmol), silver oxide (0.12 g, 0.54 mmol) and methyl acrylate (0.60 mL, 6.5 mmol) in toluene (50 mL) was stirred for 4 h. Work-up followed by flash chromatography eluting with 4:1 v/v ether/hexane afforded the product (1.58 g, 81%) as a colourless oil, which comprised a 1:1 mixture of diastereomers. Found: C, 69.90; H, 8.90; N, 3.60. $C_{21}H_{31}O_4N$ requires: C, 69.80; H, 8.65; N, 3.90%; δ (^{1}H, 250 MHz): 5.38 and 5.36 (m, 1H, cyclohexenyl olefinic-H), 5.08 (m, 1H, olefinic-H), 4.42 and 4.22 (2×m, 2×1H, CH_2O), 3.71 and 3.70 (s, 3H, OMe), 3.16 (m, 1H, $CHCO_2Me$), 2.96 (m, 1H, NHC*H*), 2.46–1.90 (m, 15H, NH, C*H*$_2$CH$_2$O, C*H*$_2$CHCO$_2$Me and aliphatic-H), 1.68 and 1.59 (2×s, 2×3H, 2×CH_3) and 1.36 (m, 1H, aliphatic-H); ν_{max} (film): 2914, 1774, 1729, 1436, 1373, 1290, 1211, 1169 and 1110 cm^{-1}; *m*/*z* (%): 361 (M$^+$, 19), 292 (37), 258 (7), 248 (18), 198 (100), 166 (34) and 140 (24).

2.3.10. Methyl 2-[2-(4-isopropylphenyl)-1-methylethyl]-6-oxo-2-phenyl-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9j). A mixture of imine **7j** (1.00 g, 3.6 mmol), triethylamine (0.56 mL, 4.0 mmol), silver oxide (0.08 g, 0.36 mmol) and methyl acrylate (0.40 mL, 4.4 mmol) in toluene (40 mL) was stirred for 5 h. Work-up followed by flash chromatography eluting with ether afforded the product (0.95 g, 72%) as a 3:1 mixture of diastereomers, which crystallised from dichloromethane/hexane as colourless plates, mp 75–82 °C. Found: C, 70.30; H, 8.20; N, 3.70. $C_{21}H_{29}O_4N$ requires: C, 70.20; H, 8.15; N, 3.70%; δ (^{1}H, 500 MHz): 7.14–7.03 (m, 4H, phenyl-H), 4.44 and 4.26 (2×m, 2×1H, OC*H*$_2$), 3.75 and 3.69 (s, 3H, OMe), 3.25 and 3.11 (m, 1H, $CHCO_2Me$), 3.01 (m, 1H, C*H*$_2$CHCO$_2$Me) and 2.88–2.84 (m, 2H, NHC*H* and aliphatic-H), 2.49–2.11 (m, 6H, NH, C*H*$_2$CH$_2$O, C*H*$_2$CHCO$_2$Me and aliphatic-H), 1.93 (m, 1H, aliphatic-H) 1.24 (d, 2×3H, *J* 6.9 Hz, 2×CH_3) and 0.94 and 0.86 (d, 3H, *J* 6.5 Hz, CH_3); δ (^{13}C, 125 MHz): 178.35 and 174.41 (lactone-CO), 146.46 and 146.29 (ester-CO), 137.24 and 136.86, 129.49 and 128.95, and 126.19 and 125.93 (Ar-C), 68.96 and 68.45 (NHCH), 65.53 and 65.33 (spiro-C), 65.13 and 64.82 (CH_2O), 51.72 and 51.56 (CO_2CH_3), 47.33 and 47.17 ($CHCO_2Me$), 41.16 and 40.91, 40.63 and 40.36, 38.26 and 38.12 (C*H*$_2$CHCO$_2$Me, *C*H$_2$CH$_2$O and aliphatic-CH$_2$), 36.25 and 35.68 (aliphatic-CH), 33.55 (aliphatic-CH), 23.94 (2×CH_3), and 17.0 and 16.92 (CH_3); ν_{max} (film): 2959, 1774, 1728, 1508, 1457, 1436, 1374, 1293 and 1170 cm^{-1}; *m*/*z* (%): 359 (M$^+$, 3), 344 (2), 315 (37), 301 (6), 198 (87), 166 (31) and 154 (100).

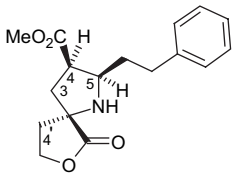
2.3.11. Methyl 2-(2-methyl-4-phenylbutyl)-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9k). A mixture of imine **7k** (1.00 g, 3.85 mmol), triethylamine (0.60 mL, 4.2 mmol), silver oxide (0.089 g, 0.38 mmol) and methyl acrylate (0.40 mL, 4.6 mmol) in toluene (40 mL) was stirred for 4 h. Work-up followed by flash chromatography eluting with ether afforded the product (0.91 g, 68%) as a colourless oil, which comprised a 1:1 mixture of diastereomers. Found: C, 69.80; H, 8.00; N, 4.30. $C_{20}H_{27}O_4N$ requires: C, 69.55; H, 7.90; N, 4.05%; δ (^{1}H, 250 MHz): 7.30–7.15 (m, 5H, phenyl-H), 4.37 and 4.20 (2×m, 2×1H, OC*H*$_2$), 3.69 and 3.63 (s, 3H, OMe), 3.39 (m, 1H, NHC*H*), 3.12 (m, 1H, $CHCO_2Me$), 2.64–2.60 (m, 2H, aliphatic-H), 2.40 (dd, 1H, *J* 6.6 and 13.2 Hz, C*H*$_2$CHCO$_2$Me), 2.22–2.15 (m, 3H, NH and C*H*$_2$CH$_2$O), 2.07 (dd, 1H, *J* 8.1 and 13.2 Hz, C*H*$_2$CHCO$_2$Me), 1.67–1.43 (m, 5H, aliphatic-H) and 1.01 and 0.96 (d, 3H, *J* 6.3 Hz, CH_3); ν_{max} (film): 2950, 1773, 1731, 1496, 1455, 1374, 1288, 1213 and 1172 cm^{-1}; *m*/*z* (%): 345 (M$^+$, 3), 314 (3.5), 301 (32), 242 (20), 198 (21), 154 (100) and 91 (12).

2.3.12. Methyl 6-oxo-2-(2-phenylpropyl)-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9l). A mixture of imine **7l** (2.16 g, 9.35 mmol), triethylamine (1.40 mL, 10.2 mmol), silver oxide (0.21 g, 0.93 mmol) and methyl acrylate (1.00 mL, 11.2 mmol) in toluene (60 mL) was stirred for 6 h. Work-up followed by flash chromatography eluting with ether separated the 1:1 isomer mixture (combined yield 2.12 g, 71%).

First eluting isomer: Crystallised from ether as colourless plates, mp 90–92 °C. Found: C, 68.00; H, 7.50; N, 4.40. $C_{18}H_{23}O_4N$ requires: C, 68.10; H, 7.30; N, 4.40%; δ (^{1}H, 250 MHz): 7.29–7.18 (m, 5H, phenyl-H), 4.35 and 4.20 (2×m, 2×1H, CH_2O), 3.71 (s, 3H, OMe), 2.98–2.94 (m, 3H, NHC*H*, $CHCO_2Me$ and aliphatic-H), 2.40 (m, 1H, C*H*$_2$CHCO$_2$Me), 2.19–2.02 (m, 4H, NH, C*H*$_2$CHCO$_2$Me and C*H*$_2$CH$_2$O), 1.73–1.63 (m, 2H, aliphatic-H) and 1.27 (d, 3H, *J* 7.0 Hz, CH_3); ν_{max} (film): 2959, 1773, 1730, 1452, 1436, 1373, 1215, 1173, 1109, 1076 and 1021 cm^{-1}; *m*/*z* (%, ES): 318 (M$^+$+1, 100).

Second eluting isomer: Crystallised from ether as colourless plates, mp 83–85 °C. Found: C, 68.10; H, 7.50; N, 4.40. $C_{18}H_{23}O_4N$ requires: C, 68.10; H, 7.30; N, 4.40%; δ (^{1}H, 250 MHz): 7.33–7.19 (m, 5H, phenyl-H), 4.34 and 4.16 (2×m, 2×1H, CH_2O), 3.71 (s, 3H, OMe), 3.20–3.11 (m, 2H, NHC*H* and $CHCO_2Me$), 2.86 (m, 1H, aliphatic-H), 2.42 (dd, 1H, *J* 6.3 and 13.2 Hz, C*H*$_2$CHCO$_2$Me), 2.19 (b, 1H, NH), 2.16–2.02 (m, 3H, C*H*$_2$CHCO$_2$Me and C*H*$_2$CH$_2$O), 1.86 and 1.67 (2×m, 2×1H, aliphatic-H) and 1.25 (d, 3H, *J* 6.9 Hz, CH_3); ν_{max} (film): 2959, 1773, 1730, 1437, 1374, 1214, 1173, 1118, 1078 and 1019 cm^{-1}; *m*/*z* (%, ES): 318 (M$^+$+1, 100).

2.3.13. Methyl 6-oxo-2-(2-phenylethyl)-7-oxa-1-azaspiro-[4.4]nonane-3-carboxylate (9m). A mixture of imine **7m** (1.00 g, 4.6 mmol), triethylamine (0.70 mL, 5.62 mmol), silver oxide (0.11 mg, 0.46 mmol) and methyl acrylate (0.50 mL, 5.5 mmol) in toluene (30 mL) was stirred for 6 h. Work-up followed by flash chromatography eluting with ether afforded the product (1.20 g, 86%), which crystallised from dichloromethane/hexane as colourless plates, mp 63–65 °C. Found: C, 67.05; H, 7.05; N, 4.55. $C_{17}H_{21}O_4N$ requires: C, 67.30; H, 7.00; N, 4.60%; δ (^{1}H, 500 MHz): 7.29–7.25 (m, 2H, phenyl-H), 7.20–7.17 (m, 3H, phenyl-H), 4.39 (ddd, 1H, *J* 3.8, 7.8 and 9.1 Hz, CH_2O), 4.21 (m, 1H, CH_2O), 3.69 (s, 3H, OMe), 3.30 (ddd, 1H, *J* 5.2, 7.6 and 8.8 Hz, NHC*H*), 3.14 (q, 1H, *J* 7.5 Hz, $CHCO_2Me$), 2.81 and 2.67 (2×m, 2×1H, aliphatic-H), 2.46 (dd, 1H, *J* 7.3 and 13.2 Hz, C*H*$_2$CHCO$_2$Me), 2.28–2.21 (m, 2H, NH and C*H*$_2$CH$_2$O), 2.14 (m, 1H, C*H*$_2$CH$_2$O), 2.08 (dd, 1H, *J* 8.0 and 13.2 Hz, C*H*$_2$CHCO$_2$Me) and 1.86–1.74 (m, 2H, aliphatic-H); ν_{max} (film): 2949, 1772, 1729, 1496, 1455, 1436, 1373, 1278, 1214, 1172 and 1074 cm^{-1}; *m*/*z* (%, ES): 304 (M$^+$+1, 100). In the NOE 4′-H proton was overlapped with aliphatic protons.



NOE data for **9m**:

Signal irradiated	Enhancement (%)			
	5-H	4-H	3-H	Aliphatic-H
5-H		6.6	—	4.8
4-H	4.3		5.2	—

2.3.14. Methyl 2-(1-*tert*.-butoxycarbonyl)amino-2-phenylethyl-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9n). A mixture of imine **7n** (1.23 g, 3.7 mmol), triethylamine (0.57 mL, 4.0 mmol), silver oxide (0.085 g, 0.37 mmol) and methyl acrylate (0.40 mL, 4.4 mmol) in toluene (30 mL) was stirred for 3 h. Wok-up followed by flash chromatography eluting with ether separated the 1:1 isomer mixture (combined yield 0.72 g, 46%).

First eluting isomer: Crystallised from dichloromethane/hexane as colourless plates, mp 65–67 °C. $[\alpha]_D^{18}$ +10.98 (*c* 1.02, $CHCl_3$). Found: C, 63.20; H, 7.40; N, 6.50. $C_{22}H_{30}O_6N_2$ requires: C, 63.15; H, 7.20; N, 6.70%; δ (^{1}H, 250 MHz): 7.31–7.19 (m, 5H, phenyl-H), 6.04 (d, 1H, *J* 10.3 Hz, amide-NH), 4.38 (m, 1H, CH_2O), 4.21–4.07 (m, 2H, CH_2O and amide NHC*H*), 3.72 (s, 3H, OMe), 3.68 (m, 1H, NHC*H*), 3.20 (m, 1H, $CHCO_2Me$), 2.87–2.55 (m, 3H, C*H*$_2$CHCO$_2$Me and aliphatic-H), 2.24–2.05 (m, 4H, C*H*$_2$CH$_2$O, NH and C*H*$_2$CHCO$_2$Me) and 1.36 (s, 3×H, 3×CH_3); ν_{max} (film): 3346, 2977, 1767, 1733, 1700, 1653, 1558, 1539, 1506, 1496, 1390, 1225 and 1172 cm^{-1}; *m*/*z* (%): 419 (M$^+$+1, 1.5), 374 (4), 345 (11), 227 (29), 198 (100), 166 (34), 120 (80) and 91 (41).

Second eluting isomer: Crystallised from dichloromethane/hexane as colourless needles, mp 178–180 °C. $[\alpha]_D^{18}$ −13.3 (*c* 0.96, $CHCl_3$). Found: C, 62.90; H, 7.20; N, 6.40. $C_{22}H_{30}O_6N_2$ requires: C, 63.15; H, 7.20; N, 6.70%; δ (^{1}H, 250 MHz): 7.31–7.18 (m, 5H, phenyl-H), 4.50 (d, 1H, *J* 9.2 Hz, amide-NH), 4.43 (ddd, 1H, *J* 4.4, 7.8 and 9.2 Hz, CH_2O), 4.24 (dt, 1H, *J* 6.8 and 9.0 Hz, CH_2O), 3.93 (m, 1H, amide NHC*H*), 3.70 (s, 3H, OMe), 3.40 (m, 1H, NHC*H*), 3.10 (q, 1H, *J* 7.1 Hz, $CHCO_2Me$), 3.01–2.98 (m, 2H, aliphatic-CH_2), 2.50–2.09 (m, 5H, NH, C*H*$_2$CHCO$_2$Me and C*H*$_2$CH$_2$O) and 1.38 (s, 3×H, 3×CH_3); ν_{max} (film): 3371, 2978, 2949, 1775, 1754, 1730, 1700, 1518, 1448, 1430, 1367, 1316 and 1273 cm^{-1}; *m*/*z* (%): 418 (M$^+$, <1), 345 (7), 301 (10), 227 (20), 198 (100), 166 (29), 120 (84) and 91 (47).

2.3.15. Methyl 2-[2-(1,3-benzodioxol-5-yl)-1-methylethyl]-6-oxo-7-oxa-1-azaspiro[4.4]nonane-3-carboxylate (9o). A mixture of imine **7o** (1.37 g, 5.0 mmol), triethylamine (0.80 mL, 10.2 mmol), silver oxide (0.12 g, 0.5 mmol) and methyl acrylate (0.56 mL, 6.3 mmol) in toluene (30 mL) was stirred for 4 h. Work-up followed by flash chromatography eluting with ether separated the 3:1 mixture of isomers (combined yield 1.45 g, 80%).

First eluting isomer: Crystallised from dichloromethane/hexane as colourless plates, mp 85–87 °C. Found: C, 63.00; H, 6.45; N, 3.90. $C_{19}H_{23}O_6N$ requires: C, 63.15; H, 6.40; N, 3.90%; δ (^{1}H, 250 MHz): 6.73–6.62 (m, 4H, phenyl-H), 5.92 (s, 2H, OCH_2O), 4.46 (ddd, 1H, *J* 4.7, 7.6 and 9.0 Hz, CH_2O), 4.27 (m, 1H, CH_2O), 3.70 (s, 3H, OMe), 3.09 (m, 1H, $CHCO_2Me$), 2.94 (dd, 1H, *J* 3.5 and 13.4 Hz, C*H*$_2$CHCO$_2$Me), 2.82 (m, 1H, NHC*H*), 2.62–2.08 (m, 6H, NH, C*H*$_2$CHCO$_2$Me, C*H*$_2$CH$_2$O and aliphatic-H), 1.89 (m, 1H, aliphatic-H) and 0.86 (d, 3H, *J* 6.7 Hz, CH_3); ν_{max} (film): 2915, 1774, 1727, 1502, 1489, 1440, 1373, 1294 and 1212 cm^{-1}; *m*/*z* (%): 361 (M$^+$, 13), 317 (32), 198 (85), 166 (42), 154 (100), 135 (82) and 94 (41).

Second eluting isomer: Crystallised from dichloromethane/hexane as colourless plates, mp 90–92 °C. Found: C, 62.80; H, 6.35; N, 3.60. $C_{19}H_{23}O_6N$ requires: C, 63.15; H, 6.40; N, 3.90%; δ (^{1}H, 250 MHz): 6.74–6.54 (m, 4H, phenyl-H), 5.93 (s, 2H, OCH_2O), 4.42 (ddd, 1H, *J* 4.1, 7.8 and 9.1 Hz, CH_2O), 4.23 (m, 1H, CH_2O), 3.76 (s, 3H, OMe), 3.24 (ddd, 1H, *J* 4.8, 6.9 and 8.3 Hz, $CHCO_2Me$), 2.99 (m, 1H, NHC*H*), 2.89 (m, 1H, aliphatic-H), 2.44 (dd, 1H, *J* 4.8 and 13.4 Hz, C*H*$_2$CHCO$_2$Me), 2.35–2.10 (m, 4H, C*H*$_2$CHCO$_2$Me, C*H*$_2$CH$_2$O and aliphatic-H), 1.90 (m, 1H, aliphatic-H), 1.68 (b, 1H, NH) and 0.94 (d, 3H, *J* 6.5 Hz, CH_3); ν_{max} (film): 2951, 1772, 1726, 1503, 1489, 1440, 1443 and 1188 cm^{-1}; *m*/*z* (%): 361 (M$^+$, 16), 317 (38), 225 (19), 198 (100), 166 (39), 154 (58), 135 (63) and 94 (35).

2.3.16. Methyl 2-(8,8-dimethyl-1,2,3,4,5,6,7,8-octahydro-2-naphthalenyl)-6-oxo-7-thia-1-azaspiro[4.4]nonane-3-carboxylate (10a). A mixture of imine **8a** (1.00 g, 3.4 mmol), DBU (0.50 mL, 3.4 mmol), silver oxide (0.079 g, 0.34 mmol) and methyl acrylate (0.37 mL, 4.0 mmol) in toluene (40 mL) was stirred for 4 h. Work-up followed by flash chromatography eluting with 3:2 v/v ether/hexane afforded the product (0.44 g, 34%) as a colourless gum, which comprised a 1:1 mixture of diastereomers. Found: C, 67.10; H, 8.35; N, 3.50; S, 8.30. $C_{21}H_{31}O_3NS$ requires: C, 66.80; H, 8.30; N, 3.70; S, 8.50%; δ (^{1}H, 250 MHz): 3.68 and 3.67 (s, 3H, OMe), 3.26–3.20 (m, 3H, CH_2S and $CHCO_2Me$), 2.95 (m, 1H, NHC*H*) and 2.30–1.40 (m, 18H, NH, C*H*$_2$CHCO$_2$Me, C*H*$_2$CH$_2$S and aliphatic-H) and 0.95 (s, 2×3H, 2×CH_3); ν_{max} (film): 2926, 1731, 1698, 1436, 1199 and 1168 cm^{-1}; *m*/*z* (%): 378 (M$^+$+1, 34), 349 (6), 334 (11), 316 (37), 290 (36), 187 (74), 128 (69) and 94 (25).

2.3.17. Methyl 2-[4-(4-methyl-3-pentenyl)-3-cyclohexen-1-yl]-6-oxo-7-thia-1-azaspiro[4.4]nonane-3-carboxylate (10b). A mixture of imine **8b** (1.20 g, 4.1 mmol), DBU (0.62 mL, 4.1 mmol), silver oxide (0.095 g, 0.41 mmol) and methyl acrylate (0.45 mL, 4.9 mmol) in toluene (50 mL) was stirred for 3 h. Work-up followed by flash chromatography eluting with 3:2 v/v ether/hexane afforded the product (1.00 g, 64%) as a pale yellow oil, which comprised a 1:1 mixture of diastereomers. Found: C, 67.00; H, 8.45; N, 3.90; S, 8.30. $C_{21}H_{31}O_3NS$ requires: C, 66.80; H, 8.30; N, 3.70; S, 8.50%; δ (^{1}H, 250 MHz): 5.38 and 5.36 (m, 1H, cyclohexenyl olefinic-H), 5.08 (m, 1H, olefinic-H), 3.69 and 3.68 (s, 3H, OMe), 3.28–2.92 (m, 4H, CH_2S, $CHCO_2Me$ and NHC*H*), 2.27–1.89 (m, 15H, NH, C*H*$_2$CH$_2$S, C*H*$_2$CHCO$_2$Me and aliphatic-H), 1.68 and 1.60 (s, 2×3H, 2×CH_3) and 1.35 (m, 1H, aliphatic-H); ν_{max} (film): 2915,

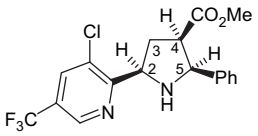
1731, 1697, 1436, 1374, 1272, 1199 and 1168 cm^{-1}; *m/z* (%): 378 (M^++1, 9), 349 (67), 316 (49), 280 (100), 187 (45), 128 (53) and 69 (59).

2.3.18. Methyl 2-[2-(4-isopropylphenyl)-1-methylethyl]-6-oxo-2-phenyl-7-thia-1-azaspiro[4.4]nonane-3-carboxylate (10c). A mixture of imine **8c** (1.57 g, 5.4 mmol), DBU (0.80 mL, 5.42 mmol), silver oxide (0.125 g, 0.54 mmol) and methyl acrylate (0.60 mL, 6.5 mmol) in toluene (60 mL) was stirred for 5 h. Work-up followed by flash chromatography eluting with 2:1 v/v ether/hexane separated the 3:1 mixture of diastereomers (combined yield 1.31 g, 64%).

First eluting isomer: Crystallised from dichloromethane/hexane as a colourless amorphous powder, mp 75–77 °C. Found: C, 67.40; H, 8.05; N, 3.75; S, 8.50. $C_{21}H_{29}O_3NS$ requires: C, 67.15; H, 7.80; N, 3.75; S, 8.55%; δ (^{1}H, 250 MHz): 7.13–7.08 (m, 4H, phenyl-H), 3.67 (s, 3H, OMe), 3.32–3.23 (m, 2H, CH_2S), 3.06–2.89 (m, 2H, $CHCO_2Me$ and aliphatic-H), 2.87–2.82 (m, 2H, NHC*H* and aliphatic-H), 2.48 (dd, 1H, *J* 8.8 and 13.3 Hz, CH_2CHCO$_2$Me), 2.29–2.22 (m, 4H, NH, CH_2CH$_2$S and aliphatic-H), 2.10 (m, 1H, CH_2CHCO$_2$Me), 1.90 (m, 1H, aliphatic-H), 1.24 (d, 2×3H, *J* 6.9 Hz, 2×CH_3) and 0.85 (d, 3H, *J* 6.6 Hz, CH_3); ν_{max} (film): 2958, 1731, 1698, 1436, 1199 and 1170 cm^{-1}; *m/z* (%): 376 (M^++1, <1), 347 (30), 314 (20), 288 (81), 187 (58), 128 (62) and 91 (100).

Second eluting isomer: Pale yellow oil. Found: C, 67.20; H, 7.90; N, 3.45; S, 8.40. $C_{21}H_{29}O_3NS$ requires: C, 67.15; H, 7.80; N, 3.75; S, 8.55%; δ (^{1}H, 250 MHz): 7.27–7.01 (m, 4H, phenyl-H), 3.73 (s, 3H, OMe), 3.28–3.17 (m, 3H, CH_2S, and $CHCO_2Me$), 3.03 (dd, 1H, *J* 6.7 and 8.8 Hz, NHC*H*), 2.95–2.84 (m, 2H, CH_2CHCO$_2$Me and aliphatic-H), 2.32–2.04 (m, 6H, NH, CH_2CH$_2$S, CH_2CHCO$_2$Me and aliphatic-H), 1.93 (m, 1H, aliphatic-H), 1.23 (d, 2×3H, *J* 6.9 Hz, 2×CH_3) and 0.94 (d, 3H, *J* 6.5 Hz, CH_3); *m/z* (%): 376 (M^++1, 1), 347 (68), 314 (37), 288 (100), 214 (25), 187 (61) and 91 (16).

2.3.19. Methyl 5-[2-chloro-4-(trifluoromethyl)phenyl]-2-phenylpyrrolidine-3-carboxylate (17). A mixture of imine **15** (0.30 g, 1.0 mmol), triethylamine (0.15 mL, 1.1 mmol), silver oxide (0.023 g, 0.1 mmol) and methyl acrylate (0.11 mL, 1.2 mmol) in toluene (10 mL) was stirred for 5 h. Work-up followed by flash chromatography eluting with ether afforded the product (0.17 g, 44%), which crystallised from dichloromethane/hexane as colourless plates, mp 145–147 °C. Found: C, 56.00; H, 4.25; N, 7.20; Cl, 9.30. $C_{18}H_{16}O_2N_2ClF_3$ requires: C, 56.20; H, 4.20; N, 7.30; Cl, 9.20%; δ (^{1}H, 500 MHz): 8.83 (d, 1H, *J* 0.9 Hz, pyridyl-H), 7.94 (s, 1H, pyridyl-H), 7.39 and 7.33 (2×m, 2×2H, phenyl-H), 7.28 (m, 1H, phenyl-H), 4.83 (apparent t, 1H, *J* 8.2 Hz, NHC*H*-pyridyl), 4.68 (d, 1H, *J* 8.1 Hz, NHC*H*-phenyl), 3.89 (b, 1H, NH), 3.51 (dt, 1H, *J* 6.6 and 8.1 Hz, $CHCO_2Me$), 3.18 (s, 3H, OMe), 2.71 (m, 1H, CH_2CHCO$_2$Me) and 2.14 (ddd, 1H, *J* 6.6, 9.0 and 13.0 Hz, CH_2CHCO$_2$Me); ν_{max} (film): 1732, 1603, 1456, 1436, 1322, 1200, 1162 and 1134 cm^{-1}; *m/z* (%): 385 (M^+, 0.7), 383 (M^+, 2), 367 (1.5), 365 (4), 300 (32), 298 (100), 284 (31), 282 (90), 223 (13), 221 (41) and 91 (20).



NOE data for **17**:

Signal irradiated	Enhancement (%)				
	5-H	4-H	3-H	2-H	Ar-H
5-H		9.4	—	2.0	8.4
4-H	8.3		5.1	1.2	2.0
2-H	1.6	1.6	4.8		—

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Synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-diones

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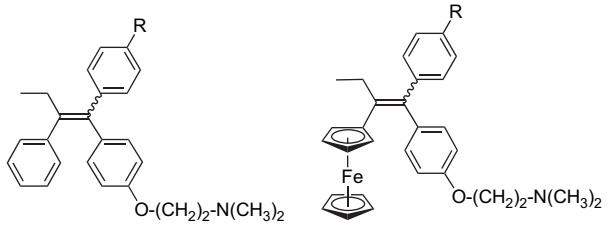
Abstract—A squarate-based synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-diones is described. When refluxed in dioxane at 100 °C, heated with silica gel as a solvent free grinded solid mixture at 125 °C or stirred with silica gel in ethyl acetate at room temperature, 4-ferrocenylethynyl-4-hydroxy-2-cyclobutenones, prepared from ethynylferrocene and 3-cyclobutene-1,2-diones, afforded 2-ferrocenylidene-4-cyclopentene-1,3-diones as the major or single product of the reaction. In some cases, ferrocenyl quinones also resulted from these reactions as the minor products. The major or exclusive formation of 2-ferrocenylidene-4-cyclopentene-1,3-diones is attributed to the radical-stabilizing ability of the ferrocenyl group.

1. Introduction

The great interest in ferrocenyl-substituted organic compounds is associated both with the peculiar chemical behavior of the ferrocene systems and with the unusual properties it imparts on the organic moiety.[1] Due to its unique structure, different membrane-permeation properties, and anomalous metabolism, ferrocene is often incorporated into a compound in order to obtain unexpected or enhanced biological activities.[2,3] A successful example is hydroxyferrocifen, which was obtained by replacing the phenyl ring of hydroxytamoxifen by a ferrocenyl group (Fig. 1).[3] Hydroxyferrocifen is the first molecule shown to be active against both hormone-dependent and hormone-independent breast cancer cells.[3] In contrast, hydroxytamoxifen, the active metabolite of tamoxifen, is active only against hormone-dependent cancer cells.[4] Notably, the integration of a ferrocenyl group into the structure generates surprising antiproliferative effects on both type of cancer cells. It appears so far that ferrocene derivatives act via mechanisms different from those of cisplatin and thus may lend themselves to treatment of a wider range of cancers. In general, the antitumor effect of ferrocene compounds is attributed to the redox properties of the central iron atom, in that only the oxidation state +3 (as in ferrocenium cations), which is readily produced by biological oxidation, exhibits inhibitory effects.[5] Therefore, in recent years, considerable interest has been devoted to the synthesis of new ferrocene derivatives, which could be potential antitumor substances.[6,7]



Tamoxifen (R = H)
Hydroxytamoxifen (R = OH)

Ferrocifen (R = H)
Hydroxyferrocifen (R = OH)

Figure 1. Structures of tamoxifens and ferrocifens.

The rapid spread of cancer has sparked an intense chemical search for new structure leads, which may be of use in designing novel antitumor drugs. In this regard, the 2-methylene-4-cyclopentene-1,3-dione pharmacophore (**1**) has occupied a unique position in the design and synthesis of novel biologically active agents that exert remarkable anticancer activities (Fig. 2). Inayama and co-workers synthesized a series of 2-arylidene-4-cyclopentene-1,3-diones (**2**) and examined their antitumor activity.[8] All compounds exhibited a high degree of activity, but the 3-methoxy-4-hydroxybenzylidene derivatives possessed the greatest potency.[8] Recently, using this innovative pharmacophore, Hori and co-workers have prepared new derivatives of 2-hydroxyarylidene-4-cyclopentene-1,3-diones as new candidates for antitumor agents.[9] Their comprehensive evaluation of these agents with respect to protein tyrosine kinase (PTK) inhibition, mitochondrial inhibition, antitumor activity, and hepatotoxicity demonstrates that PTK inhibitors TX-1123 and TX-1925 (Fig. 2) are more promising

Keywords: Ferrocenylidenecyclopentenediones; Ferrocenyl quinones; Alkynylcyclobutenones; Cyclobutenediones; Cyclobutenols; Rearrangement; Radicals.
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URL: http://www.chem.metu.edu.tr/academic/zora/index.htm

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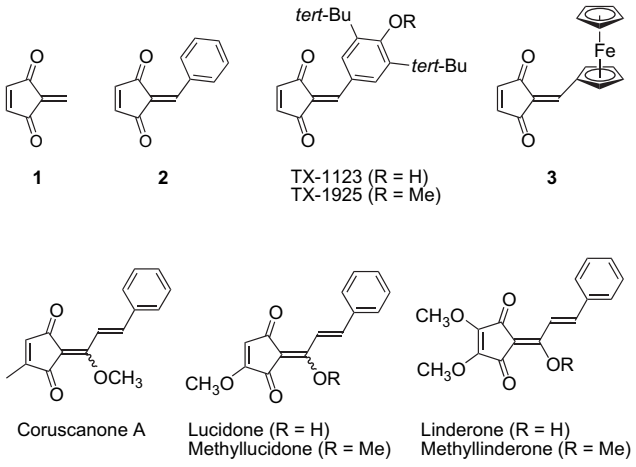


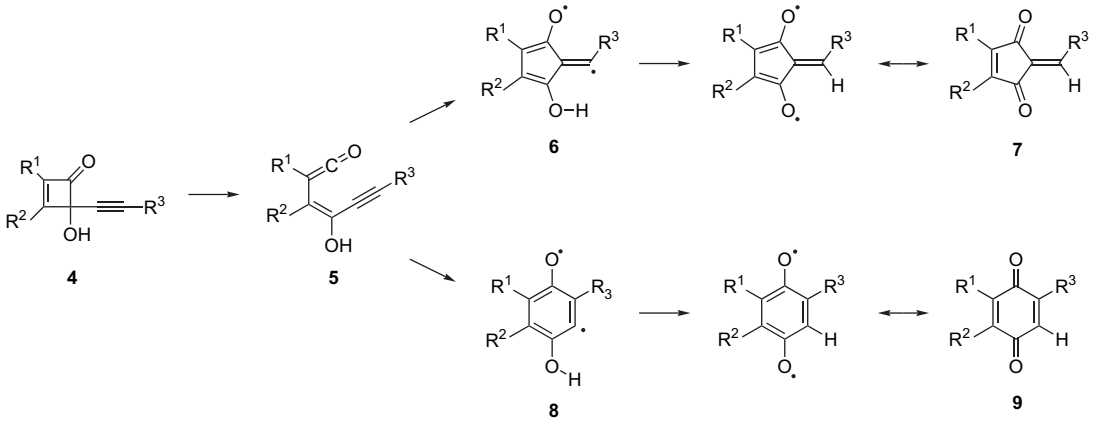
Figure 2. Structures of 2-methylene-4-cyclopentene-1,3-dione (**1**) and related pharmacophores and molecules.

candidates for antitumor agents than well known compound tyrphostin AG17.[9] Naturally occurring lucidone, linderone, and their methyl derivatives methyllucidone and methyllinderone,[10,11] and coruscanone A[12] also contain a 2-methylene-4-cyclopentene-1,3-dione (**1**) pharmacophore in their structures and show farnesyl protein transferase inhibition, antitumor, and/or antifungal activities.

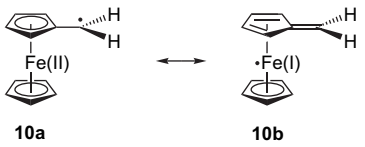
Our attention was then directed toward the synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-dione derivatives, such as **3**, since the incorporation of the essential structural features of 2-methylene-4-cyclopentene-1,3-dione (**1**) pharmacophore with a ferrocenyl moiety could provide compounds with enhanced antitumor activities. Surprisingly, 2-ferrocenylidene-4-cyclopentene-1,3-diones (**3**) are not known. The development of a general synthetic entry to such compounds is therefore of considerable interest since it could lead to a new source of biologically active compounds.

Recently, as shown by Moore and co-workers,[13] 4-alkynyl-4-hydroxycyclobutenones (**4**) have emerged as valuable reagents in organic synthesis since such cyclobutenones undergo a remarkably selective electrocyclic ring opening to give the corresponding conjugated ketenes **5** (Scheme 1). Ketenes **5** then experience five- and/or six-membered ring closure to afford diradicals **6** and/or **8**, which finally lead to 2-alkylidene-4-cyclopentene-1,3-diones (**7**) and/or benzoquinones (**9**) after an intramolecular transfer of the H atom. The selectivity of the rearrangement to give either cyclopentenediones **7** or benzoquinones **9** is significantly influenced by the R^3 substituent in that radical-stabilizing groups, such as alkoxy, phenyl, and trimethylsilyl, favor exclusively, or in part, the cyclopentenedione formation.[14,15] As suggested by Moore,[13b,c] the aromatic stabilization associated with six-membered ring formation is apparently outweighed by direct stabilization of the vinyl radical by the adjacent R^3 substituent when five-membered ring formation takes place. Recently, Engels and co-workers have theoretically studied the substituent effects on the cyclization of 1,3-hexadiene-5-ynone derivatives to the corresponding five- and six-membered diradicals at the density functional theory (DFT) level (B3LYP/6-31G*).[16] They have found that, in addition to a radical-stabilizing group such as Ph as the alkyne substituent (R^3), electron donor groups such as OH and OMe at the other positions are required to make five-membered ring formation as the major pathway. The nature of certain electronic effects of a ferrocenyl substituent was studied by Nesmeyanov et al.[17] It was found that the ferrocenyl substituent exhibits a strong positive inductive effect and a weak positive conjugation effect. Recently, Creary et al. examined the quantitative ability of the ferrocenyl group to stabilize free radicals by employing the experimental methylenecyclopropane rearrangement probe.[18] They found that a ferrocenyl group is 1.6 times better at stabilizing an α radical than a phenyl group. Computational studies have also been carried out in order to gain further insight into the radical-stabilizing ability of ferrocenyl group.[19] DFT (B3LYP/LANL2DZ) calculations on ferrocenyl-substituted methyl radical **10** showed that the radical-stabilizing ability of the ferrocenyl group can be explained by a spin delocalization mechanism involving the Fe atom and a major contribution from an η^4-form, as represented by **10b** (Scheme 2), where the iron is formally a 17-electron system in the +1 oxidation state. Moreover, calculations at the same level indicated that ferrocenylmethyl radical **10** is more stable than the benzyl radical by 1.5 kcal mol^{-1}, in agreement with the experimental results.[19]

In light of these results, it is expected that the thermal rearrangement of alkynylcyclobutenones **4** bearing a ferrocenyl



Scheme 1. Mechanism for the formation of 2-alkylidenecyclopentenediones **7** and benzoquinones **9** from 4-alkynylcyclobutenones **4**.



Scheme 2. Stabilization of ferrocenylmethyl radical.

group as the R^3 substituent should produce 2-ferrocenylidene-4-cyclopentene-1,3-dione derivatives as the major product of the reaction. This methodology, however, has not been utilized for the synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-diones, presumably due to the scarce availability of the starting ferrocenylcyclobutenones. As part of our general involvement in ferrocene-containing potential pharmaceuticals, we have investigated the synthesis of ferrocenylcyclobutenones and their rearrangements to 2-ferrocenylidene-4-cyclopentene-1,3-diones.[20] We herein report the results of this study.

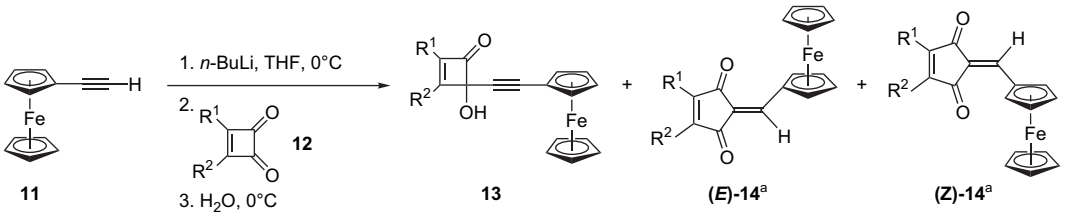
2. Results and discussion

Initially, starting materials were prepared. The synthesis of ethynylferrocene **11** was accomplished from acetylferrocene in two steps according to a well known literature procedure[21] (acetylferrocene is readily available in large quantities from ferrocene according to a standard protocol).[22] Treatment of acetylferrocene with phosphorus oxychloride in DMF led to (2-formyl-1-chlorovinyl)ferrocene, which upon base-induced elimination using aqueous sodium hydroxide in dioxane provided ethynylferrocene **11** in good yield.[21] Cyclobutenediones **12A–D** were prepared from squaric acid according to Liebeskind's procedure.[23] For the synthesis of diphenylcyclobutenedione **12E**, squaric acid was first reacted with thionyl chloride to afford semisquaric chloride.[24] Friedel–Crafts reaction of semisquaric chloride with ferrocene in the presence of $AlCl_3$ produced cyclobutenedione **12E**.[25]

We next synthesized 4-ferrocenylethynylcyclobutenones **13** as shown in Table 1. Treatment of ethynylferrocene **11** with *n*-butyllithium produced in situ lithioethynylferrocene that was further reacted with cyclobutenediones **12** to yield the corresponding cyclobutenones **13**. It should be noted that 4-ferrocenylethynyl-substituted cyclobutenones **13**, especially 2-phenyl-substituted cyclobutenones **13C** and **13E**, were found to be quite reactive and, during the isolation, they partly decomposed and/or rearranged to the corresponding 2-ferrocenylidenecyclopentenediones **14** in varying amounts. Interestingly, 2-methyl-substituted cyclobutenones **13B** and **13D** were insoluble in hexane and it was possible to obtain these compounds in pure form by filtrating their hexane solution. The easy isolation of these derivatives prevented in part their rearrangement to the corresponding cyclopentenediones, and allowed us to have their pure samples for spectroscopic identification. Notably, 2-phenyl-substituted cyclobutenones **13C** and **13E** were highly reactive since they started more rapidly to decompose and/or undergo rearrangement, and it was not possible to obtain pure samples for characterization. That is why, after synthesis, 2-phenyl-substituted cyclobutenones **13C** and **13E** were isolated as crude products and immediately subjected to rearrangement. Moreover, it was observed that, during the chromatographic purification, silica gel accelerated the conversion of cyclobutenones **13** to cyclopentenediones **14** to some extent.

Subsequently, we investigated the rearrangements of 4-ferrocenylethynylcyclobutenones **13** to 2-ferrocenylidenecyclopentenediones **14**. The results are summarized in Table 2. In fact, for these conversions, we employed three different procedures. Firstly, we used a typical thermolysis procedure, which, in general, is the most commonly used protocol for such rearrangements. For this purpose, cyclobutenones **13** were heated in refluxing dioxane at 100 °C for 4 h (Method A). Recently, to run reactions on the surface of solids has attracted considerable interest since, in this way, reactions can be accelerated or new chemistry may occur.[26] We found that when heated with silica gel as a solvent free grinded solid mixture in an oven at 125 °C for a short reaction time, such as 15 min (Method B), cyclobutenones **13** were quickly rearranged to cyclopentenediones **14**. More importantly, stirring a mixture of cyclobutenones **13** and silica gel in ethyl

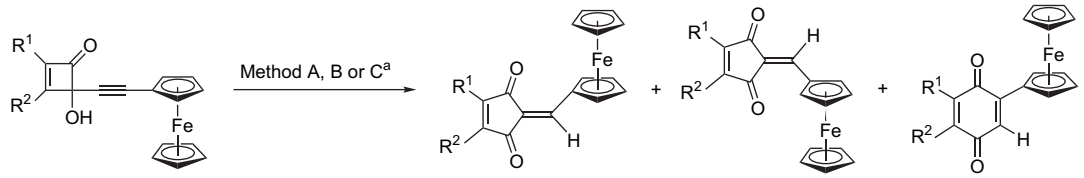
Table 1. Synthesis of 4-ferrocenylethynyl-4-hydroxy-2-cyclobutenones (**13**)



Entry	Starting compound	R^1	R^2	Products (Yields, %)[b]
1	**11A+12A**	*i*-PrO	*i*-PrO	**13A** (65)+**14A** (5)
2	**11A+12B**	Me	*i*-PrO	**13B** (34)+(*E*)-**14B** (19)+(*Z*)-**14B** (9)
3	**11A+12C**	Ph	*i*-PrO	**13C**[c]+(*E*)-**14C** (35)
4	**11A+12D**	Me	Me	**13D** (38)+**14D** (22)
5	**11A+12E**	Ph	Ph	**13E**[c]+**14E** (37)

[a] When R^1 and R^2 are the same, this structure presents compound **14**.
[b] Isolated yields.
[c] For this compound, yield calculation could not be made since, during isolation, it was continuously decomposed and/or rearranged to the corresponding cyclopentenedione. That is why this compound was isolated as a crude product and immediately subjected to rearrangement.

Table 2. Synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-diones (**14**)



13 **(*E*)-14**[b] **(Z)-14**[b] **15**

Entry	Starting compound	R^1	R^2	Method[a]	Products (Yields, %)[c]
1	**13A**	*i*-PrO	*i*-PrO	A	**14A** (70)+**15A** (2)
2	**13A**	*i*-PrO	*i*-PrO	B	**14A** (67)
3[d]	**13A**	*i*-PrO	*i*-PrO	C	**14A** (34)
4	**13B**	Me	*i*-PrO	A	(*E*)-**14B** (61)+(*Z*)-**14B** (3)+**15B** (4)
5	**13B**	Me	*i*-PrO	B	(*E*)-**14B** (45)+(*Z*)-**14B** (32)
6	**13B**	Me	*i*-PrO	C	(*E*)-**14B** (51)+(*Z*)-**14B** (6)
7	**13C**	Ph	*i*-PrO	A	(*E*)-**14C** (53)[e]
8	**13C**	Ph	*i*-PrO	C	(*E*)-**14C** (56)[e]
9	**13D**	Me	Me	A	**14D** (55)+**15D** (8)
10	**13D**	Me	Me	B	**14D** (71)+**15D** (5)
11	**13D**	Me	Me	C	**14D** (74)+**15D** (8)
12	**13E**	Ph	Ph	A	**14E** (58)[f]
13	**13E**	Ph	Ph	C	**14E** (45)[f]

[a] Method A: dioxane, 100 °C, 4 h; Method B: SiO_2, 125 °C, 15 min; Method C: SiO_2, ethyl acetate, 25 °C, 24 h.
[b] When R^1 and R^2 are the same, this structure presents compound **14**.
[c] Isolated yields.
[d] For this reaction, reaction time was 48 h.
[e] For this compound, yield was calculated from ethynylferrocene (**11**) since, in this reaction, crude cyclobutenone **13C**, obtained from **11**, was used.
[f] For this compound, yield was calculated from ethynylferrocene (**11**) since, in this reaction, crude cyclobutenone **13E**, obtained from **11**, was used.

acetate at room temperature for overnight (Method C) also afforded cyclopentenediones **14**, which is a clear indicative of the high reactivity of 4-ferrocenylethynyl-substituted cyclobutenones **13**. To the best of our knowledge, these last two procedures (Methods B and C) have not been used previously for effecting such rearrangements. In terms of chemical yields, all methods we used appear to be comparable with each other.

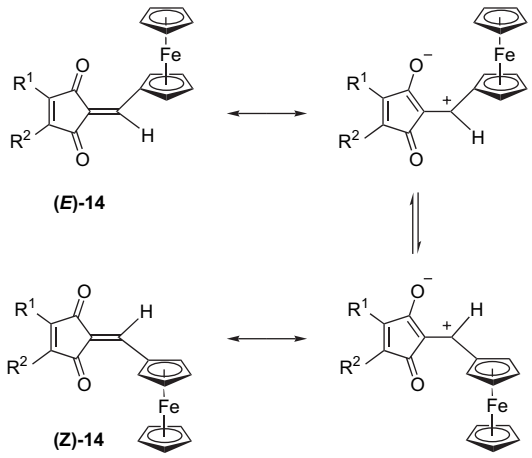
As can be seen in Table 2, all protocols produced the expected cyclopentenediones **14** as the major or single product of the reaction. From unsymmetrically substituted cyclobutenones **13B** and **13C** (Table 2, entries 4–8), mostly or exclusively *E* isomers of cyclopentenediones ((*E*)-**14**) were obtained. In reactions with **13B**, *Z* isomer ((*Z*)-**14**) was also observed but in minor amounts except the one case (Table 2, entry 5), in which *Z* isomer was the significant proportion of the product. In some cases, ferrocenyl quinones were also resulted from these reactions as the minor products.[27] On the basis of the mechanism in Scheme 1 as suggested by Moore,[13] the most or exclusive formation of 2-ferrocenylidenecyclopentenediones **14**, as compared to quinones, clearly shows the radical-stabilizing ability of the ferrocenyl group, a result consistent with the findings of Creary as well.[18,19]

As mentioned before, the reactions of Methods B and C were performed in the presence of silica gel. To verify the silica gel effect, the reactions of **13A** in entries 2 and 3 of Table 2 were repeated in the absence of silica gel. First reaction gave a very low yield of cyclopentenedione **14A**. In the second reaction, conversion to **14A** was almost insignificant. Both results clearly demonstrate that the use of silica gel in these reactions, i.e., in Methods B and C, is vital. The effect of silica gel should be similar to that of a weak Lewis and/or Brønsted acid.

It is interesting to note that 2-ferrocenylidenecyclopentenediones **14** and ferrocenyl quinones **15** can be easily differentiated from each other via their respective ^{1}H NMR spectra. The vinyl proton in quinones **15** appears at 6.67–6.80 ppm while that in cyclopentenediones **14** resonates at 7.22–7.69 ppm since the latter is conjugated to the two carbonyl groups and, as expected, it is more deshielded. In addition, during mass analysis under FAB conditions, ferrocenyl quinones **15** were reduced to the corresponding hydroquinones, as indicated by the MS and HRMS results, but such reductions were not observed for cyclopentenediones **14**. We also realized that ferrocenyl-substituted cyclopentenediones **14** and quinones **15** can be recognized by their colors since cyclopentenediones **14** are in claret red or purple color while quinones **15** are in green color.

The major or exclusive isomer of differently substituted cyclopentenediones **14B** and **14C** was assigned as the *E* isomer. As shown by Moore,[13] in the conversion of diradical intermediate **6** to alkylidenecyclopentenedione **7** (Scheme 1), the H atom migration occurs intramolecularly, which translates to the indicated *E* stereochemistry of the major or exclusive isomer of **14B** and **14C**. The formation of the minor *Z* isomer in some cases may not actually represent a new reaction pathway since it is a secondary product of the reaction and results from the initially formed *E* isomer through partial isomerization or equilibration. It was already shown that the treatment of *E* isomer of a 2-benzylidene-4-cyclopentene-1,3-dione derivative with silica gel resulted in its facile equilibration with the *Z* isomer.[13b] Similarly, when heated with silica gel as a grinded solid mixture at

125 °C for 1 h, pure (*E*)-**14B** equilibrated with its *Z* isomer. A possible mechanism for this isomerization is given in Scheme 3. During the *E*–*Z* isomerization, a positive charge develops at the exo β-carbon atom adjacent to ferrocenyl group (Scheme 3), but it is well stabilized since the ferrocenyl group is much more effective at carbocation stabilization than it is at radical stabilization.[18] In addition, the ferrocenyl group is a better carbocation stabilizing group than the phenyl group.[28]



Scheme 3. A possible mechanism for the *E*–*Z* isomerization of 2-ferrocenylidenecyclopentenediones **14**.

3. Conclusion

In summary, we have described a squarate-based synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-diones (**14**), which are the first examples of these kind, containing a ferrocene moiety. When refluxed in dioxane at 100 °C, heated with silica gel as a solvent free grinded solid mixture at 125 °C or stirred with silica gel in ethyl acetate at room temperature, 4-ferrocenylethynyl-4-hydroxy-2-cyclobutenones (**13**) afford 2-ferrocenylidenecyclopentenediones **14** as the major or single product of the reaction, accompanied by minor amounts of ferrocenyl quinones in some cases. The formation of 2-ferrocenylidene-4-cyclopentene-1,3-diones is attributed to the radical-stabilizing ability of the ferrocenyl group, which has not been utilized before in such reactions.

4. Experimental

4.1. General consideration

Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were recorded on a Bruker Spectrospin Avance DPX400 Ultrashield (400 MHz) spectrometer. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference. Coupling constants (*J* values) are reported in hertz (Hz) and spin multiplicities are indicated by the following symbols: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). DEPT ^{13}C NMR information is given in parenthesis as C, CH, CH_2, and CH_3. Infrared spectra were recorded on a Perkin–Elmer 1600 Series FT-IR spectrometer. Band positions are reported in reciprocal centimeters (cm^{-1}). Band intensities are reported relative to the most intense band and are listed as: br (broad), vs (very strong), s (strong), m (medium), w (weak), and vw (very weak). Mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained on a VG-7070E magnetic sector instrument using fast atom bombardment (FAB); *m*/*z* values are reported, followed by the relative intensity in parentheses. The matrix used for FAB was ethylene glycol or a mixture of dithiothritol and dithioerithitol. Flash chromatography was performed using thick-walled glass columns and 'flash grade' silica (Merck 230–400 mesh). Routine thin layer chromatography (TLC) was effected by using precoated 0.25 mm silica gel plates purchased from Merck. The relative proportion of solvents in mixed chromatography solvents refers to the volume:volume ratio. Ethynylferrocene (**11**)[21] and cyclobutenediones **12A**–**E** were synthesized according to the well known literature procedures.[23–25] All other commercially available reagents and reactants were obtained in reagent grade and used without purification. All reaction solvents were distilled for purity. Diethyl ether, THF, and dioxane were distilled from sodium/benzophenone ketyl. The inert atmosphere was created by slight positive pressure (ca. 0.1 psi) of argon.

4.2. General procedure for synthesis of 4-ferrocenylethynyl-4-hydroxy-2-cyclobutenones (13) (Table 1)

To a solution of ethynylferrocene (**11**)[21] (252 mg, 1.2 mmol) in THF (15 mL) at 0 °C under argon was added via syringe *n*-butyllithium (0.65 mL of a 1.7 M hexane solution, 1.1 mmol) over a period of 15 min. The mixture was stirred for 45 min at the same temperature, and then transferred via cannula to a solution of the corresponding cyclobutenedione **12**[23–25] (1.0 mmol) in THF (15 mL) at −78 °C under argon. The reaction mixture was stirred at −78 °C for 3 h and then quenched with water (10 mL) at −78 °C. The mixture was allowed to warm to room temperature and diluted with diethyl ether (50 mL). The layers were separated and the aqueous layer was extracted with ether (2×50 mL). After drying over $MgSO_4$, the combined organic layers were removed on a rotary evaporator. Final purification was achieved by flash chromatography on silica gel using 9:1 hexane/ethyl acetate followed by 4:1 hexane/ethyl acetate as the eluent. The products given in Table 1 were isolated with the indicated yields.

4.3. Spectral data for cyclobutenones 13

4.3.1. 4-Ferrocenylethynyl-4-hydroxy-2,3-diisopropoxy-2-cyclobutenone (13A). R_f=0.30 in 4:1 C_6H_{14}/EtOAc; off yellow solid; 265.3 mg (65%); ^{1}H NMR ($CDCl_3$): δ 5.01 (septet, 1H, *J*=6.1 Hz), 4.87 (septet, 1H, *J*=6.1 Hz), 4.41 (s, 2H), 4.17 (s, 7H), 3.10 (br s, 1H), 1.45 (d, 3H, *J*=6.1 Hz), 1.43 (d, 3H, *J*=6.1 Hz), 1.29 (d, 3H, *J*=6.1 Hz), 1.28 (d, 3H, *J*=6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 181.0 (C), 164.8 (C), 134.3 (C), 88.3 (C), 80.0 (C), 79.7 (C), 78.2 (CH), 74.5 (CH), 72.0 (CH), 70.4 (CH), 69.3 (CH), 63.9 (C), 23.1 (CH_3, two methyl carbons overlap), 22.9 (CH_3, two methyl carbons overlap); IR (CH_2Cl_2): 3358 (w), 3323 (br), 2975 (m), 2928 (m), 2223 (w), 1773 (s), 1627 (vs), 1458 (w), 1388 (s), 1322 (s), 1261 (s), 1096 (s) cm^{-1}; MS (FAB): 409 ($[M+H]^+$, 55), 408 ($[M]^+$, 100), 395 (11), 324 (19), 311 (6), 213 (18), 199 (7), 137 (17), 136 (16), 43 (11), 41 (9); HRMS (FAB) calcd for $C_{22}H_{24}FeO_4$: 408.1034. Found: 408.1024.

4.3.2. 4-Ferrocenylethynyl-4-hydroxy-3-isopropoxy-2-methyl-2-cyclobutenone (13B). R_f=0.25 in 4:1 C_6H_{14}/EtOAc; brownish yellow solid; 123.8 mg (34%); ^{1}H NMR ($CDCl_3$): δ 5.08 (septet, 1H, J=6.1 Hz), 4.40 (s, 2H), 4.17 (s, 7H), 3.34 (s, 1H), 1.68 (s, 3H), 1.50 (d, 3H, J=6.1 Hz), 1.46 (d, 3H, J=6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 187.6 (C), 180.3 (C), 124.9 (C), 89.4 (C), 84.2 (C), 79.9 (C), 78.5 (CH), 72.0 (CH), 70.4 (CH), 69.4 (CH), 63.8 (C), 23.4 (CH_3), 23.1 (CH_3), 7.0 (CH_3); IR (CH_2Cl_2): 3559 (w), 3308 (br), 2976 (w), 2926 (vw), 2223 (w), 1764 (s), 1623 (vs), 1463 (vw), 1397 (s), 1312 (s), 1097 (s) cm^{-1}; MS (FAB): 365 ([M+H]$^+$, 81), 364 ([M]$^+$, 87), 347 (82), 322 (100), 305 (25), 295 (16), 257 (96), 255 (13), 210 (11), 183 (6), 157 (8), 121 (20), 85 (9); HRMS (FAB) calcd for $C_{20}H_{20}FeO_3$: 364.0762. Found: 364.0775.

4.3.3. 4-Ferrocenylethynyl-4-hydroxy-2,3-dimethyl-2-cyclobutenone (13D). R_f=0.17 in 4:1 C_6H_{14}/EtOAc; brown solid; 121.6 mg (38%); ^{1}H NMR ($CDCl_3$): δ 4.39 (s, 2H), 4.18 (s, 2H), 4.16 (s, 5H), 2.58 (s, 1H), 2.20 (s, 3H), 1.75 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 189.8 (C), 177.2 (C), 151.1 (C), 89.2 (C), 87.1 (C), 80.4 (C), 72.0 (CH), 70.6 (CH), 69.4 (CH), 63.9 (C), 10.9 (CH_3), 8.4 (CH_3); IR (CH_2Cl_2): 3568 (w), 3392 (br), 3099 (vw), 2960 (vw), 2219 (m), 1765 (vs), 1637 (s), 1432 (w), 1380 (w), 1303 (w), 1259 (w), 1176 (w), 1099 (m) cm^{-1}; MS (FAB): 321 ([M+H]$^+$, 68), 320 ([M]$^+$, 100), 303 (76), 275 (50), 255 (90), 253 (11), 183 (10), 155 (14), 121 (13), 115 (4), 85 (5); HRMS (FAB) calcd for $C_{18}H_{16}FeO_2$: 320.0500. Found: 320.0489.

4.4. General procedures for the synthesis of 2-ferrocenylidene-4-cyclopentene-1,3-diones (14) (Table 2)

4.4.1. Method A. A dioxane (15 mL) solution of the corresponding cyclobutenone **13** (0.50 mmol) was heated to reflux at 100 °C under argon for a period of 4 h. The mixture was then allowed to cool to room temperature and the solvent was removed on a rotary evaporator. Final purification was achieved by flash chromatography on silica gel using 9:1 hexane/ethyl acetate as the eluent. The products given in Table 2 according to Method A were isolated with the indicated yields.

4.4.2. Method B. A ground mixture of the corresponding cyclobutenone **13** (0.10 mmol) and silica gel (0.5 g) were heated on a watch glass in an oven at 125 °C for 15 min. After cooling to room temperature, the residue was loaded onto a silica gel flash column and purified by using 9:1 hexane/ethyl acetate as the eluent. The products given in Table 2 according to Method B were isolated with the indicated yields.

4.4.3. Method C. A mixture of the corresponding cyclobutenone **13** (0.10 mmol) and silica gel (0.5 g) in ethyl acetate (10 mL) was stirred at room temperature under argon for 24 h (note that for the reaction in Table 2, entry 3, reaction time was 48 h). After the solvent was removed on a rotary evaporator, the residue was loaded onto a silica gel flash column and purified by using 9:1 hexane/ethyl acetate as the eluent. The products given in Table 2 according to Method C were isolated with the indicated yields.

4.5. Spectral data for cyclopentenediones 14 and ferrocenyl quinones 15

4.5.1. 2-Ferrocenylidene-4,5-diisopropoxy-4-cyclopentene-1,3-dione (14A). R_f=0.40 in 9:1 C_6H_{14}/EtOAc; claret red oily solid; 142.8 mg (70%, Method A), 27.3 mg (67%, Method B), 13.9 mg (34%, Method C); ^{1}H NMR ($CDCl_3$): δ 7.22 (s, 1H), 5.50 (septet, 1H, J=6.2 Hz), 5.44 (septet, 1H, J=6.2 Hz), 5.14 (pseudo t, 2H, J=1.7 Hz), 4.56 (pseudo t, 2H, J=1.7 Hz), 4.13 (s, 5H), 1.36 (d, 6H, J=6.2 Hz), 1.34 (d, 6H, J=6.2 Hz); ^{13}C NMR ($CDCl_3$): δ 187.7 (C), 186.1 (C), 150.9 (C), 147.1 (C), 139.0 (CH), 121.6 (C), 76.2 (C), 74.9 (CH), 74.7 (CH), 74.2 (CH), 73.4 (CH), 70.4 (CH), 23.5 (CH_3), 23.4 (CH_3); IR (CH_2Cl_2): 2982 (w), 2933 (vw), 1668 (vs), 1620 (vs), 1461 (vw), 1377 (m), 1305 (s), 1102 (s), 1029 (s) cm^{-1}; MS (FAB): 409 ([M+H]$^+$, 36), 408 ([M]$^+$, 75), 367 (12), 366 (9), 324 (41), 301 (27), 259 (100), 186 (9), 135 (10), 121 (8), 103 (18), 85 (18), 45 (34); HRMS (FAB) calcd for $C_{22}H_{24}FeO_4$: 408.1024. Found: 408.1035.

4.5.2. (*E*)-2-Ferrocenylidene-4-isopropoxy-5-methyl-4-cyclopentene-1,3-dione ((*E*)-14B). R_f=0.58 in 9:1 C_6H_{14}/EtOAc; claret red solid; mp 99.5–100.3 °C; 111.0 mg (61%, Method A), 16.4 mg (45%, Method B), 18.6 mg (51%, Method C); ^{1}H NMR ($CDCl_3$): δ 7.25 (s, 1H), 5.61 (septet, 1H, J=6.1 Hz), 5.21 (s, 2H), 4.60 (s, 2H), 4.15 (s, 5H), 1.93 (s, 3H), 1.36 (d, 6H, J=6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 191.0 (C), 189.5 (C), 163.8 (C), 140.7 (CH), 134.7 (C), 122.6 (C), 76.1 (C), 74.5 (CH) (ferrocenyl CH and isopropoxy CH overlap), 73.8 (CH), 70.5 (CH), 23.7 (CH_3), 7.6 (CH_3); IR (CH_2Cl_2): 2980 (vw), 1712 (w), 1668 (vs), 1605 (vs), 1492 (w), 1376 (vs), 1319 (m), 1247 (w), 1127 (m), 1088 (s), 1024 (s) cm^{-1}; MS (FAB): 365 ([M+H]$^+$, 73), 364 ([M]$^+$, 100), 322 (35), 299 (16), 257 (100), 155 (12), 119 (26), 85 (30); HRMS (FAB) calcd for $C_{20}H_{20}FeO_3$: 364.0762. Found: 364.0775.

4.5.3. (*Z*)-2-Ferrocenylidene-4-isopropoxy-5-methyl-4-cyclopentene-1,3-dione ((*Z*)-14B). R_f=0.45 in 9:1 C_6H_{14}/EtOAc; claret red solid; 5.5 mg (3%, Method A), 11.7 mg (32%, Method B), 2.2 mg (6%, Method C); ^{1}H NMR ($CDCl_3$): δ 7.39 (s, 1H), 5.58 (septet, 1H, J=6.1 Hz), 5.20 (s, 2H), 4.64 (s, 2H), 4.18 (s, 5H), 1.97 (s, 3H), 1.40 (d, 6H, J=6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 192.3 (C), 188.0 (C), 166.3 (C), 141.0 (CH), 130.0 (C), 122.2 (C), 76.2 (C), 74.6 (CH), 74.4 (CH), 73.8 (CH), 70.5 (CH), 23.6 (CH_3), 7.5 (CH_3).

4.5.4. (*E*)-2-Ferrocenylidene-4-isopropoxy-5-phenyl-4-cyclopentene-1,3-dione ((*E*)-14C). R_f=0.48 in 4:1 C_6H_{14}/EtOAc; purple solid; mp 130.5–131.4 °C; 112.9 mg (53%, Method A), 23.9 mg (56%, Method C); ^{1}H NMR ($CDCl_3$): δ 7.97 (d, 2H, J=7.2 Hz), 7.45–7.35 (m, 4H), 5.91 (septet, 1H, J=6.1 Hz), 5.27 (pseudo t, 2H, J=1.7 Hz), 4.65 (pseudo t, 2H, J=1.7 Hz), 4.15 (s, 5H), 1.41 (d, 6H, J=6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 189.6 (C), 189.4 (C), 162.7 (C), 142.3 (CH), 132.1 (C), 130.0 (CH), 129.4 (CH), 128.5 (CH), 122.7 (C), 76.1 (C), 75.7 (CH), 74.9 (CH), 74.1 (CH), 70.6 (CH), 23.8 (CH_3); IR (CH_2Cl_2): 2982 (vw), 1712 (w), 1668 (vs), 1617 (s), 1593 (m), 1491 (vw), 1376 (m), 1322 (w), 1268 (m), 1126 (w), 1088 (w), 1023 (m) cm^{-1}; MS (FAB): 427 ([M+H]$^+$, 87), 426 ([M]$^+$, 100), 384 (47), 361

(12), 320 (30), 319 (91), 245 (4), 189 (4), 149 (4), 121 (5), 85 (4); HRMS (FAB) calcd for $C_{25}H_{22}FeO_3$: 426.0918. Found: 426.0908.

4.5.5. 2-Ferrocenylidene-4,5-dimethyl-4-cyclopentene-1,3-dione (14D). R_f=0.49 in 4:1 C_6H_{14}/EtOAc; purple solid; mp 170.5–171.6 °C; 88.0 mg (55%, Method A), 22.7 mg (71%, Method B), 23.7 mg (74%, Method C); ^{1}H NMR ($CDCl_3$): δ 7.40 (s, 1H), 5.24 (pseudo t, 2H, J=1.7 Hz), 4.64 (pseudo t, 2H, J=1.7 Hz), 4.13 (s, 5H), 2.02 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 194.7 (C), 193.5 (C), 154.0 (C), 150.2 (C), 142.8 (CH), 121.1 (C), 76.0 (C), 74.6 (CH), 74.1 (CH), 70.6 (CH), 9.6 (CH_3), 9.5 (CH_3); IR (CH_2Cl_2): 2982 (w), 2933 (vw), 1712 (w), 1668 (vs), 1604 (vs), 1492 (w), 1376 (vs), 1326 (s), 1248 (m), 1126 (s), 1088 (s), 1023 (s) cm^{-1}; MS (FAB): 321 ([M+H]$^+$, 51), 320 ([M]$^+$, 100), 256 (23), 255 (84), 149 (14), 121 (9), 85 (9), 69 (6); HRMS (FAB) calcd for $C_{18}H_{16}FeO_2$: 320.0500. Found: 320.0514.

4.5.6. 2-Ferrocenylidene-4,5-diphenyl-4-cyclopentene-1,3-dione (14E). R_f=0.42 in 4:1 C_6H_{14}/EtOAc; purple solid; mp 198.3–199.2 °C; 129.1 mg (58%, Method A), 20.0 mg (45%, Method C); ^{1}H NMR ($CDCl_3$): δ 7.69 (s, 1H), 7.46–7.40 (m, 4H), 7.38–7.31 (m, 6H), 5.34 (s, 2H), 4.72 (s, 2H), 4.21 (s, 5H); ^{13}C NMR ($CDCl_3$): δ 193.2 (C), 192.0 (C), 151.0 (C), 147.5 (C), 146.2 (CH), 130.6 (CH), 130.5 (CH), 130.3 (C), 130.1 (C), 130.0 (CH), 129.5 (C), 128.8 (CH), 76.2 (C), 75.1 (CH), 74.8 (CH), 70.8 (CH), note that CH peaks of phenyl groups overlap; IR (CH_2Cl_2): 2982 (w), 2933 (vw), 1712 (w), 1668 (vs), 1616 (vs), 1606 (vs), 1492 (w), 1376 (vs), 1326 (m), 1248 (w), 1127 (m), 1088 (s), 1024 (s) cm^{-1}; MS (FAB): 446 ([M+2H]$^+$, 39), 445 ([M+H]$^+$, 100), 444 ([M]$^+$, 81), 379 (67), 377 (9), 323 (6), 279 (5), 202 (5), 135 (8), 119 (15), 85 (13); HRMS (FAB, [M]$^+$) calcd for $C_{28}H_{20}FeO_2$: 444.0813. Found: 444.0825; HRMS (FAB, [M+H]$^+$) calcd for $C_{28}H_{21}FeO_2$: 445.0891. Found: 445.0914.

4.5.7. 5-Ferrocenyl-2,3-diisopropoxy-1,4-benzoquinone (15A). R_f=0.41 in 9:1 C_6H_{14}/EtOAc; green solid; 4.1 mg (2%, Method A); ^{1}H NMR ($CDCl_3$): δ 6.67 (s, 1H), 4.91 (s, 2H), 4.89 (septet, 1H, J=6.2 Hz), 4.74 (septet, 1H, J=6.2 Hz), 4.57 (s, 2H), 4.13 (s, 5H), 1.33 (d, 6H, J=6.2 Hz), 1.31 (d, 6H, J=6.2 Hz); ^{13}C NMR ($CDCl_3$): δ 184.8 (C), 184.0 (C), 147.2 (C), 145.8 (C, two quaternary carbons overlap), 125.5 (CH), 76.3 (C), 76.2 (CH, two isopropoxy CH overlap), 72.5 (CH), 70.9 (CH), 70.1 (CH), 23.1 (CH_3), 23.0 (CH_3); IR (CH_2Cl_2): 2975 (w), 2928 (vw), 1637 (s), 1567 (s), 1453 (w), 1378 (w), 1261 (vs), 1181 (m), 1101 (s), 1049 (w) cm^{-1}; MS (FAB): 410 ([M+2H]$^+$, 100), 409 ([M+H]$^+$, 14), 408 ([M]$^+$, 16), 368 (18), 325 (23), 291 (29), 259 (24), 213 (17), 186 (8), 121 (8); HRMS (FAB) calcd for $C_{22}H_{24}FeO_4$: 408.1024. Found: 408.1015.

For *5-ferrocenyl-2-isopropoxy-3-methyl-1,4-hydroquinone* (formed by reduction of **15A** during mass analysis), HRMS (FAB) calcd for $C_{22}H_{26}FeO_4$: 410.1180. Found: 410.1199.

4.5.8. 5-Ferrocenyl-2-isopropoxy-3-methyl-1,4-benzoquinone (15B). R_f=0.59 in 9:1 C_6H_{14}/EtOAc; green solid; 7.3 mg (4%, Method A); ^{1}H NMR ($CDCl_3$): δ 6.67 (s, 1H), 4.92 (s, 2H), 4.91 (septet, 1H, J=6.1 Hz), 4.58 (s, 2H), 4.12 (s, 5H), 1.96 (s, 3H), 1.31 (d, 6H, J=6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 187.7 (C), 183.5 (C), 154.8 (C), 148.8 (C), 131.0 (C), 126.0 (CH), 76.6 (C), 76.3 (CH), 72.5 (CH), 70.9 (CH), 70.3 (CH), 23.4 (CH_3), 9.9 (CH_3); IR (CH_2Cl_2): 2975 (vw), 2928 (vw), 1646 (vs), 1580 (vs), 1453 (vw), 1378 (w), 1317 (vw), 1256 (s), 1181 (vs), 1096 (s), 1016 (m) cm^{-1}; MS (FAB): 366 ([M+2H]$^+$, 75), 365 ([M+H]$^+$, 68), 364 ([M]$^+$, 100), 323 (27), 322 (19), 257 (45), 229 (9), 186 (3), 149 (6), 121 (6), 85 (4); HRMS (FAB) calcd for $C_{20}H_{20}FeO_3$: 364.0762. Found: 364.0775.

For *5-ferrocenyl-2-isopropoxy-3-methyl-1,4-hydroquinone* (formed by reduction of **15B** during mass analysis), HRMS (FAB) calcd for $C_{20}H_{22}FeO_3$: 366.0918. Found: 366.0912.

4.5.9. 5-Ferrocenyl-2,3-dimethyl-1,4-benzoquinone (15D). R_f=0.53 in 9:1 C_6H_{14}/EtOAc; green solid; 12.8 mg (8% yield, Method A), 1.6 mg (5%, Method B), 2.6 mg (8%, Method C); ^{1}H NMR ($CDCl_3$): δ 6.80 (s, 1H), 4.91 (s, 2H), 4.55 (s, 2H), 4.11 (s, 5H), 1.94 (s, 3H), 1.93 (s, 3H); IR (CH_2Cl_2): 3680 (w), 3586 (vw), 2919 (w), 1641 (vs), 1623 (s), 1585 (s), 1453 (w), 1378 (w), 1317 (m), 1247 (s), 1030 (m) cm^{-1}; MS (FAB): 322 ([M+2H]$^+$, 72), 321 ([M+H]$^+$, 88), 320 ([M]$^+$, 100), 287 (5), 255 (42), 253 (4), 209 (4), 177 (4), 155 (14), 119 (24), 85 (23); HRMS (FAB, [M]$^+$) calcd for $C_{18}H_{16}FeO_2$: 320.0500. Found: 320.0489.

For *5-ferrocenyl-2,3-dimethyl-1,4-hydroquinone* (formed by reduction of **15D** during mass analysis), HRMS (FAB) calcd for $C_{18}H_{18}FeO_2$: 322.0656. Found: 322.0667.

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Synthesis of new, BODIPY-based sensors and labels

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Abstract—New, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dye based thiol-reactive fluorescent label, fluorescent amino acid, and fluoroionophore compounds with 540–560 nm emission are described. Combination of a BODIPY dye with a nitronyl nitroxide or an imino nitroxide or a bifunctional pyrroline nitroxide furnished a nitric oxide, a redox sensitive molecule and a double (spin and fluorescence) label, respectively.

1. Introduction

Fluorescent probes and sensors have attracted attention because of their high sensitivity and exceptional ease of handling relative to their radioactive counterparts.[1] Mapping of the distribution of different ions within biological and environmental systems is a typical challenge. Binding of the target analyte to a synthetic fluorophore (sensor) can result in either amplification or quenching of the fluorescence.[2,3] These fluorescent probes are either constructed as fluorophore–spacer–receptor or as intrinsic fluorescent probes. In the former case, the receptor and fluorophore are separated by an alkyl chain, whereas in the latter case the receptor is part of a π-electron system.[4,5] The optical properties of intrinsic probes (ICT probes) are determined by a strong solvent-dependent behavior. In fluorophore–spacer–receptor systems only long-range electronic interactions are possible, the most common is photoinduced electron transfer (PET). There are many examples of crown-,[6] cryptand-,[7] podand-,[8] 2,2′-dipyridyl-,[9] and calixarene-based[10] PET sensor molecules as selective for sodium, magnesium, potassium, calcium, and transition metal ions. In these sensors the photoinduced electron transfer quenches the luminescence in the absence of the analyte. Binding the analyte (a metal ion or proton) inhibits the PET and switches on the emission.[11] It has been reported very recently that the combination of ICT and PET switches resulted in a molecular half-subtractor with reconfigurable logic gates.[12] Fluorophores covalently bound to a nitroxide give a unique, redox-sensitive sensor.[13] In these donor–acceptor molecules the paramagnetic nitroxide as an acceptor quenches the fluorescence, however, when nitroxide is reduced to hydroxylamine, the fluorescence increases. This feature of these donor–acceptor pairs was experienced with naphthyl,[13] coumarin,[14] dansyl,[15] aminophthalimide,[16] and BODIPY[17] donors. A sterically hindered amine (e.g., nitroxide precursor) covalently bound to a fluorophore offers the detection of reactive oxygen species (ROS) as formation of the nitroxide quenches the fluorescence.[18]

Another utilization of fluorescence spectroscopy is fluorescent labeling of biomolecules such as proteins, lipids, and DNA. Numerous fluorophores are known as covalent and noncovalent labels. The covalent probes can have a variety of reactive groups, for coupling with amine, sulfhydryl, hydroxyl, or histidine side chains in proteins.[19] In the case of proteins the introduction of a fluorescent, unnatural amino acid by conventional solid phase synthesis[20] or by nonsense suppression technique[21] is also a well-known approach. However, there is still a challenge to synthesize selective, long wavelength emitting probes for modifying biomolecules.[22,23]

Among the aforementioned examples, the BODIPY dyes[24] were used as fluorophores with many advantages, including high extinction coefficient, high quantum yield, narrow emission bandwidth, and therefore, good signal/noise ratio, insensitivity to pH and solvent polarity, and greater chemical and photochemical stabilities in solution and in solid state. Absorption/emission wavelengths can be tuned by the modification of the pyrrole core;[25,26] therefore, visible and infrared regions of the spectrum can be spanned. This property was used in a BODIPY-based, oxidation-sensitive fluorescent lipid peroxidation probe.[27]

In the present account, we wish to describe BODIPY-based labels for modifying proteins and BODIPY-based sensor molecules containing crown ether or nitroxide group to detect metal ions, reducing agents, or nitrogen-monoxide by fluorescence.

Keywords: Fluorescence; Crown ethers; Amino acid; Nitroxides; Double sensors.
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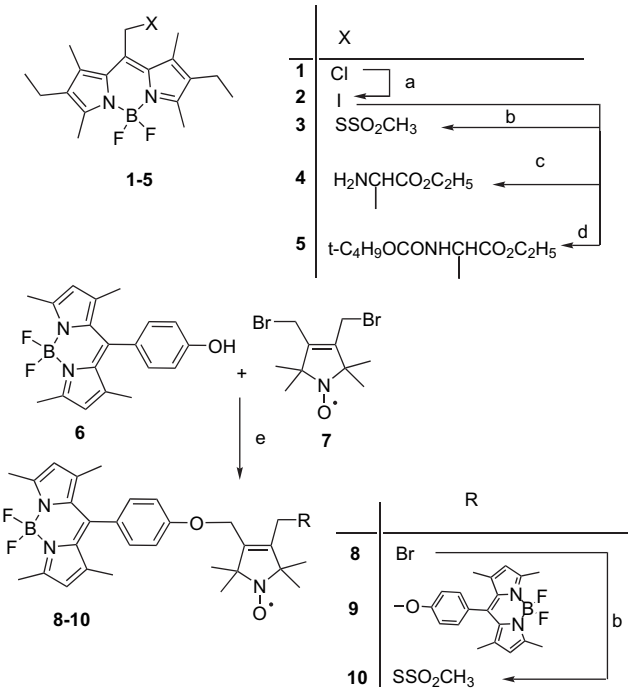
2. Results and discussion

2.1. Synthesis of fluorescent labels

Compound 4,4-difluoro-8-chloromethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**1**) is readily available from 3-ethyl-2,4-dimethylpyrrole and chloroacetyl chloride[28] and it was quite obvious that this derivative can be functionalized further by nucleophilic substitution. Chloromethyl compound **1** was converted to the more reactive iodomethyl derivative **2** by treatment with NaI in THF. Compound **2** was transformed to methanethiosulfonate derivative (MTS) **3** by a substitution reaction with $NaSSO_2CH_3$ in aq acetone. The resulting compound is a thiol-specific, reversible fluorescent dye with 555 nm emission (Table 1), capable of forming S–S bond with a cysteine SH group, and analogously to the frequently used spin labeling technique[29] compound **3** is a good candidate for site-directed fluorescent labeling of a peptide.[30] The BODIPY dye containing fluorescent D,L-amino acid was obtained by a modified O'Donnell reaction.[31,32] Treatment of carbanion, generated from dibenzylideneglycine ethyl ester with NaHMDS at −78 °C, with compound **2** yielded the fluorescent amino acid ester **4** after acid catalyzed hydrolysis of the imine. The treatment of compound **4** with di-*tert*-butyldicarbonate in THF gave the more stable *N*-Boc protected D,L-amino acid ester **5**. Compounds **3** and **5** are capable of fluorescent modification of the peptide by labeling at the cysteine side-chain or by incorporation of the fluorescent amino acid by solid phase peptide synthesis. Our further idea was the combination of spin and fluorescence labeling, i.e., to synthesize a double (spin and fluorescence) label capable of simultaneous labeling at the same site of a protein. A possible synthetic approach for this was the treatment of phenol containing BODIPY dye **6**[12] with a paramagnetic homobifunctional alkylating nitroxide **7**.[33] Fortunately, this could be accomplished under mild conditions to yield allylic bromide **8** and the two fluorophores containing **9** as a by-product. Compound **8** was then converted to methanethiosulfonate **10** by treatment with $NaSSO_2CH_3$ in aq acetone. The product was an SH-specific double (fluorescent and spin) label with a green emission (508 nm), a reduced quantum yield (Φ=0.22) and 2.5 ns lifetime, which is about half of the regular BODIPY lifetime[34] (Scheme 1).

2.2. Synthesis of fluorescent sensors

Fluoroionophores, by changing their optical properties upon complexation with certain ions have great potential for practical applications. It was obvious that compound **2** is a good candidate to modify different ion recognition sites. Alkylation of aza-15-crown-5 or aza-18-crown-6 in acetone in the presence of K_2CO_3 or Na_2CO_3 with compound **2** furnished fluoroionophores **11** or **12**, respectively (Scheme 2). During the study of these ionophores the absorption and emission spectra were recorded in acetonitrile. The ion-free fluoroionophores **11** and **12** gave sharp absorption maxima at 534 nm and emission maxima at 547 and 552 nm, respectively. The spectral response to the addition of ions was measured using the corresponding perchlorate salts and perchloric acid. The absorption spectra of compound **11** shifted to 554 nm upon addition of Ca^{2+} and H^+, and to 556 nm upon addition of Mg^{2+} ions. Addition of Li^+, Na^+, K^+ ions to solution of **11** causes only 1–3 nm red shift with some hypsochromic effect. In the case of compound **12** a small hypsochromic shift and some bathochromic shift occurred, except upon protonation, which resulted in a 19 nm bathochromic shift (Fig. 1). The fluorescence

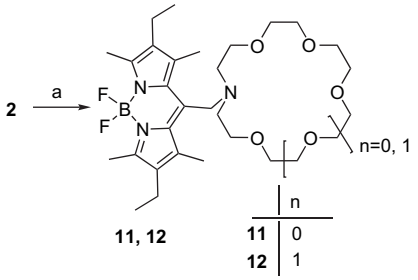


Scheme 1. Reagents and conditions: (a) NaI (2 equiv), THF, reflux, 45 min, 75%; (b) $NaSSO_2CH_3$ (2 equiv), water/acetone, reflux, 30 min, 43–27%; (c) $Ph_2NCH_2CO_2Et$ (1 equiv), THF, NaHMDS (1.1 equiv), −78 °C, 15 min, then **2** (1 equiv), 1 h, then, warm to 0 °C, quench with aq NH_4Cl, EtOH/aq H_2SO_4, rt, 40 min, 38%; (d) Boc_2O (1.0 equiv), THF, 40 °C, 30 min, 76%; (e) **6** (1.5 equiv), acetone, K_2CO_3 (1.5 equiv), 18-crown-6 (0.05 equiv), 10 min, then **7** (1 equiv), reflux, 30 min, 22% for **8** and 30% for **9**.

Table 1. Optical properties of the synthesized new BODIPY derivatives

Compound	λ_{abs} (nm)	ε (L×mol^{-1}×cm^{-1})	λ_{ex} (nm)	λ_{em} (nm)	Φ
3	543	3.37×10^4	543	555	0.80
5	529	3.54×10^4	530	543	0.86
10	498	4.35×10^4	497	508	0.22
11	534	5.87×10^4	530	547	0.001
12	534	7.70×10^4	533	552	0.01
15	499	2.73×10^4	505	518	0.006
20	499	3.43×10^4	510	523	0.005

Dissolved in acetonitrile and referred to fluorescein in 0.1 M NaOH at 496 nm, n=3, accuracy ±10%.



Scheme 2. Reagents and conditions: (a) 1-aza-15-crown-15 for **11**, 1-aza-18-crown-6 for **12** (1 equiv), Na_2CO_3 for **12** or K_2CO_3 for **11** (1.5 equiv), acetone, reflux, 30 min–2 h, 64–77%.

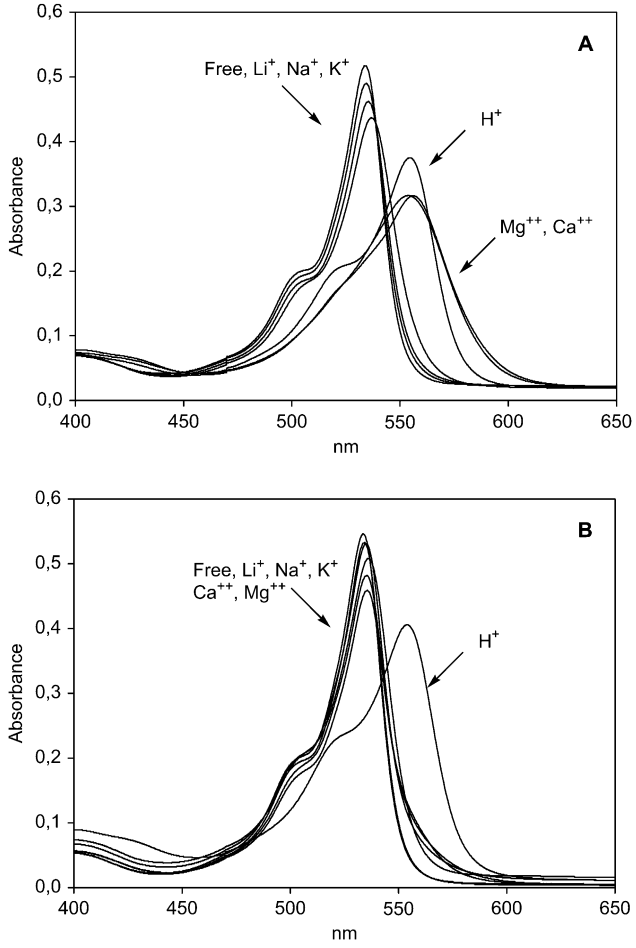


Figure 1. (**A**) Absorption spectra of **11** in acetonitrile and its complexes with 1000 equiv perchlorate salts (1 equiv=1.1 nM); (**B**) absorption spectra of **12** in acetonitrile and its complexes with 1000 equiv perchlorate salts (1 equiv=1.1 μM).

enhancement (quantum yield, Φ/Φ_0) was also studied in acetonitrile solution by the addition of 20 and 10,000 equiv ions (Fig. 2). Upon protonation of compound **11** regardless of the ratio of crown compound/H^+ a 75-fold increase was observed with a red shift to 571 nm. The emission was also red shifted with the addition of a large excess (10,000 equiv) of Ca^{2+} and Mg^{2+} ions with a 17- and 108-fold fluorescence quantum yield increase. This observation, with a fluorescence shift of 1-aza-15-crown-5 when Ca^{2+} is complexed, is in good agreement with Wu et al.'s findings.[35] The addition of Na^+ and Li^+ to an acetonitrile solution of compound **11** caused a 2–3 nm bathochromic shift with 73-fold and 82-fold increase in fluorescence quantum yield when 10,000 equiv ions were added, however only a three-fold fluorescence increase was experienced upon addition of a large excess of K^+ ions. Additions of 20 equiv of ions increased the fluorescence quantum yield of compound **11** to 2–6 times. Compound **12** proved to be more selective, although protonation caused a 17-fold enhancement of fluorescence, but with a red shift to 580 nm. The addition of 20 equiv of K^+ ions caused a six-fold increase, while a large excess of K^+ ions induced a 56-fold enhancement with a small (3 nm) bathochromic shift. Titration of compound **11** with Li^+ and Mg^{2+} indicated the formation of 1:1 complex and the association constants are 3140 and 440 M^{-1}

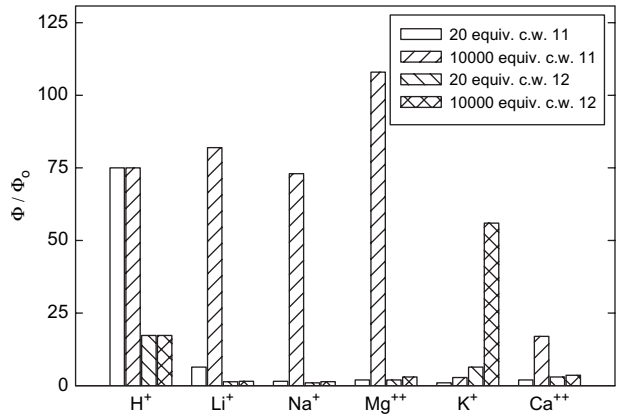


Figure 2. The fluorescence response of aza-crown ethers **11** and **12** to various cations. The first and third bars represent the integrated emissions of compounds **11** and **12** in the presence of 20 equiv of the cations of interest, the second and fourth bars represent the integrated emissions of compounds **11** and **12** in the presence of 10,000 equiv of the cations of interest, respectively (1 equiv=1 μM). The response was normalized with respect to integrated emission of free dye (Φ_0); excitation was provided at 530 nm for **11** and 533 nm for **12**; the emission was integrated from 536 to 700 nm in case of **11** and 539 to 700 nm in case of **12**; the slit width was 3 nm.

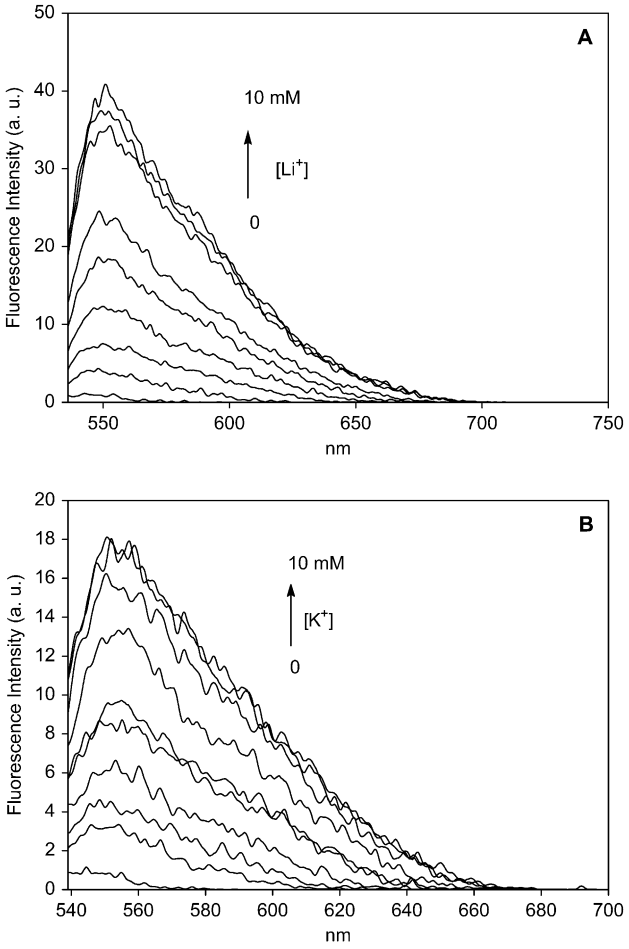
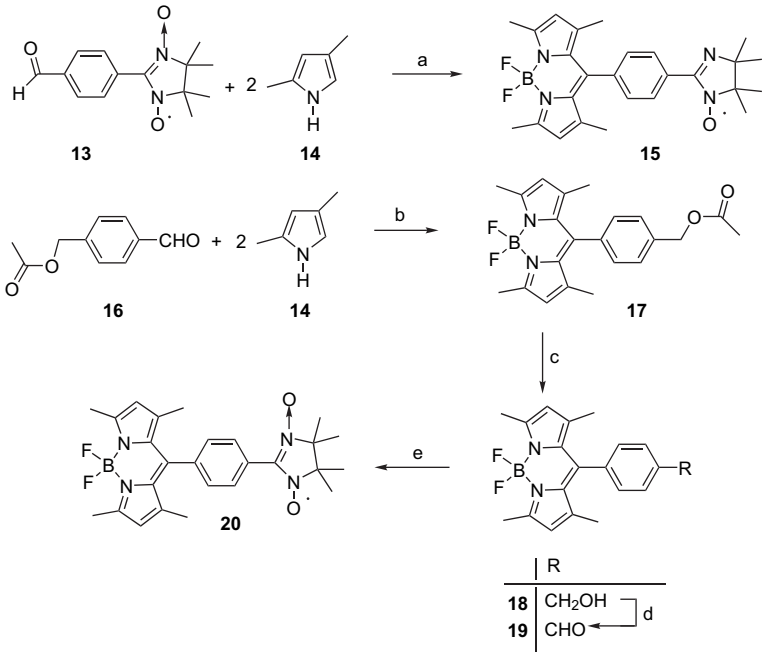


Figure 3. (**A**) Fluorescence emission spectra of compound **11** (1 μM) in acetonitrile with free Li^+ concentrations of 0, 25, 50, 100, 200, 500, 2000, 5000, and 10,000 μM, λ_{ex}=530 nm; (**B**) fluorescence emission spectra of compound **12** (1 μM) in acetonitrile with free K^+ concentrations of 0, 25, 50, 100, 200, 250, 500, 2000, 5000, and 10,000 μM, λ_{ex}=533 nm.

for Li^+ and Mg^{2+}, respectively. Although there is a significant difference between the association constants of **11** and Mg^{2+} versus **11** and Li^+, compound **11** cannot be regarded as a selective fluoroionophore, because Li^+, Na^+, Mg^{2+}, Ca^{2+} all increases the fluorescence. In contrast to compound **11**, compound **12** exhibits good selectivity toward K^+ and the association constant is 6570 M^{-1} in acetonitrile estimated by titration and no other ions caused significant increase in fluorescence quantum yield (except H^+, but this with a red shift) (Fig. 3). An investigation of the sensing mechanism is in progress, however, the changes in spectral band positions are comparatively small, in agreement with a PET signaling mechanism. Binding of a cation alters the redox potential of the aza-crown ether by weakening the nitrogen donor strength thus inhibiting the quenching process and, therefore, increasing the fluorescence. The fluorophore–σ-spacer–receptor arrangement with an electronically decoupled amino nitrogen atom also supports the PET mechanism of sensing rather than an ICT process, as demonstrated earlier by Rurack et al.[36]

From our laboratory, we have demonstrated that nitroxide covalently linked to a BODIPY dye is a good redox sensor reagent. Wang et al. demonstrated that a pyrene fluorophore attached to an imino nitroxide has optical and gate properties, e.g., reduction of nitroxide to *N*-hydroxylamine and protonation of imino nitrogen resulted in fluorescence enhancement.[37] It seemed a real challenge to combine nitronyl nitroxide with the BODIPY-type dye. The reaction of nitronyl nitroxide aldehyde **13**[38] with 2 equiv 2,4-dimethylpyrrole **14** in the presence of TFA in CH_2Cl_2 and after treatment with DDQ, $BF_3\cdot Et_2O$, and *i*-Pr_2EtN and oxidation with PbO_2 gave imino nitroxide **15** instead of the required nitronyl nitroxide. Because the synthetic pathway resulted in the loss of the 3-oxo group, we decided to introduce the nitronyl nitroxide ring in the last step. Reaction of 4-acetoxymethyl benzaldehyde **16**[39] with pyrrole **14** in an acid catalyzed reaction, followed by treatment with DDQ, $BF_3\cdot Et_2O$, and *i*-Pr_2EtN gave compound **17**. Deacetylation of this compound with NaOMe yielded alcohol **18**. Oxidation of compound **18** with activated MnO_2 furnished aldehyde **19**. Treatment of compound **19** with 2,3-bis-hydroxylamine-2,3-dimethylbutane monosulfate salt in the presence of Et_3N in methanol yielded the desired nitronyl nitroxide **20** after oxidation with $NaIO_4$ (Scheme 3).

We have investigated the spectral properties of compounds **15** and **20**. The addition of 2-(*N*,*N*-diethylamino)-diazenolate-2-oxide (diethylamine NONOate), as a solid, water soluble NO source to a solution (0.1 M phosphate buffer solution (PBS)/acetonitrile, 50:1) of nitronyl nitroxide **20** yielded a small (~20%) increase in quantum yield (Fig. 4). In the UV–vis spectrum the $S_0\rightarrow S_1$ band does not change, only bands at 230, 266, and 363 nm disappear, while a new band at 237 nm appears. The significant change is observed only in EPR spectra, the 5 line EPR spectra of nitronyl nitroxide change to the 7 line EPR spectra of an imino nitroxide **15**, as happens with any other nitronyl nitroxide when subjected to nitrogen oxide (Fig. 4).[40] The EPR spectral data are: a_{N1}=4.3 G, a_{N2}=9.2 G, 7 lines and they were identical with the EPR spectra of the directly prepared compound **15**. However, the resulting imino nitroxide **15** has interesting optical properties. Titration of compound **15** in a 0.1 M phosphate buffer with ascorbic acid as a reducing agent, results in about 80% increase in fluorescence quantum yield after adding excess ascorbic acid. However, dissolving compound **15** in TFA/acetonitrile/water (pH=1.5) the fluorescence quantum yield increases five times, without any



Scheme 3. Reagents and conditions: (a) **13** (1 equiv), **14** (2 equiv), CH_2Cl_2, TFA (1.1 equiv), rt, 8 h, then DDQ (1 equiv), 30 min, $BF_3\cdot Et_2O$ (10 equiv) and *i*-Pr_2EtN (15 equiv), rt, 40 min, then the crude product was oxidized with PbO_2 (2 equiv), $CHCl_3$, reflux, 15 min, 10%; (b) **16** (1 equiv), **14** (2 equiv), CH_2Cl_2, TFA (1.1 equiv), rt, 8 h, then DDQ (1 equiv), 40 min, $BF_3\cdot Et_2O$ (10 equiv) and *i*-Pr_2EtN (15 equiv), rt, 30 min, 35%; (c) NaOMe (0.3 equiv), MeOH, rt, 40 min, 59%; (d) MnO_2 (15 equiv), CH_2Cl_2, rt, 2 h, 64%; (e) 2,3-bishydroxylamino-2,3-dimethylbutane monosulfate (1 equiv), MeOH, Et_3N (1.5 equiv), 12 h, rt, then oxidation with aq $NaIO_4$ (10 equiv), rt, 5 min, 38%.

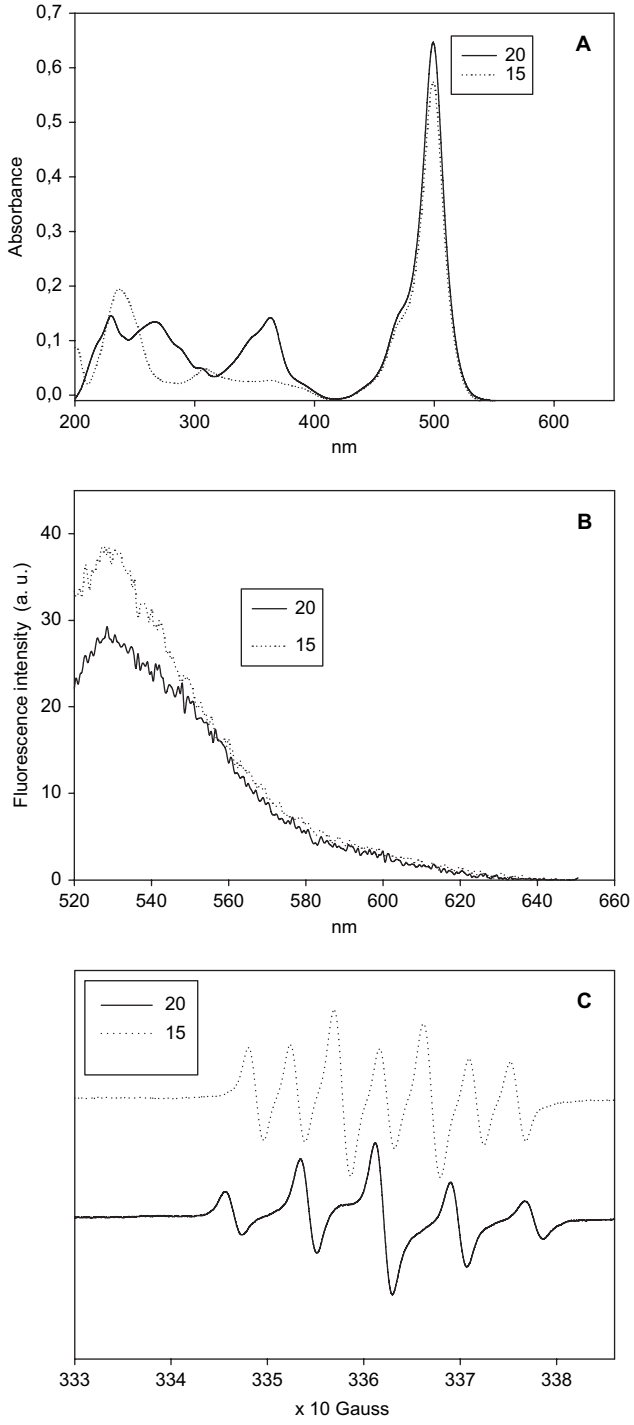


Figure 4. (**A**) Absorption; (**B**) emission spectra of compounds **20** (24 μM) and **15** in phosphate buffer solution (PBS)/acetonitrile 50:1, (pH=7.4). Spectra of **15** were generated by addition of 1 equiv diethylamine NONOate; (**C**) EPR spectra of compounds **20** (427 μM) and **15** in acetonitrile/PBS 1:1 mixture. The spectrum of **15** was generated by addition of 1.07 equiv diethylamine NONOate.

reducing agent. Titration of acidic solution of compound **15** with ascorbic acid resulted in further increases of quantum yield (up to 10 times). This confirms that compound **15** has got a redox center (nitroxide) and a proton sensitive receptor (imidazole nitrogen). Only the reduction of nitroxide and protonation of imidazole nitrogen restore the fluorescence of the BODIPY dye (Fig. 5).

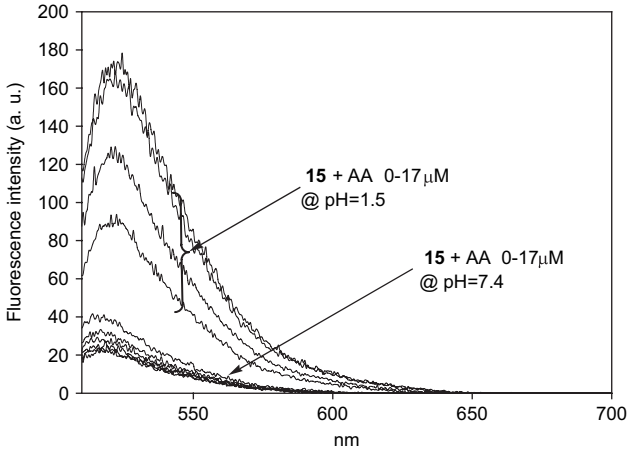


Figure 5. Titration of compound **15** (10 μM) in 0.1 M PBS (pH=7.4)/acetonitrile 50:1 with ascorbic acid (AA) 0, 1.7, 3.4, 6.8, 10.2, 13.6, and 17 μM (final concentration). The upper four plot: titration of compound **15** (10 μM) in water/TFA (pH=1.5)/acetonitrile 50:1 with ascorbic acid (AA) 0, 6.8, 13.6, and 17 μM (final concentration), λ_{ex}: 505 nm.

3. Conclusion

In conclusion, we have reported the synthesis and preliminary study of new BODIPY-based sensor molecules, a thiol-specific fluorescent label, a fluorescent amino acid, and a double (spin and fluorescence) label. Among the fluoroionophores, 1-aza-18-crown-6 in combination with a BODIPY dye is superior to 1-aza-15-crown-5 owing to its larger association constant and potassium-selective spectral change. The imino nitroxide attached BODIPY dye is a H^+- and redox sensitive molecule and a good candidate for Boolean logic gate application. Further work with new labels and sensors is in progress.

4. Experimental

4.1. General

Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, and S) were performed on a Carlo Erba EA 1110 CHNS elemental analyzer. Mass spectra were recorded on an Automass Multi instrument in the EI mode (70 eV, direct inlet) or on a VG TRIO-2 instrument with thermospray technique. ESR spectra were obtained from 10^{-5} M solutions ($CHCl_3$), using a Magnettech MS200 spectrometer. Preparative flash column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm). The UV spectra were taken with a Specord 40 (Jena Analytic) in acetonitrile using 1 cm quartz cells and solute concentrations (2–0.5)$\times 10^{-5}$ M. The molar extinction coefficients (ε) at absorption maximum were obtained from slope of absorbance versus concentration using five solutions of different concentrations. Fluorescence spectra of compounds dissolved in acetonitrile were measured with Perkin–Elmer LS50B spectrofluorimeter, with 3 nm slits, with correction of instrumental factors by means of a rhodamine B quantum counter and correction files supplied by the manufacturer. Quantum yields were referred to fluorescein dissolved in 0.1 M NaOH

(Φ'=0.95). The values were calculated from equation $\Phi=(I/I')(A'/A)(n/n')\Phi'$, where I', A', and Φ' are the integrated emission, absorbance (at the excitation wavelength), and quantum yield of the reference sample, respectively. n' is the refractive index of the solvent used for reference sample. I, A, n, and Φ are related to sample with the same definitions applied to reference sample. The fluorescence data of all final compounds are listed in Table 1. Lifetimes were measured with ISS K2 multifrequency phase fluorimeter and referred to glycogen. The association constant (K_a) of complexes were estimated by Eq. 1, which is a linear plot $F_0/(F-F_0)$ versus $1/C$.

$$F_0/(F-F_0)=\left(\frac{\Phi_M\varepsilon_M}{\Phi_C\varepsilon_C-\Phi_M\varepsilon_M}\right)\times\left(\frac{1}{K_aC}+1\right) \quad (1)$$

F_0 denotes the fluorescence intensity of metal free complex at a selected wavelength, F the fluorescence intensity of metal–fluoroionophore complex, C the metal ion concentration, Φ_M and Φ_C are the quantum yields of free and metal–fluoroionophore complex, ε_M and ε_C are the molar extinction coefficients. Qualitative TLC was carried out on commercially prepared plates (20×20×0.02 cm) coated with Merck Kieselgel GF_{254}. ^{1}H NMR spectra of diamagnetic compounds were recorded with Varian Unity Inova 400 WB spectrometer; chemical shifts were referenced to TMS. $NaSSO_2CH_3$,[41] compounds **1**, **6**, **7**, **13**, **16**, and 2,3-bishydroxylamine-2,3-dimethylbutane monosulfate salt[42] were prepared as described earlier and all other reagents and compounds were purchased from Aldrich or Fluka.

4.1.1. 4,4-Difluoro-8-iodomethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (2). A solution of compound **1** (1.76 g, 5.0 mmol) and NaI (1.50 g, 10.0 mmol) was stirred and refluxed in dry THF (20 mL) for 45 min. After cooling, the solution was diluted with Et_2O (20 mL), washed with water (20 mL), and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/Et_2O) to give the title compound as a dark purple solid 1.66 g (75%), mp 145–147 °C, R_f: 0.63 (hexane/Et_2O, 2:1). EA: calcd $C_{18}H_{24}BF_2IN_2$: C, 48.68; H, 5.45; N, 6.31. Found: C, 48.55; H, 5.39; N, 6.20. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.69 (s, 2H, CH_2I), 2.51, 2.48 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=6.8 Hz, 4H, 2×CH_2CH$_3$), 1.04 (t, J=6.8 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1605, 1560, 1510 (C=C) cm^{-1}. MS (EI) m/z: 444 (M$^+$, 24), 425 (9), 410 (5), 317 (100).

4.2. General procedure for the synthesis of methanethiosulfonates (3 and 10)

A solution of compound **2** (444 mg, 1.0 mmol) or compound **8** (585 mg, 1.0 mmol) and $NaSSO_2CH_3$ (270 mg, 2.0 mmol) was dissolved in acetone (10 mL) and water (3 mL), and the mixture was heated at reflux until the consumption of the starting halogen compound (~30 min). After cooling, the acetone was evaporated off, water (5 mL) was added, and the residue was partitioned between water and EtOAc (20 mL). The organic phase was separated, dried ($MgSO_4$), filtered, and evaporated, and flash column chromatography (hexane/EtOAc) purification afforded methanethiosulfonates **3** or **10**.

4.2.1. 4,4-Difluoro-8-methanesulfonylmethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (3). Yield 184 mg (43%), red solid, mp 186–189 °C, R_f: 0.22 (hexane/EtOAc, 2:1). EA: calcd for $C_{19}H_{27}BF_2N_2O_2S_2$: C, 53.27; H, 6.35; N, 6.54; S, 14.97. Found: C, 53.10; H, 6.41; N, 6.33; S, 14.88. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.70 (s, 2H, CH_2S), 3.37 (s, 3H, SO_2CH_3), 2.51, 2.48 (two s, each 6H, 4×ArCH_3), 2.37 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.04 (t, J=7.2 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1600, 1560, 1505 cm^{-1} (C=C). MS (EI) m/z: 428 (M$^+$, 59), 413 (2), 396 (30), 317 (100).

4.2.2. 3-Methanethiosulfonylmethyl-2,2,5,5-tetramethyl-4-[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl-4′-oxy)ylmethyl]-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (10). Yield 166 mg (27%), red solid, mp 148–150 °C, R_f: 0.14 (hexane/EtOAc, 2:1). EA: calcd for $C_{30}H_{37}BF_2N_3O_4S_2$: C, 58.42; H, 6.05; N, 6.82; S, 10.40. Found: C, 58.26; H, 6.13; N, 6.73; S, 10.23. IR (Nujol) ν: 1650, 1560, 1535, 1505 (C=C) cm^{-1}. MS (thermospray) m/z: 617 (M+H)$^+$. EPR (in $CHCl_3$): triplet, a_N=14.6 G.

4.2.3. D,L-2-Amino-3-(4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-yl)-propionic acid ethyl ester (4). To a solution of *N*-(diphenylmethylene)glycine ethyl ester (267 mg, 1.0 mmol) in dry THF (10 mL), NaHMDS (1.0 M THF solution) (1.1 mL, 1.1 mmol) was added at −78 °C in one portion and the mixture was stirred for 15 min at this temperature, then compound **2** (444 mg, 1.0 mmol) dissolved in dry THF (10 mL) was added dropwise. After 1 h stirring at −78 °C the mixture was allowed to warm to 0 °C and quenched with satd aq NH_4Cl solution (10 mL). After evaporation of THF, water (10 mL) was added and the aq phase was extracted with $CHCl_3$ (2×20 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated. The crude residue was dissolved in EtOH (10 mL) and 5% aq H_2SO_4 (5 mL) was added and the mixture was allowed to stand at rt for 40 min. The solution was diluted with water (15 mL) and the solution was concentrated to half in vacuo. The aq phase was washed with EtOAc (2×10 mL) to remove the benzophenone and other by-products, and organic phase was discarded. The aq phase pH was adjusted to 8 by addition of solid K_2CO_3 and extracted with $CHCl_3$ (2×20 mL), then dried ($MgSO_4$), filtered, and evaporated to give 159 mg (38%) of a thick purple oil, R_f: 0.48 ($CHCl_3$/Et_2O, 2:1). EA: calcd for $C_{22}H_{32}BF_2N_3O_2$: C, 63.02; H, 7.69; N, 10.02. Found: C, 62.97; H, 7.55; N, 10.00. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.22 (br s, 1H, CH), 3.96 (q, J=7 Hz, 2H, OCH_2), 3.51 (d, J=6 Hz, 2H, ArCH$_2$), 2.48, 2.47 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.27 (t, J=7 Hz, 3H, OCH$_2$CH_3), 1.04 (t, J=7.2 Hz, 6H, 2×CH$_2$CH_3). IR (neat) ν: 3400 (NH$_2$), 1740 (C=O), 1615, 1560, 1540, 1500 (C=C) cm^{-1}. MS (EI) m/z: 419 (M$^+$, 11), 418 (4), 318 (45), 182 (80), 41 (100).

4.2.4. D,L-2-*tert*-Butoxycarbonylamino-3-(4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-yl)propionic acid ethyl ester (5). To a solution of compound **4** (155 mg, 0.37 mmol) in THF (10 mL) was added *tert*-butoxycarbonyl anhydride (81 mg, 0.37 mmol)

and the mixture was stirred at 40 °C for 30 min. After cooling, Et_2O (10 mL) was added, the organic phase was washed with brine (10 mL), then the organic phase was separated, dried ($MgSO_4$), filtered, and evaporated, and compound **5** (130 mg, 76%) was obtained after flash column chromatography purification as a red solid, mp 170–172 °C, R_f: 0.55 (hexane/EtOAc, 2:1). EA: calcd for $C_{27}H_{40}BF_2N_3O_4$: C, 62.43; H; 7.76; N, 8.09. Found: C, 62.38; H, 7.69; N, 8.00. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=5.18 (br s, 1H, *H*N), 4.44 (br s, 1H, C*H*), 3.96 (q, J=7 Hz, 2H, OCH_2), 3.51 (d, J=6 Hz, 2H, ArCH_2), 2.48, 2.47 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.36 (s, 9H, C(CH_3)$_3$), 1.27 (t, J=7 Hz, 3H, OCH$_2$CH_3). 1.04 (t, J=7.2 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 3330 (NH), 1745, 1680 (C=O), 1560, 1540, 1505 (C=C) cm^{-1}. MS (EI) *m/z*: 519 (M^+, 2), 463 (1), 317 (4), 287 (7), 57 (100).

4.2.5. 3-Bromomethyl-2,2,5,5-tetramethyl-4-[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl-4′-oxy)-ylmethyl]-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (8) and 2,2,5,5-tetramethyl-3,4-bis[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl-4′-oxy)-ylmethyl]-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (9). A solution of compound **6** (510 mg, 1.5 mmol), 18-crown-6 (19 mg), and powdered K_2CO_3 (207 mg, 1.5 mmol) and acetone were stirred at 40 °C for 10 min, then compound **7** (326 mg, 1.0 mmol) was added dropwise, dissolved in acetone (5 mL), and the mixture was stirred and heated at reflux for 30 min. After cooling, the acetone was evaporated off and the residue was dissolved in $CHCl_3$ (20 mL), the organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered, and evaporated, and the residue was purified by flash column chromatography (hexane/Et_2O followed by hexane/EtOAc). The first two bands are the remains of starting materials **6** and **7**, respectively, and then compound **8** R_f: 0.44 (hexane/EtOAc, 2:1), then compound **9** R_f: 0.21 (hexane/EtOAc, 2:1) eluted. The yield of compound **8** is 128 mg (22%), red solid, mp 195–197 °C. EA: calcd for $C_{29}H_{34}BBrF_2N_3O_2$: C, 59.97; H, 5.87; N, 7.19. Found: C, 59.88; H, 5.78; N, 7.10. MS (EI) *m/z*: 586/584 (M^+, 10/10), 542/540 (25/25), 340 (58), 186 (100). IR (Nujol) ν: 1660, 1625, 1570, 1550, 1510 (C=C) cm^{-1}. Yield of compound **9** is 253 mg (30%), red solid, mp 183–185 °C. EA: calcd for $C_{48}H_{52}B_2F_4N_5O_3$: C, 68.21; H, 6.21; N, 8.29. Found: C, 68.15; H, 6.15; N, 8.25. IR (Nujol) ν: 1650, 1555, 1535, 1505 (C=C) cm^{-1}. MS (thermospray) *m/z*: 845 (M+H)$^+$.

4.3. General procedure for the alkylation of aza-crown ethers (11 and 12)

A solution of compound **2** (444 mg, 1.0 mmol), 1-aza-15-crown-5 (219 mg, 1.0 mmol), and powdered K_2CO_3 (207 mg, 1.5 mmol) or 1-aza-18-crown-6 (263 mg, 1.0 mmol) and Na_2CO_3 (159 mg, 1.5 mmol) was stirred and refluxed in acetone (10 mL) until compound **2** (30 min for **12** and 2 h for **11**) was consumed. After cooling, the inorganic salts were filtered off, washed with $CHCl_3$ (5 mL), and the solvents were evaporated under reduced pressure. The residue was purified by flash column chromatography ($CHCl_3$/Et_2O and $CHCl_3$/MeOH) to yield the title compounds.

4.3.1. 13-(4,4-Difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-methylenyl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (11). Yield 412 mg (77%), red solid, mp 148–150 °C, R_f: 0.34 ($CHCl_3$/Et_2O, 2:1). EA: calcd for $C_{28}H_{44}BF_2N_3O_4$: C, 62.80; H, 8.28; N, 7.85. Found: C, 62.82; H, 8.30; N, 7.66. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.99 (s, 2H, ArCH_2N), 3.61, 3.56 (two s, 16H, 8×CH_2), 2.89 (s, 4H, 2×NCH_2), 2.48, 2.40 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.03 (t, J=7.6 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1645, 1540, 1505 (C=C) cm^{-1}.

4.3.2. 16-(4,4-Difluoro-1,3,5,7-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-methylenyl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (12). Yield 370 mg (64%), red solid, mp 84–86 °C, R_f: 0.85 ($CHCl_3$/MeOH, 9:1). EA: calcd for $C_{30}H_{48}BF_2N_3O_5$: C, 62.18; H, 8.35; N, 7.25. Found: C, 62.15; H, 8.31; N, 7.12. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.02 (s, 2H, ArCH_2N), 3.62, 3.55 (two s, 20H, 10×CH_2), 2.93 (s, 4H, 2×NCH_2), 2.47, 2.40 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.03 (t, J=7.6 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1640, 1550, 1505 (C=C) cm^{-1}.

4.3.3. 2-[(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl)-4-yl]-4,4,5,5-tetramethyl-4,5-dihydro-1*H*-imidazol-1-yloxyl radical (15). To a deoxygenated solution of aldehyde **13** (1.0 mmol, 261 mg) and 2,4-dimethylpyrrole (2.0 mmol, 190 mg) in CH_2Cl_2 (200 mL), TFA (125 mg, 1.1 mmol) was added and the mixture was stirred overnight under N_2 at rt. The red solution was treated with DDQ (227 mg, 1.0 mmol), stirred for 30 min, then *i*-Pr_2EtN (2.0 mL, 11.6 mmol) and $BF_3 \cdot Et_2O$ (1.25 mL, 10.0 mmol) were added at 0 °C, and the mixture was stirred at rt for further 40 min. After washing with satd aq $NaHCO_3$, the organic phase was separated, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in $CHCl_3$ (10 mL), PbO_2 (478 mg, 2.0 mmol) was added, and the mixture was stirred and refluxed for 15 min. The PbO_2 was filtered off, the solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography (hexane/EtOAc), collecting the first red/purple fraction afforded the imino nitroxide linked to the BODIPY dye, 46 mg (10%), orange-red solid, mp 195 °C (decomposes on heating), R_f: 0.57 (hexane/EtOAc, 2:1). EA: calcd for $C_{26}H_{30}BF_2N_4O$: C, 67.40; H, 6.53; N, 12.09. Found: C, 67.51; H, 6.48; N, 12.00. IR (Nujol) ν: 1560, 1540, 1505 (C=C) cm^{-1}. MS (EI) *m/z*: 463: (M^+, 1), 391 (1), 349 (5), 41 (100). EPR (in $CHCl_3$): 7 lines, a_{N1}=4.3 G, a_{N2}=9.0 G and (in acetonitrile/PBS 1:1) 7 lines, a_{N1}=4.3 G, a_{N2}=9.2 G.

4.3.4. 4,4-Difluoro-8-(4-acetoxymethylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (17). To a deoxygenated solution of aldehyde **16** (1.78g, 10.0 mmol) and 2,4-dimethylpyrrole (20.0 mmol, 1.90 g) in CH_2Cl_2 (500 mL), TFA (114 mg, 1.0 mmol) was added and the mixture was stirred overnight under N_2 at rt. The red solution was treated with DDQ (2.27 g, 10.0 mmol), stirred for 30 min, then *i*-Pr_2EtN (25.7 mL, 0.15 mol) and $BF_3 \cdot Et_2O$ (12.5 mL, 0.1 mol) were added at 0 °C, and the mixture was stirred at rt for further 40 min. After washing with satd aq $NaHCO_3$, the organic phase was separated, dried

($MgSO_4$), filtered, and concentrated. The residue was purified by flash column chromatography (hexane/EtOAc), collecting the first brownish fraction afforded the acetoxy derivative **17**, 1.38 g (35%), brownish-red solid, mp 86–88 °C (decomposes on heating), R_f: 0.60 (hexane/EtOAc, 2:1). EA: calcd for $C_{22}H_{23}BF_2N_2O_2$: C, 66.69; H, 5.85; N, 7.07. Found: C, 66.70; H, 5.77; N, 7.01. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.45 (d, J=7.6 Hz, 2H, Ar*H*), 7.25 (d, J=7.6 Hz, 2H, Ar*H*), 5.96 (s, 2H, pyrrole *H*), 5.09 (s, 2H, CH_2O), 2.54 (s, 6H, 2×CH_3), 2.13 (s, 3H, COCH_3), 1.36 (s, 6H, 2×CH_3). IR (Nujol) ν: 1735 (C=O), 1630, 1560, 1540, 1510 (C=C) cm^{-1}. MS (EI) *m*/*z*: 396 (M$^+$, 18), 350 (6), 256 (74), 162 (82), 43 (100).

4.3.5. 4,4-Difluoro-8-(4-hydroxymethylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (18). To a solution of ester **17** (792 mg, 2.0 mmol) in MeOH (10 mL), NaOMe solution (0.6 mmol, freshly made from 14 mg Na metal and 5 mL MeOH) was added and the mixture was allowed to stand at rt for 40 min. Then the solution was diluted with water (10 mL) and extracted with $CHCl_3$ (2×15 mL). The combined organic phase was dried ($MgSO_4$), filtered, evaporated, and the residue was purified by flash column chromatography ($CHCl_3$/Et_2O) to yield the alcohol **18** as a dark red solid with a greenish shine, 417 mg (59%), mp 194–195 °C, R_f: 0.73 ($CHCl_3$/Et_2O, 2:1). EA: calcd for $C_{20}H_{21}BF_2N_2O$: C, 67.82; H, 5.98; N, 7.91. Found: C, 67.81; H, 5.88; N, 7.83. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.47 (d, J=7.2 Hz, 2H, Ar*H*), 7.26 (d, J=7.6 Hz, 2H, Ar*H*), 5.96 (s, 2H, pyrrole *H*), 4.79 (s, 2H, CH_2O), 2.54 (s, 6H, 2×CH_3), 1.36 (s, 6H, 2×CH_3). IR (Nujol) ν: 3300 (OH), 1645, 1560, 1545, 1505 (C=C) cm^{-1}. MS (EI) *m*/*z*: 354: (M$^+$, 100), 334 (52), 287 (36), 91 (75), 77 (67).

4.3.6. 4,4-Difluoro-8-(4-formylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (19). To a solution of alcohol **18** (400 mg, 1.13 mmol) in CH_2Cl_2 (10 mL), activated MnO_2 (1.46 g, 17.0 mmol) was added and the mixture was stirred at rt until the consumption of alcohol was complete (2 h, as monitored by TLC). The mixture was filtered through Celite, the filtrate was evaporated, and then the solid residue was further purified by flash column chromatography (hexane/EtOAc) to yield the aldehyde as a red powder, 254 mg (64%), mp 174–176 °C, R_f: 0.66 (hexane/EtOAc, 2:1). EA: calcd for $C_{20}H_{19}BF_2N_2O$: C, 68.21; H, 5.44; N, 7.95. Found: C, 68.19; H, 5.35; N, 7.89. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=10.10 (s, 1H, C*H*O), 8.02 (d, J=8 Hz, 2H, Ar*H*), 7.49 (d, J=8 Hz, 2H, Ar*H*), 5.98 (s, 2H, pyrrole *H*), 2.59 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.34 (s, 3H, CH_3). IR (Nujol) ν: 1700 (C=O), 1655, 1560, 1540, 1505 (C=C) cm^{-1}. MS (EI) *m*/*z*: 352: (M$^+$, 100), 332 (47), 287 (16), 136 (53).

4.3.7. 2-[(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl)-4-yl]-4,4,5,5-tetramethyl-4,5-dihydro-1*H*-imidazol-3-oxo-1-yloxyl radical (20). To a deoxygenated solution in MeOH (10 mL) of 2,3-bis-hydroxylamino-2,3-dimethylbutane monosulfate (1.0 mmol, 246 mg) and Et_3N (150 mg, 1.5 mmol), aldehyde **19** (352 mg, 1.0 mmol) was added and stirred overnight under N_2. After evaporation of the solvent, the residue was partitioned between $CHCl_3$ (15 mL) and water (20 mL) containing $NaIO_4$ (2.13g, 10.0 mmol), and the mixture was vigorously shaken for 5 min. The organic phase was then separated, dried ($MgSO_4$), filtered, and evaporated, and after flash chromatography (hexane/EtOAc) we got the title compound as dark brown crystals with a greenish shine, 182 mg (38%), mp 280 °C, decomp., R_f: 0.18 (hexane/EtOAc, 2:1). EA: calcd for $C_{26}H_{30}BF_2N_4O_2$: C, 65.15; H, 6.31; N, 11.69. Found: C, 65.12; H, 6.22; N, 11.52. MS (EI) *m*/*z*: 479 (M$^+$, 1), 463 (3), 391 (5), 349 (21), 217 (67), 84 (100). IR (Nujol) ν: 1555, 1535, 1505 (C=C) cm^{-1}. EPR (in $CHCl_3$): 5 lines, a_N=7.4 G.

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Stereodivergent synthesis of 1,4-bifunctional compounds by regio- and diastereoselective Pd-catalyzed allylic substitution reaction

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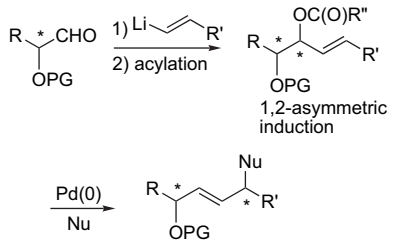
Abstract—Highly stereoselective synthesis of 1,4-bifunctional compounds was accomplished via 1,2-asymmetric induction to α-oxyaldehyde and α-oxyketone followed by regio- and diastereoselective Pd-catalyzed allylic substitution reaction. We found that trifluoroacetate is a suitable leaving group for the allylic substitution reaction. Various nucleophiles containing carbon, nitrogen, and sulfur can be applied to the method. Both 1,4-*syn*- and 1,4-*anti*-adducts were synthesized with high stereoselectivity by using stereodivergent reduction of the propargyl alcohols followed by allylic substitution reaction.

1. Introduction

A combination of asymmetric alkenylation of α-oxyaldehydes and 1,3-chirality transfer reaction affords an attractive methodology to synthesize olefins flanked by two stereogenic centers, which are synthetically useful chiral building blocks.[1] To make this strategy practical, both high diastereoselectivity in the first step and efficient regio- and diastereoselectivity in the following step are essential. Pd-catalyzed allylic alkylation is known to proceed stereospecifically with various nucleophiles, thereby being a promising candidate for the 1,3-chirality transfer reaction, if the regioselectivity is completely controlled.[2] We have recently reported a novel method to synthesize 1,4-bifunctional compounds via diastereoselective alkenylation of α-hydroxy aldehydes followed by Pd-catalyzed allylic substitution (Scheme 1).[3] We also succeeded in a stereodivergent synthesis of the diastereoisomers of the 1,4-bifunctional compounds by modification of this strategy.[4] In these reactions, the stereogenic center at the protected chiral secondary alcohol not only works as a stereocontroller in the first step but also controls the regiochemistry in the diastereoselective allylic substitution reaction.[5]

In this paper, we reported full details of this methodology including generality of this method by applying it to various substrates and nucleophiles. Extension of the method to α-oxyketone is also described.



Scheme 1.

2. Results and discussion

2.1. Pd-catalyzed 1,3-chirality transfer reaction

Three kinds of allylic esters *anti*-**3a–c** were prepared by alkenylation[6] of aldehyde **1**[7] followed by acylation of the resulting alcohol (Scheme 2). We investigated efficiency of Pd-catalyzed 1,3-chirality transfer reaction using these substrates and also compared the reaction of the *anti*- and *syn*-allylic trifluoroacetates **3c** (Table 1).

In the reaction, the choice of the acyl group was crucial.[8] With increase of the inductive effect of the acyl group, the yield was remarkably increased. Although no reaction proceeded in the allylic acetate *anti*-**3a** even under refluxing conditions (entry 1), more reactive allylic trichloroacetate *anti*-**3b** afforded product *anti*-**4** in 32% yield accompanied

Keywords: Catalytic reaction; 1,4-Bifunctional compound; Stereoselective reaction; 1,3-Asymmetric transfer.
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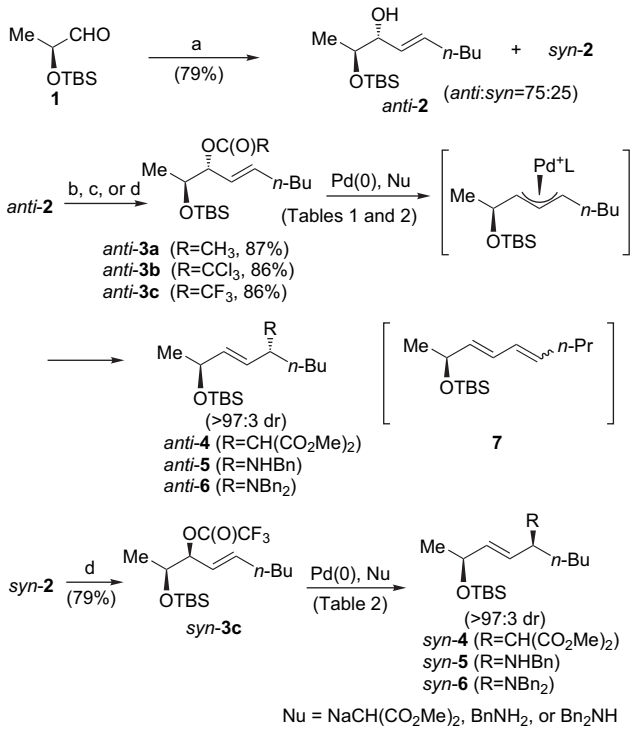


Scheme 2. Reagents and conditions: (a) *t*-BuLi, (*E*)-1-bromo-1-hexene, Et_2O, −65 °C; (b) Ac_2O, DMAP, pyridine, rt; (c) trichloroacetyl chloride, pyridine, rt; (d) $(CF_3CO)_2O$, pyridine, 0 °C.

Table 1. Pd-catalyzed allylic substitution reaction of *anti*-**3a–c** with dimethyl sodiomalonate (Nu=$NaCH(CO_2Me)_2$)[a]

Entry	Substrate	Nu (equiv)	Conditions	Yield (%)[b]	
				anti-**4**	*anti*-**2**
1	*anti*-**3a**	3	Reflux, 5 h	0	0
2	*anti*-**3b**	3	Reflux, 2 h	32	5
3	*anti*-**3c**	3	rt, 0.5 h	74	23
4	*anti*-**3c**	2	rt, 0.5 h	93	tr
5	*anti*-**3c**	1.5	rt, 5 h	72	14
6[c]	*anti*-**3c**	3	rt to reflux, 6 h	0	78

[a] The reactions were carried out using $Pd(PPh_3)_4$ (10 mol %) in THF under Ar.
[b] Isolated yield.
[c] Without Pd catalyst.

by 5% of allylic alcohol *anti*-**2** produced by nucleophilic attack to the ester moiety (entry 2). Trifluoroacetate[9] *anti*-**3c** was the best ester of all to give *anti*-**4** in 74% even at rt, but *anti*-**2** was increased to 23% yield (entry 3). Production of *anti*-**2** was restricted by using 2 equiv of dimethyl sodiomalonate (entry 4). However, when the nucleophile was reduced to 1.5 equiv, the reaction time was prolonged and the yield of *anti*-**4** was decreased (entry 5). The allylic substitution did not occur in the absence of Pd catalyst; instead, nucleophilic attack took place at the ester moiety (entry 6). This means that the reaction is not non-catalytic S_N2' displacement. In all cases, nucleophilic attack took place exclusively at the distal position to the TBSO group with retention of the stereochemistry via double stereoinversion, giving the 1,4-*anti*-adduct as the sole product. Thus, we have found that trifluoroacetate is effective for the allylic substitution under mild conditions.

Next, 1,3-chirality transfer of *anti*- and *syn*-**3c** was investigated using the optimized conditions (Scheme 2, Table 2).

Table 2. Pd-catalyzed allylic substitution reaction of *anti*-**3a–c** with dimethyl sodiomalonate and amines[a]

Entry	Substrate	Product	Conditions	Yield (%)[b]
1	*anti*-**3c**	*anti*-**4**	rt, 30 min	93 (tr)[c]
2	*anti*-**3c**	*anti*-**5**	rt, 30 min	83 (10)[c]
3	*anti*-**3c**	*anti*-**6**	reflux, 23 h	34 (45)[d]
4	*syn*-**3c**	*syn*-**4**	rt, 30 min	98 (tr)[e]
5	*syn*-**3c**	*syn*-**5**	rt, 30 min	84 (15)[e]
6	*syn*-**3c**	*syn*-**6**	rt to reflux, 3 h	46 (23)[d]

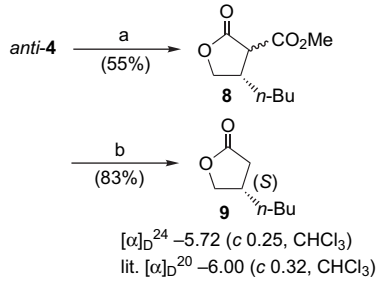
[a] The reactions were carried out using *anti*- or *syn*-**3c** (1 equiv), nucleophile (2 equiv), and $Pd(PPh_3)_4$ (10 mol %) in THF under Ar.
[b] Isolated yield.
[c] Yield in parentheses is the yield of *anti*-**2**.
[d] Yield in parentheses is the yield of **7**.
[e] Yield in parentheses is the yield of *syn*-**2**.

Benzylamine also exclusively attacked distal to the TBSO group at rt, giving the 1,4-*anti*-isomer *anti*-**5** (entry 2).

The reaction was stereospecific and no 1,4-*syn*-isomer was produced from *anti*-**2**. In the case of bulky dibenzylamine, the reaction became sluggish and no product arising from the allylic substitution reaction was formed at rt. When the reaction mixture was refluxed, the starting material disappeared to give the adduct *anti*-**6** in 34% yield along with 45% of diene **7** (ca. 2.7:1 *E*/*Z* mixture) probably formed by β-hydride elimination (entry 3). Although other Pd catalysts were examined, the yields were not better than that of $Pd(PPh_3)_4$ [$Pd_2(dba)_3\cdot CHCl_3$, dppe, 18 h (*anti*-**4**: 9%, **7**: 64%); $(\eta^3\text{-}C_3H_5PdCl)_2$, dppe, 44 h (*anti*-**4**: 0%, **7**: 34%); $Pd_2(dba)_3\cdot CHCl_3$, dppf, 3 h (*anti*-**4**: 29%, **7**: 41%); $Pd(PPh_3)_2Cl_2$, 48 h (*anti*-**4**: 37%, **7**: 14%)].[10]

The reaction of the *syn*-adduct *syn*-**3c** with dimethyl sodiomalonate and benzylamine afforded the corresponding 1,4-*syn*-compounds *syn*-**4** and *syn*-**5**, respectively, in good yields with high regio- and diastereoselectivity (entries 4 and 5). The reaction of *syn*-**3c** with dibenzylamine was sluggish, but the product *syn*-**6** was obtained stereospecifically in 46% yield (entry 6). Thus, two kinds of chiral 1,4-bifunctional compounds *anti*- and *syn*-**4–6** were synthesized stereodivergently.

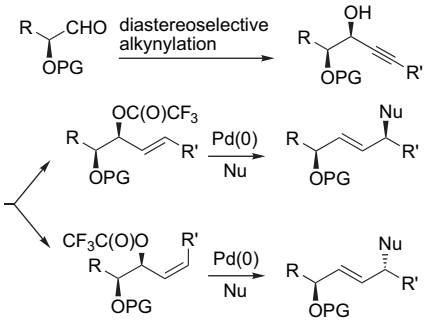
To confirm the absolute configuration, *anti*-**4** was converted to a known compound as shown in Scheme 3. Ozonolysis of the double bond followed by $NaBH_4$ reduction led to *anti*-**4** into γ-lactone **8**. Subsequent demethoxycarboxylation afforded the known γ-lactone **9**.[11] By comparison with the specific rotation, the absolute configuration was determined to be *S*, thereby being consistent with our speculation.[12]



Scheme 3. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C then $NaBH_4$, EtOH–H_2O, rt; (b) NaCl, aq DMSO, 130–150 °C.

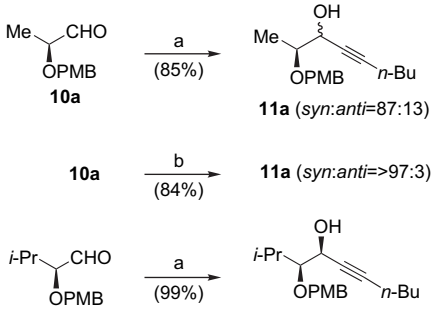
2.2. Stereodivergent synthesis of 1,4-asymmetric compounds

Diastereoselective alkylation affords a new strategy for the stereodivergent synthesis of 1,4-asymmetric compounds. Since propargylic alcohol can be converted to (*E*)- and (*Z*)-allylic alcohols selectively, two diastereoisomers of chiral 1,4-bifunctional compounds could be synthesized by the regio- and diastereoselective allylic substitution reaction (Scheme 4).



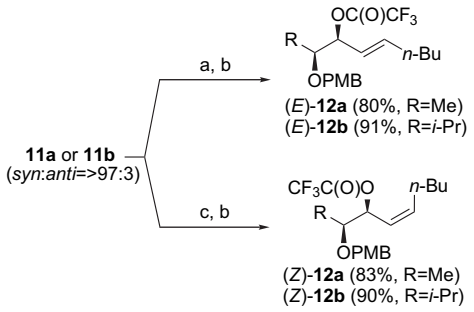
Scheme 4.

Chelation-controlled alkynylation of α-oxyaldehyde **10a**[13] was performed with 1-hexynyllithium under Mead's conditions (Scheme 5).[14] As expected, the *syn*-adduct was obtained mainly (87:13 dr), but the selectivity was unsatisfactory probably due to the small difference in size between Me and H. To improve the diastereoselectivity, we employed Carreira's asymmetric alkynylation using (1*S*,2*R*)-(+)-*N*-methylephedrin (NME) as a chiral ligand.[15] The reaction proceeded in 84% yield and **11a** was obtained with very high diastereoselectivity (>97:3 dr). In the case of α-oxyaldehyde **10b**[16] bearing an isopropyl group, the chiral reagent was not required and alkynylation proceeded under Mead's conditions almost quantitatively with high diastereoselectivity (>97:3 dr).



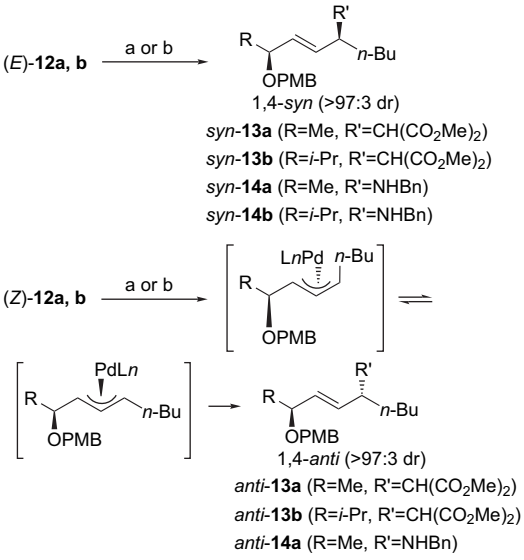
Scheme 5. Reagents and conditions: (a) *n*-BuLi, 1-hexyne, $ZnBr_2$, ether, −78 to 0 °C; (b) 1-hexyne, $Zn(OTf)_2$, (1*R*,2*S*)-(+)-NME, Et_3N, toluene, rt.

The propargylic alcohols **11a** and **11b** were reduced with $LiAlH_4$ in THF and the resulting (*E*)-allylic alcohols were converted to trifluoroacetate (*E*)-**12a** (80%) and (*E*)-**12b** (91%), respectively. On the other hand, the corresponding (*Z*)-isomers were synthesized by hydrogenation on the Lindlar catalyst in MeOH followed by trifluoroacetylation, giving (*Z*)-**12a** and (*Z*)-**12b** in 83% and 90% yield, respectively (Scheme 6).



Scheme 6. Reagents and conditions: (a) $LiAlH_4$, THF, reflux; (b) $(CF_3CO)_2O$, pyridine, Et_2O, rt; (c) H_2, Lindlar catalyst, MeOH, rt.

The results of allylic substitution reactions of trifluoroacetates (*E*)- and (*Z*)-**12a**,**b** are summarized in Scheme 7 and Table 3.



Scheme 7. Reagents and conditions: (a) $Pd(PPh_3)_4$ (10 mol %), $NaCH(CO_2Me)_2$, THF, rt; (b) $Pd(PPh_3)_4$ (10 mol %), $BnNH_2$, THF, rt.

Upon treatment with dimethyl sodiomalonate and benzylamine in the presence of 10 mol % of $Pd(PPh_3)_4$, the nucleophilic substitution proceeded absolutely distal to the *p*-methoxybenzyloxy group in (*E*)-**12a**,**b**, giving 1,4-*syn* isomers *syn*-**13a**,**b** and *syn*-**14a**,**b** in 80–98% yields with high diastereoselectivity by the double inversion via the π-allylpalladium complex (entries 1–4). Bulkiness of the

Table 3. Nucleophilic substitution of allylic trifluoroacetates (*E*)-**12a**,**b** and (*Z*)-**12a**,**b**[a]

Entry	Product	R	R′	Yield (%)[b]	dr[c]
1	*syn*-**13a**	Me	$CH(CO_2Me)_2$	96	>97:3
2	*syn*-**14a**	Me	NHBn	84	>97:3
3	*syn*-**13b**	*i*-Pr	$CH(CO_2Me)_2$	98	>97:3
4	*syn*-**14b**	*i*-Pr	NHBn	80	>97:3
5	*anti*-**13a**	Me	$CH(CO_2Me)_2$	93	>97:3
6	*anti*-**14a**	Me	NHBn	85	>97:3
7	*anti*-**13b**	*i*-Pr	$CH(CO_2Me)_2$	97	>97:3

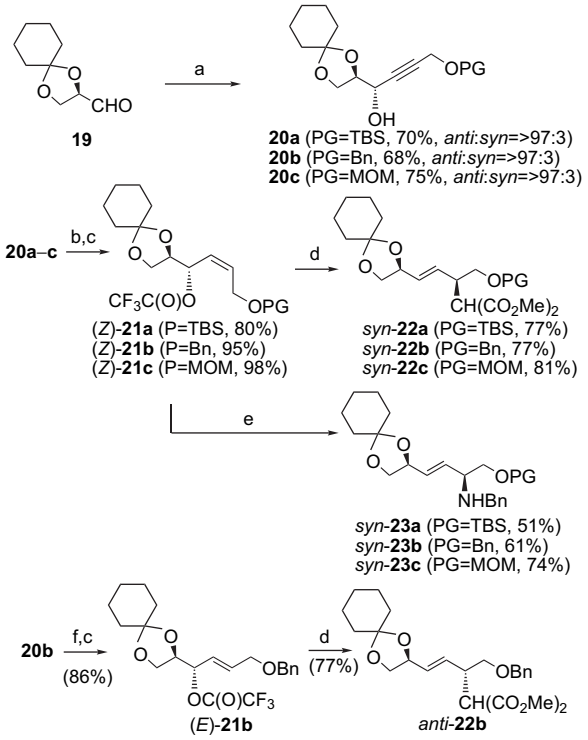
[a] The reactions were carried out using (*E*)- or (*Z*)-**12a**,**b** (1 equiv), nucleophile (2 equiv), and $Pd(PPh_3)_4$ (10 mol %) in THF under Ar.
[b] Isolated yield.
[c] Determined by ^1H NMR spectral data.

substituent R and geometry of the double bond did not influence the reactivity of the allylic substitution reaction. The reaction of the (*Z*)-**12a**,**b** also proceeded regio- and diastereoselectively to give 1,4-*anti* isomers, *anti*-**13a**,**b** and *anti*-**14a** having *trans*-olefin, via π–σ–π isomerization[17] to a thermodynamically more stable π-allyl complex (entries 5–7). The yields ranged from 85% to 97%, wherein the regio- and diastereoselectivity were very high to give the almost sole product. Thus, starting from the common propargylic alcohols, 1,4-*syn* and 1,4-*anti* isomers were synthesized stereodivergently.

Next, the reactions of (*E*)-**12b** with other nucleophiles were examined. The results are summarized in Table 4.

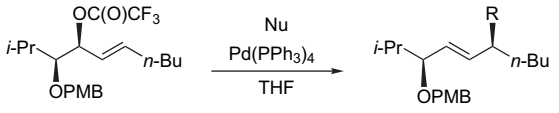
The allylic substitution reaction with malononitrile took place with high regio- and diastereoselectivity to give **15** in 95% yield (entry 1), but the yield of the adduct **16** with Meldrum's acid was low (24%) (entry 2). Morpholine was a good nucleophile even though a secondary amine, giving **17** in 90% yield (entry 3). Sulfur nucleophile, sodium *p*-toluenesulfinate, also afforded the sulfone **18** in 94% yield (entry 4).

Cyclohexylidene acetal-protected aldehyde **19**[18] afforded a route to synthesize highly functionalized compounds (Scheme 8). In this case, diastereoselectivity of chelation-controlled alkynylation was insufficient.[19] However, use of Carreira's conditions furnished *anti*-adducts **20a**–**c** with high diastereoselectivity (>97:3 dr). After conversion to trifluoroacetate (*Z*)-**21a**–**c**, the nucleophilic substitution was carried out under the regular conditions. The reaction of (*Z*)-**21a**–**c** with dimethyl sodiomalonate proceeded in 77–81% yield without being influenced by the protecting group (PG). On the other hand, yield of the reactions with benzylamine varied from 51% to 74% depending on the size of the protecting group. The yield is decreased when the protecting group becomes bulky. Presumably, benzylamine is a weak nucleophile and was affected by the steric hindrance around the reaction site.

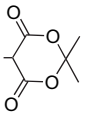
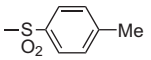


Scheme 8. Reagents and conditions: (a) *O*-protected propargyl alcohol, $Zn(OTf)_2$, (1*S*,2*R*)-(+)-NME, Et_3N, toluene, rt; (b) H_2, Lindlar catalyst, MeOH, rt; (c) $(CF_3CO)_2O$, pyridine, Et_2O, rt; (d) $Pd(PPh_3)_4$ (10 mol %), $NaCH(CO_2Me)_2$, THF, rt; (e) $Pd(PPh_3)_4$ (10 mol %), $BnNH_2$, THF, rt; (f) Red-Al®, ether, rt.

Table 4. Nucleophilic substitution of (*E*)-**12b** with various nucleophiles[a]



(*E*)-**12b** **15–18**

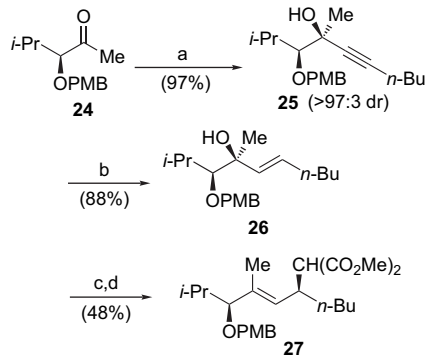
Entry	Product	R	Conditions	Yield (%)[b]	dr[c]
1	**15**	–CH(CN)$_2$	$CH_2(CN)_2$, NaH, rt, 40 min	95	>97:3
2	**16**		Meldrum's acid, NaH, rt, 33.5 h	24	>97:3
3	**17**		Morpholine, rt, 45 min	90	>97:3
4	**18**		TolS(O)ONa, MeOH, rt, 10 min	94	>97:3

[a] The reactions were carried out using (*E*)-**12b** (1 equiv), nucleophile (2 equiv), and $Pd(PPh_3)_4$ (10 mol %) in THF under Ar.
[b] Isolated yield.
[c] Determined by ^1H NMR spectral data.

Stereodivergent conversion was demonstrated using the benzyl ether **20b**. After *E*-selective reduction of **20b** with Red-Al® and subsequent trifluoroacetylation, the resulting (*E*)-**21b** underwent Pd-catalyzed allylic alkylation to furnish 1,4-*anti*-compound *anti*-**22b** in good overall yield. Even in the highly oxygenated and sterically congested substrates, stereodivergent synthesis was accomplished. In all the cases, nucleophiles were introduced with high regio- and diastereoselectivity.

Finally, we examined alkynylation of α-hydroxyketone followed by 1,3-chirality transfer (Scheme 9). Methylketone **24** was alkynylated with magnesium acetylide generated from 1-hexyne with EtMgBr, giving *syn*-adduct **25** as the sole product. *E*-Selective reduction of the triple bond with $LiAlH_4$ led **25** to the allylic alcohol **26**. Trifluoroacetate of **26** was subjected to the Pd-catalyzed 1,3-chirality transfer reaction, but the reaction required refluxing conditions. The adduct **27** with dimethyl sodiomalonate was produced in 48% yield in two steps with high *E*- and diastereoselectivity.

In conclusion, we have developed a strategy to synthesize 1,4-asymmetric compounds by a combination of 1,2-asymmetric addition to α-oxyaldehydes and ketones followed by Pd-catalyzed 1,3-asymmetric transfer. The strategy could be applied to other α-substituted aldehydes and ketones and become a useful tool for synthesis of natural products.



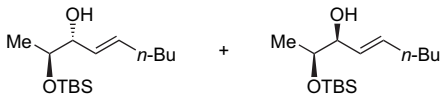
Scheme 9. Reagents and conditions: (a) 1-hexyne, EtMgBr, THF, −40 °C; (b) $LiAlH_4$, THF, reflux; (c) $(CF_3CO)_2O$, pyridine, rt; (d) $Pd(PPh_3)_4$, $NaCH(CO_2Me)_2$, reflux.

3. Experimental

3.1. General

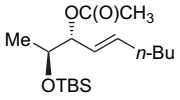
Melting points are uncorrected. NMR spectra were recorded in $CDCl_3$ solution at 500 MHz (^1H) and 75 or 67.8 MHz (^{13}C). IR absorption spectra (FT: diffuse reflectance spectroscopy) were recorded with KBr powder, and only noteworthy absorptions (cm^{-1}) are listed. Column chromatography was carried out using Merck silica gel 60 (70–230 mesh) or Kanto Chemical silica gel 60N (63–210 μm). All air- or moisture-sensitive reactions were carried out in flame-dried glassware under an atmosphere of N_2 or Ar. All solvents were dried and distilled according to standard procedures. All organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated with a rotary evaporator under reduced pressure. Experimental procedure of *syn*-**13a** and *syn*-**14a**, and ^1H and ^{13}C NMR spectra of *anti*-**4**–**6**, **13a**, **14a** and *syn*-**4**–**6**, **13a**, **14a** were reported in the Supporting Information of Ref. 4.

3.1.1. (*E*,2*S*,3*R*)- and (*E*,2*S*,3*S*)-2-(*tert*-Butyldimethylsilyloxy)-4-nonen-3-ol (*anti*-2 and *syn*-2).



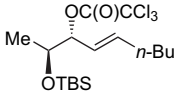
t-BuLi (1.47 M in *n*-pentane, 2.72 mL, 4.00 mmol) was added to a solution of (*E*)-1-bromo-1-hexene (420 mg, 2.00 mmol) in Et_2O (10 mL) with stirring at −78 °C under Ar and the stirring was continued at −65 °C for 10 min. A solution of **1** (376 mg, 2.00 mmol) in Et_2O (1 mL) was added to the mixture with stirring at −78 °C and the whole was stirred at this temperature for 30 min. The reaction was quenched with saturated NH_4Cl, water, and brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (20:1) to give *anti*-**2** and *syn*-**2** (431 mg, 79%, *anti*:*syn*=75:25). *anti*-**2**: Colorless oil. $[\alpha]_D^{19}$ +8.61 (*c* 1.13, $CHCl_3$). ^1H NMR δ: 0.08 (s, 6H, $Si(CH_3)_2$), 0.89 (t, *J*=7.0 Hz, 3H, CH_2CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 1.07 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.28–1.40 (m, 4H, $CH_2\times2$), 2.05 (q, *J*=6.7 Hz, 2H, CH=CHCH_2), 2.22 (d, *J*=3.7 Hz, 1H, OH), 3.81 (qd, *J*=6.1, 3.7 Hz, 1H, TBSOC*H*), 3.97 (dt, *J*=7.3, 3.7 Hz, 1H, C*H*OH), 5.41 (dd, *J*=15.3, 7.3 Hz, 1H, C*H*=CHCH$_2$), 5.69 (dt, *J*=15.3, 6.7 Hz, 1H, CH=C*H*CH$_2$). ^{13}C NMR δ: −4.9, −4.5, 13.9, 17.6, 18.0, 22.2, 25.8 (3C), 31.3, 32.1, 71.6, 76.5, 128.1, 133.8. IR: 3458 (OH), 2929 (CH), 2858 (CH). MS (FAB) *m*/*z* 294 (MNa^+). HRMS (FAB) calcd for $C_{15}H_{32}NaO_2Si$ (MNa^+): 295.2069; found: 295.2062. *syn*-**2**: Colorless oil. $[\alpha]_D^{25}$ +23.2 (*c* 1.03, $CHCl_3$). ^1H NMR δ: 0.07 (s, 6H, $Si(CH_3)_2$), 0.87 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 0.89 (s, 9H, $C(CH_3)_3$), 1.11 (d, *J*=6.7 Hz, 3H, TBSOCHCH_3), 1.26–1.39 (m, 4H, $CH_2\times2$), 2.03 (qd, *J*=6.7, 1.8 Hz, 2H, CH=CHCH_2), 2.57 (d, *J*=3.4 Hz, 1H, OH), 3.62 (quint, *J*=6.7 Hz, 1H, TBSOC*H*), 3.72 (td, *J*=6.7, 3.4 Hz, 1H, C*H*OH), 5.37 (ddt, *J*=15.6, 6.7, 1.8 Hz, 1H, C*H*=CHCH$_2$), 5.71 (dt, *J*=15.6, 6.7 Hz, 1H, CH=C*H*CH$_2$). ^{13}C NMR δ: −4.9, −4.3, 13.9, 18.0, 19.9, 22.2, 25.8 (3C), 31.2, 32.0, 72.2, 77.3, 129.1, 134.1. IR: 3438 (OH), 2956 (CH), 2929 (CH), 2958 (CH). Anal. Calcd for $C_{15}H_{32}O_2Si$: C, 66.11; H, 11.84. Found: C, 66.24; H, 11.67.

3.1.2. (*E*,2*S*,3*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-nonen-3-yl acetate (*anti*-3a).



Acetic anhydride (0.14 mL, 1.50 mmol) was added to a mixture of *anti*-**2** (136 mg, 0.500 mmol) and DMAP (61.1 mg, 0.500 mmol) in pyridine (1 mL) with stirring at 0 °C and the stirring was continued at rt for 30 min. The reaction was quenched with saturated NH_4Cl at 0 °C and the mixture was extracted with Et_2O. The extract was washed with saturated NH_4Cl, water, and brine prior to drying and solvent evaporation. The residue was purified by flash chromatography on silica gel with hexane–EtOAc (50:1) to give *anti*-**3a** (137 mg, 87%) as a colorless oil. $[\alpha]_D^{22}$ −31.9 (*c* 1.08, $CHCl_3$). ^1H NMR δ: 0.04 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$), 0.89 (t, *J*=7.0 Hz, 3H, CH_2CH_3), 0.89 (s, 9H, $C(CH_3)_3$), 1.07 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.25–1.39 (m, 4H, $CH_2\times2$), 2.05 (s, 3H, $COCH_3$), 2.05 (dq, *J*=6.7, 1.8 Hz, 2H, CH=CHCH_2), 3.90 (qd, *J*=6.1, 3.7 Hz, 1H, TBSOC*H*), 5.03 (dd, *J*=7.9, 3.7 Hz, 1H, C*H*OAc), 5.48 (ddt, *J*=15.6, 7.9, 1.8 Hz, 1H, C*H*=CHCH$_2$), 5.71 (dt, *J*=15.6, 6.7 Hz, 1H, CH=C*H*CH$_2$). ^{13}C NMR δ: −4.7, −4.5, 14.0, 18.1, 19.9, 21.5, 22.3, 25.8 (3C), 31.1, 32.1, 69.6, 79.0, 124.1, 136.5, 170.0. IR: 2958 (CH), 2929 (CH), 2858 (CH), 1740 (C=O). MS (FAB) *m*/*z* 337 (MNa^+). HRMS (FAB) calcd for $C_{17}H_{34}NaO_3Si$ (MNa^+): 337.2175; found: 337.2183.

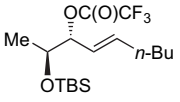
3.1.3. (*E*,2*S*,3*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-nonen-3-yl trichloroacetate (*anti*-3b).



Trichloroacetyl chloride (45 μL, 0.400 mmol) was added to a mixture of *anti*-**2** (54.5 mg, 0.200 mmol) in pyridine (0.4 mL) with stirring at 0 °C and the stirring was continued at rt for 15 min. The reaction was quenched with saturated

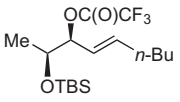
NH_4Cl at 0 °C and the mixture was extracted with Et_2O. The extract was washed with saturated NH_4Cl, water, and brine prior to drying and solvent evaporation. The residue was purified by flash chromatography on silica gel with hexane–EtOAc (50:1) to give *anti*-**3b** (71.5 mg, 86%) as a colorless oil. $[\alpha]_D^{23}$ −8.95 (*c* 0.90, $CHCl_3$). ^1H NMR δ: 0.07 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$), 0.88 (s, 9H, $C(CH_3)_3$), 0.89 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 1.16 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.26–1.40 (m, 4H, $CH_2\times2$), 2.08 (dq, *J*=6.7, 1.8 Hz, 2H, CH=CHCH_2), 3.99 (qd, *J*=6.1, 4.3 Hz, 1H, TBSOC*H*), 5.15 (dd, *J*=7.9, 4.3 Hz, 1H, C*H*OC(O)CCl_3), 5.50 (ddt, *J*=15.6, 7.9, 1.8 Hz, 1H, C*H*=CHCH_2), 5.88 (dt, *J*=15.9, 6.7 Hz, 1H, CH=C*H*CH_2). ^{13}C NMR δ: −4.8, −4.4, 14.0, 18.0, 19.4, 22.2, 25.8 (3C), 30.9, 32.1, 69.4, 84.5, 90.4, 122.5, 138.6, 160.9. IR: 2956 (CH), 2929 (CH), 2858 (CH), 1765 (C=O). MS (FAB) *m*/*z* 439 (MNa^+). HRMS calcd for $C_{17}H_{31}NaO_3Si$ (MNa^+): 439.1006; found: 439.0985.

3.1.4. (*E*,2*S*,3*R*)-2-(*tert*-Butyldimethylsilyloxy)-4-nonen-3-yl trifluoroacetate (*anti*-3c).



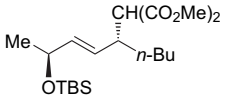
Trifluoroacetic anhydride (0.45 mL, 3.20 mmol) was added to a mixture of *anti*-**2** (437 mg, 1.60 mmol) in pyridine (3.2 mL) with stirring at 0 °C and the stirring was continued at this temperature for 5 min. The reaction was quenched with saturated NH_4Cl at 0 °C and the mixture was extracted with Et_2O. The extract was washed with saturated NH_4Cl, water, and brine prior to drying and solvent evaporation. The residue was purified by flash chromatography on silica gel with hexane–EtOAc (50:1) to give *anti*-**3c** (508 mg, 86%) as a colorless oil. $[\alpha]_D^{24}$ −30.0 (*c* 0.95, $CHCl_3$). ^1H NMR δ: 0.05 (s, 6H, $Si(CH_3)_2$), 0.88 (s, 9H, $C(CH_3)_3$), 0.89 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 1.11 (d, *J*=6.7 Hz, 3H, TBSOCHCH_3), 1.27–1.40 (m, 4H, $CH_2\times2$), 2.08 (d, *J*=6.7 Hz, 2H, CH=CHCH_2), 3.98 (qd, *J*=6.7, 3.1 Hz, 1H, TBSOC*H*), 5.20 (dd, *J*=7.9, 3.1 Hz, 1H, C*H*OC(O)CF_3), 5.51 (dd, *J*=15.9, 7.9 Hz, 1H, C*H*=CHCH_2), 5.85 (dt, *J*=15.9, 6.7 Hz, 1H, CH=C*H*CH_2). ^{13}C NMR δ: −5.0, −4.6, 14.0, 18.0, 19.4, 22.2, 25.7 (3C), 30.9, 32.1, 69.4, 83.5, 114.5 (q, J_{C-F}=285.1 Hz), 122.1, 139.3, 156.6 (q, J_{C-F}=41.9 Hz). IR: 2958 (CH), 2931 (CH), 2898 (CH), 1786 (C=O). Anal. Calcd for $C_{17}H_{31}F_3O_3Si$: C, 55.41; H, 8.48. Found: C, 55.58; H, 8.47.

3.1.5. (*E*,2*S*,3*S*)-2-(*tert*-Butyldimethylsilyloxy)-4-nonen-3-yl trifluoroacetate (*syn*-3c).



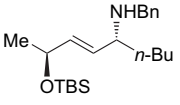
Compound *syn*-**3c** was obtained as a colorless oil (175 mg, 79%) from *syn*-**2** (164 mg, 0.600 mmol) as described for *anti*-**3c**. $[\alpha]_D^{25}$ +33.3 (*c* 1.01, $CHCl_3$). ^1H NMR δ: 0.05 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$), 0.87 (s, 9H, $C(CH_3)_3$), 0.89 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 1.12 (d, *J*=6.7 Hz, 3H, TBSOCHCH_3), 1.26–1.39 (m, 4H, $CH_2\times2$), 2.06 (qd, *J*=7.3, 1.8 Hz, 2H, CH=CHCH_2), 3.91 (quint, *J*=6.7 Hz, 1H, TBSOC*H*), 5.17 (t, *J*=6.7 Hz, 1H, C*H*OC(O)CF_3), 5.38 (ddt, *J*=15.3, 6.7, 1.8 Hz, 1H, C*H*=CHCH_2), 5.87 (dt, *J*=15.3, 7.3 Hz, 1H, CH=C*H*CH_2). ^{13}C NMR δ: −5.2, −4.7, 13.8, 17.9, 19.8, 22.1, 25.6 (3C), 30.8, 32.0, 68.9, 83.8, 114.6 (q, J_{C-F}=286.5 Hz), 123.0, 139.3, 156.8 (q, J_{C-F}=41.7 Hz). IR: 2958 (CH), 2931 (CH), 2860 (CH), 1784 (C=O). MS (EI) *m*/*z* (%): 311 ($M^+-C_4H_9$, 50.8). HRMS (EI) calcd for $C_{13}H_{22}F_3O_3Si$ ($M^+-C_4H_9$): 311.1290; found: 311.1300.

3.1.6. General procedure for Pd-catalyzed allylic substitution reaction with dimethyl sodiomalonate: dimethyl 2-[(*E*,2*S*,5*R*)-2-(*tert*-butyldimethylsilyloxy)-3-nonen-5-yl]malonate (*anti*-4).



NaH (60% in oil, 12.0 mg, 0.300 mmol) was washed with *n*-hexane under N_2 and suspended in THF (2 mL). Dimethyl malonate (38 μL, 0.33 mmol) was added dropwise to the suspension with stirring at 0 °C and the stirring was continued for 10 min at rt. The solution of dimethyl sodiomalonate in THF was added to a mixture of *anti*-**3c** (55.3 mg, 0.15 mmol) and $Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol) in THF (2 mL), and the whole was stirred at rt for 30 min. The reaction was quenched with water and THF was evaporated. The residue was extracted with EtOAc. The extract was washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (50:1) to give *anti*-**4** (53.9 mg, 93%) as a colorless oil. $[\alpha]_D^{24}$ −1.14 (*c* 0.54, $CHCl_3$). ^1H NMR δ: 0.04 (s, 6H, $Si(CH_3)_2$), 0.86 (t, *J*=7.0 Hz, 3H, CH_2CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 1.16 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.18–1.32 (m, 5H, $CH_2\times3$), 1.39–1.45 (m, 1H, C$H_2CH_2CH_2CH_3$), 2.72 (qd, *J*=9.2, 3.7 Hz, 1H, C*H*$CH(CO_2CH_3)_2$), 3.34 (d, *J*=9.2 Hz, 1H, C*H*$(CO_2CH_3)_2$), 3.67 (s, 3H, CO_2CH_3), 3.72 (s, 3H, CO_2CH_3), 4.23 (quint, *J*=6.1 Hz, 1H, TBSOC*H*), 5.34 (dd, *J*=15.3, 9.2 Hz, 1H, TBSOCHCH=C*H*), 5.53 (dd, *J*=15.3, 6.1 Hz, 1H, TBSOCH*CH*=CH). ^{13}C NMR δ: −4.8, −4.6, 14.0, 18.4, 22.5, 24.9, 26.0 (3C), 29.3, 32.4, 42.8, 52.2, 52.4, 57.3, 69.2, 127.9, 138.0, 168.4, 168.6. IR: 2956 (CH), 2929 (CH), 2858 (CH), 1761 (C=O), 1741 (C=O). Anal. Calcd for $C_{20}H_{38}O_5Si$: C, 62.14; H, 9.91. Found: C, 62.18; H, 9.76.

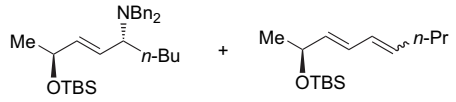
3.1.7. General procedure for Pd-catalyzed allylic substitution reaction with benzylamine: (*E*,2*S*,5*R*)-5-benzylamino-2-(*tert*-butyldimethylsilyloxy)-3-nonene (*anti*-5).



Benzylamine (33 μL, 0.30 mmol) was added to a mixture of *anti*-**3** (221 mg, 0.600 mmol) and $Pd(PPh_3)_4$ (69.3 mg, 60 μmol) in THF (12 mL) and the whole was stirred at rt for 30 min. The reaction was quenched with saturated

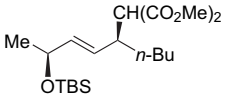
NH_4Cl. After water was added to the mixture at rt, the mixture was extracted with Et_2O. The extract was washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (5:1) to give *syn*-**5** (192 mg, 83%) as a yellow oil along with *anti*-**2** (16.9 mg, 10%). $[\alpha]_D^{23}$ +10.8 (*c* 0.76, $CHCl_3$). ^1H NMR δ: 0.070 (s, 3H, $SiCH_3$), 0.073 (s, 3H, $SiCH_3$), 0.87 (t, *J*=6.1 Hz, 3H, CH_2CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 1.24 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.24–1.31 (m, 5H, $CH_2\times3$), 1.37–1.44 (m, 1H, $CH_2CH_2CH_2CH_3$), 1.50 (br, 1H, NH), 2.99 (td, *J*=7.9, 5.5 Hz, 1H, C*H*NHBn), 3.61 (d, *J*=12.8 Hz, 1H, CH_2Ph), 3.79 (d, *J*=12.8 Hz, 1H, CH_2Ph), 4.33 (quint, *J*=6.1 Hz, 1H, TBSOC*H*), 5.36 (dd, *J*=15.3, 7.9 Hz, 1H, TBSOCHCH=C*H*), 5.56 (dd, *J*=15.3, 6.1 Hz, 1H, TBSOCHC*H*=CH), 7.22–7.33 (m, 5H, ArH). ^{13}C NMR δ: −4.8, −4.6, 14.0, 18.3, 22.7, 24.9, 25.9 (3C), 28.2, 35.8, 51.2, 59.8, 69.1, 126.8, 128.2 (2C), 128.3 (2C), 131.4, 136.9, 140.8. IR: 2956 (CH), 2929 (CH), 2858 (CH). MS (FAB) *m*/*z* 362 (MH^+). HRMS (FAB) calcd for $C_{22}H_{40}NOSi$ (MH^+): 362.2879; found: 362.2877.

3.1.8. General procedure for Pd-catalyzed allylic substitution reaction with dibenzylamine: (*E*,2*S*,5*R*)-2-(*tert*-butyldimethylsilyloxy)-5-dibenzylamino-3-nonene (*anti*-6) and (3*E*,5*EZ*,2*S*)-2-(*tert*-butyldimethylsilyloxy)-3,5-nonadiene (7).



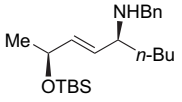
Dibenzylamine (58 μL, 0.300 mmol) was added to a mixture of *anti*-**3** (55.3 mg, 0.150 mmol) and $Pd(PPh_3)_4$ (17.3 mg, 15 μmol) in THF (3 mL) and the whole was refluxed for 23 h. The reaction was quenched with water and THF was evaporated. The residue was extracted with EtOAc. The extract was washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (50:1) to give *anti*-**6** (22.9 mg, 34%) along with **7** (17.3 mg, 45%, major:minor=73:27) each as a colorless oil. *anti*-**6**: $[\alpha]_D^{25}$ +21.2 (*c* 0.34, $CHCl_3$). ^1H NMR δ: 0.087 (s, 3H, $SiCH_3$), 0.093 (s, 3H, $SiCH_3$), 0.84 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 0.92 (s, 9H, $C(CH_3)_3$), 1.17–1.43 (m, 5H, $CH_2\times3$), 1.27 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.64–1.71 (m, 1H, $CH_2CH_2CH_2CH_3$), 2.97 (q, *J*=7.9 Hz, 1H, C*H*NBn_2), 3.31 (d, *J*=13.7 Hz, 2H, CH_2Ph), 3.78 (d, *J*=13.7 Hz, 2H, CH_2Ph), 4.37 (quint, *J*=6.1 Hz, 1H, TBSOC*H*), 5.47 (dd, *J*=15.3, 6.1 Hz, 1H, TBSOCHC*H*=CH), 5.55 (dd, *J*=15.3, 7.9 Hz, 1H, TBSOCHCH=C*H*), 7.20 (t, *J*=7.3 Hz, 2H, ArH), 7.28 (t, *J*=7.3 Hz, 4H, ArH), 7.36 (d, *J*=7.3 Hz, 4H, ArH). ^{13}C NMR δ: −4.6, −4.4, 14.2, 18.5, 22.7, 25.2, 26.0 (3C), 28.8, 32.4, 53.7 (2C), 59.5, 69.2, 126.2, 126.5 (2C), 128.0 (4C), 128.6 (4C), 138.3, 140.5 (2C). IR: 2956 (CH), 2929 (CH), 2858 (CH). MS (FAB) *m*/*z* 474 (MNa^+). HRMS (FAB) calcd for $C_{29}H_{45}NNaOSi$ (MNa^+): 474.3168; found: 474.3180. Compound **7**: ^1H NMR δ: 0.038 (s, 0.81H, $SiCH_3$), 0.042 (s, 0.81H, $SiCH_3$), 0.050 (s, 2.19H, $SiCH_3$), 0.058 (s, 2.19H, $SiCH_3$), 0.88–0.92 (m, 11.19H, $C(CH_3)_3$ and 9-H), 1.00 (t, *J*=7.3 Hz, 0.81H, 9-H), 1.12 (d, *J*=6.1 Hz, 0.81H, 1-H), 1.21 (d, *J*=6.7 Hz, 2.19H, 1-H), 1.41 (sext, *J*=7.3 Hz, 1.46H, 8-H), 2.03–2.24 (m, 2.54H, 7-H and 8-H), 3.80 (quint, *J*=6.1 Hz, 0.27H, 2-H), 4.32 (quint, *J*=6.1 Hz, 0.73H, 2-H), 5.51–5.67 (m, 2H, 3-H and 6-H), 5.96–6.12 (m, 2H, 4-H and 5-H). ^{13}C NMR (major) δ: −4.61, −4.42, 13.9, 18.4, 22.6, 24.6, 26.0 (3C), 34.8, 69.0, 128.4, 129.7, 134.1, 135.5; (minor) δ: −4.56, −4.51, 13.8, 18.4, 23.6, 25.7, 26.0 (3C), 43.1, 68.8, 128.4, 129.2, 132.4, 134.3. IR: 2958 (CH), 2929 (CH), 2858 (CH). MS (FAB) *m*/*z* 253 (M^+-H). HRMS (FAB) calcd for $C_{15}H_{29}OSi$ (M^+-H): 253.1988; found: 253.2004.

3.1.9. Dimethyl 2-[(*E*,2*S*,5*S*)-2-(*tert*-butyldimethylsilyloxy)-3-nonen-5-yl]malonate (*syn*-4).



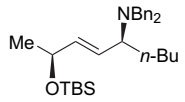
Compound *syn*-**4** was obtained as a colorless oil (56.6 mg, 98%) from *syn*-**3c** (55.3 mg, 0.150 mmol) as described for *anti*-**4**. $[\alpha]_D^{23}$ −8.02 (*c* 1.03, $CHCl_3$). ^1H NMR δ: 0.03 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$), 0.86 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.88 (s, 9H, $C(CH_3)_3$), 1.16 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.18–1.32 (m, 5H, $CH_2\times3$), 1.39–1.45 (m, 1H, $CH_2CH_2CH_2CH_3$), 2.74 (qd, *J*=9.2, 3.7 Hz, 1H, C*H*$CH(CO_2CH_3)_2$), 3.36 (d, *J*=9.2 Hz, 1H, C*H*$(CO_2Me)_2$), 3.68 (s, 3H, CO_2CH_3), 3.72 (s, 3H, CO_2CH_3), 4.24 (quint, *J*=6.1 Hz, 1H, TBSOC*H*), 5.39 (dd, *J*=15.3, 9.2 Hz, 1H, TBSOCHCH=C*H*), 5.55 (dd, *J*=15.3, 6.1 Hz, 1H, TBSOCHC*H*=CH). ^{13}C NMR δ: −4.8, −4.6, 14.0, 18.4, 22.4, 24.8, 25.9 (3C), 29.2, 32.3, 42.4, 52.2, 52.4, 57.1, 68.6, 127.6, 137.7, 168.4, 168.7. IR: 2958 (CH), 2929 (CH), 2860 (CH), 1761 (C=O), 1741 (C=O). Anal. Calcd for $C_{20}H_{38}O_5Si$: C, 62.14; H, 9.91. Found: C, 62.23; H, 9.79.

3.1.10. (*E*,2*S*,5*S*)-5-Benzylamino-2-(*tert*-butyldimethylsilyloxy)-3-nonene (*syn*-5).



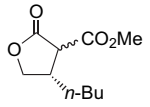
Compound *syn*-**5** was obtained as a yellow oil (45.6 mg, 84%) along with *syn*-**2** (6.3 mg, 15%) from *syn*-**3c** (55.3 mg, 0.150 mmol) as described for *anti*-**5**. $[\alpha]_D^{22}$ −28.1 (*c* 0.45, $CHCl_3$). ^1H NMR δ: 0.08 (s, 6H, $Si(CH_3)_2$), 0.86 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 0.91 (s, 9H, $C(CH_3)_3$), 1.23 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.23–1.31 (m, 5H, $CH_2\times3$), 1.37–1.42 (m, 1H, $CH_2CH_2CH_2CH_3$), 1.51 (br, 1H, NH), 2.99 (td, *J*=8.2, 5.5 Hz, 1H, C*H*NHBn), 3.64 (d, *J*=13.1 Hz, 1H, CH_2Ph), 3.83 (d, *J*=13.1 Hz, 1H, CH_2Ph), 4.32 (quint, *J*=6.1 Hz, 1H, TBSOC*H*), 5.36 (dd, *J*=15.3, 8.2 Hz, 1H, TBSOCHCH=C*H*), 5.57 (dd, *J*=15.3, 6.1 Hz, 1H, TBSOCHC*H*=CH), 7.22–7.33 (m, 5H, ArH). ^{13}C NMR δ: −4.6, −4.4, 14.2, 18.5, 22.8, 25.0, 26.0 (3C), 28.3, 35.8, 51.3, 59.8, 69.2, 126.7, 128.1 (2C), 128.3 (2C), 131.3, 136.9, 140.7. IR: 2956 (CH), 2927 (CH), 2858 (CH). MS (FAB) *m*/*z* 377 (MH^+). HRMS (FAB) calcd for $C_{22}H_{40}NOSi$ (MH^+): 362.2879; found: 362.2872.

3.1.11. (*E*,2*S*,5*S*)-2-(*tert*-Butyldimethylsilyloxy)-5-dibenzylamino-3-nonene (*syn*-6).



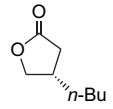
Compound *syn*-**6** was obtained as a colorless oil (31.4 mg, 46%) along with **7** (9.1 mg, 23%) from *syn*-**3c** (55.3 mg, 0.150 mmol) as described for *anti*-**6**. $[\alpha]_D^{25}$ −42.6 (*c* 0.68, $CHCl_3$). ^1H NMR δ: 0.11 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.84 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 0.95 (s, 9H, $C(CH_3)_3$), 1.17–1.43 (m, 5H, $CH_2\times3$), 1.25 (d, *J*=6.1 Hz, 3H, TBSOCHCH_3), 1.63–1.69 (m, 1H, $CH_2CH_2CH_2CH_3$), 2.98 (q, *J*=7.9 Hz, 1H, C*H*NBn_2), 3.37 (d, *J*=13.7 Hz, 2H, CH_2Ph), 3.77 (d, *J*=13.7 Hz, 2H, CH_2Ph), 4.33 (quint, *J*=6.1 Hz, 1H, TBSOC*H*), 5.48 (dd, *J*=15.3, 6.1 Hz, 1H, TBSOCHC*H*=CH), 5.56 (dd, *J*=15.3, 7.9 Hz, 1H, TBSOCHCH=C*H*), 7.20 (t, *J*=7.3 Hz, 2H, ArH), 7.28 (t, *J*=7.3 Hz, 4H, ArH), 7.36 (d, *J*=7.3 Hz, 4H, ArH). ^{13}C NMR δ: −4.5, −4.4, 14.2, 18.5, 22.7, 25.1, 26.0 (3C), 28.8, 32.3, 53.6 (2C), 59.3, 69.4, 126.3, 126.5 (2C), 128.0 (4C), 128.6 (4C), 138.3, 140.5 (2C). IR: 2956 (CH), 2929 (CH), 2858 (CH). MS (FAB) *m*/*z* 452 (MH^+). HRMS (FAB) calcd for $C_{29}H_{46}NOSi$ (MH^+): 452.3349; found: 452.3354.

3.1.12. Methyl (3*RS*,4*S*)-4-butyl-2-oxotetrahydrofuran-3-carboxylate (8).



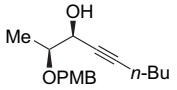
A stream of ozone was bubbled through a solution of *anti*-**4** (77.3 mg, 0.200 mmol) in CH_2Cl_2 (5 mL) at −78 °C for 40 min. Nitrogen was allowed to bubble through the solution to remove excess ozone at this temperature. Then, a solution of $NaBH_4$ (56.7 mg, 1.50 mmol) in aqueous EtOH (1:1 mixture) (1 mL) was added dropwise and the whole was stirred at rt for 12 h. The reaction was quenched with water, and the mixture was extracted with EtOAc. The extract was washed with water and brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (4:1) to give **8** (22.0 mg, 55%, major:minor=89:11) as a colorless oil. ^1H NMR δ: 0.90 (t, *J*=7.3 Hz, 3H, 4′-H), 1.23–1.37 (m, 4H, 2′-H and 3′-H), 1.46–1.58 (m, 2H, 1′-H), 2.75–2.83 (m, 0.11H, 4-H), 2.97 (sext, *J*=7.9 Hz, 0.89H, 4-H), 3.24 (d, *J*=7.9 Hz, 0.89H, 3-H), 3.54 (d, *J*=8.5 Hz, 0.11H, 3-H), 3.77 (s, 0.33H, CO_2CH_3), 3.81 (s, 2.67H, CO_2CH_3), 3.91 (t, *J*=7.9 Hz, 0.89H, 5-H), 4.15 (dd, *J*=10.4, 8.5 Hz, 0.11H, 5-H), 4.43 (t, *J*=8.5 Hz, 0.11H, 5-H), 4.51 (t, *J*=7.9 Hz, 0.89H, 5-H). ^{13}C NMR (major) δ: 13.9, 22.6, 29.1, 32.1, 40.2, 52.5, 53.1, 72.1, 168.1, 171.9. IR: 2958 (CH), 2933 (CH), 2862 (CH), 1778 (C=O), 1739 (C=O). MS (FAB) *m*/*z* 201 (MH^+). HRMS (FAB) calcd for $C_{10}H_{17}O_4$ (MH^+): 201.1127; found: 201.1127.

3.1.13. (*S*)-3-Butyl-γ-butyrolactone (9).



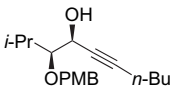
NaCl (5.8 mg, 0.100 mmol) and five drops of water were added to a solution of **8** (13.0 mg, 0.0650 mmol) in DMSO (1 mL). The mixture was heated at 130 °C for 2 h and then 150 °C for 4 h. After cooling, brine was added to the mixture and the whole was extracted with Et_2O. The extract was washed with water and brine and dried. The solvent was evaporated to give **9** (7.7 mg, 83%) as a yellow oil. $[\alpha]_D^{24}$ −5.72 (*c* 0.25, $CHCl_3$).

3.1.14. (2*S*,3*S*)-2-(4-Methoxybenzyloxy)-4-nonyn-3-ol (11a).



A flask was charged with $Zn(OTf)_2$ (800 mg, 2.20 mmol). Vacuum (7 mmHg) was applied and heated to 120 °C for 10 h. After the flask was cooled to rt, the vacuum was released. (1*S*,2*R*)-(+)-*N*-Methylephedrine (NME) (430 mg, 2.40 mmol), toluene (2.2 mL), and Et_3N (0.33 mL, 2.40 mmol) were added to the flask with stirring at rt. After 3.5 h, a solution of 1-hexyne (0.23 mL, 2.00 mmol) was added to the mixture with stirring at rt. After 15 min, a solution of **10a** (194 mg, 1.00 mmol) in toluene (0.5 mL) was added to the mixture with stirring at rt. The reaction mixture was stirred for 20 h. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc and the extract was washed with saturated NH_4Cl, water, and brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (4:1) to give **11a** (232 mg, 84%, >97:3 dr) as a pale yellow oil. $[\alpha]_D^{22}$ +49.5 (*c* 0.66, $CHCl_3$). ^1H NMR δ: 0.90 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 1.26 (d, *J*=6.4 Hz, 3H, PMBOCHCH_3), 1.37–1.53 (m, 4H, $CH_2\times2$), 2.22 (td, *J*=6.7, 1.8 Hz, 2H, C≡CCH_2), 2.66 (d, *J*=3.1 Hz, 1H, OH), 3.56 (quint, *J*=6.4 Hz, 1H, PMBOC*H*), 3.81 (s, 3H, OCH_3), 4.18–4.21 (m, 1H, C*H*OH), 4.48 (d, *J*=11.3 Hz, 1H, CH_2Ar), 4.62 (d, *J*=11.3 Hz, 1H, CH_2Ar), 6.88 (d, *J*=8.5 Hz, 2H, ArH), 7.27 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 13.5, 15.9, 18.4, 21.9, 30.5, 55.2, 66.6, 71.2, 78.1, 78.2, 86.6, 113.8 (2C), 129.4 (2C), 130.1, 159.3. IR: 3431 (OH), 2958 (CH), 2933 (CH), 2873 (CH). MS (FAB) *m*/*z* 299 (MNa^+). HRMS (FAB) calcd for $C_{17}H_{24}NaO_3$ (MNa^+): 299.1623; found: 299.1646.

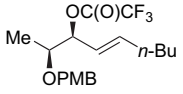
3.1.15. (3*S*,4*S*)-3-(4-Methoxybenzyloxy)-2-methyl-5-decyn-4-ol (11b).



n-BuLi (1.15 M in hexane, 33.6 mL, 38.6 mmol) was added to a solution of 1-hexyne (4.44 mL, 38.6 mmol) in Et_2O

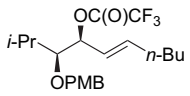
(77 mL) at 0 °C. After 30 min, $ZnBr_2$ (9.62 g, 42.7 mmol, dried at 120 °C for 4 h under 5 mmHg) was added to the mixture and the whole was stirred at 0 °C for 20 min. A solution of aldehyde **10b** (4.30 g, 19.3 mmol) in Et_2O (23 mL) was added to the mixture and the stirring was continued at −78 °C for 30 min. Then, the mixture was warmed to 0 °C over 1 h and stirred for 3 h. The reaction was quenched with saturated aqueous NH_4Cl solution and the mixture was extracted with Et_2O. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (10:1) to give **11b** (5.81 g, 99%, >97:3 dr) as a colorless oil. $[\alpha]_D^{27}$ +35.4 (*c* 1.17, $CHCl_3$). ^1H NMR δ: 0.90 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.97 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 0.99 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 1.41 (qt, *J*=6.7, 6.7 Hz, 2H, CH_2CH_3), 1.51 (tt, *J*=6.7, 6.7 Hz, 2H, C=CCH_2CH_2), 2.02 (m, 1H, $CH(CH_3)_2$), 2.23 (td, *J*=6.7, 1.8 Hz, 2H, C=CCH_2), 2.49 (br, 1H, OH), 3.26 (dd, *J*=5.5, 5.5 Hz, 1H, CHOPMB), 3.81 (s, 3H, $ArOCH_3$), 4.37 (m, 1H, CHOH), 4.64 (d, *J*=11.0 Hz, 1H, CH_2Ar), 4.79 (d, *J*=11.0 Hz, 1H, CH_2Ar), 6.89 (d, *J*=8.5 Hz, 2H, ArH), 7.31 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 13.6, 17.8, 18.5, 19.8, 22.0, 30.1, 30.6, 55.3, 63.1, 75.0, 79.8, 86.1, 87.4, 113.8 (2C), 129.6 (2C), 130.6, 159.3. IR: 3428 (OH), 2956 (CH), 2871 (CH). MS (FAB) *m/z* 327 (MNa^+). HRMS (FAB) calcd for $C_{19}H_{28}NaO_3$ (MNa^+): 327.1936; found: 327.1921.

3.1.16. (*E*,2*S*,3*S*)-2-(4-Methoxybenzyloxy)-4-nonen-3-yl trifluoroacetate [(*E*)-12a].



$LiAlH_4$ (15.2 mg, 0.400 mmol) was added to a stirred solution of **11a** (55.3 mg, 0.200 mmol) in THF (2 mL) at 0 °C. The mixture was heated at reflux for 2.5 h. Saturated Rochelle salt was gradually added to the stirred mixture. The mixture was extracted with Et_2O, and the extract was washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel hexane–EtOAc (4:1) to give the allylic alcohol (52.3 mg, 94%) as a colorless oil. $[\alpha]_D^{24}$ +50.8 (*c* 0.82, $CHCl_3$). ^1H NMR δ: 0.89 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 1.15 (d, *J*=6.7 Hz, 3H, PMBOCHCH_3), 1.25–1.40 (m, 4H, $CH_2\times2$), 2.05 (q, *J*=6.7 Hz, 2H, CH=$CHCH_2$), 2.79 (s, 1H, OH), 3.37 (quint, *J*=6.7 Hz, 1H, PMBOCH), 3.81 (s, 3H, OCH_3), 3.86 (t, *J*=6.7 Hz, 1H, CHOH), 4.39 (d, *J*=11.0 Hz, 1H, CH_2Ar), 4.61 (d, *J*=11.0 Hz, 1H, CH_2Ar), 5.39 (dd, *J*=15.3, 6.7 Hz, 1H, CH=$CHCH_2$), 5.76 (dt, *J*=15.3, 6.7 Hz, 1H, CH=$CHCH_2$), 6.89 (d, *J*=8.5 Hz, 2H, ArH), 7.27 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 14.0, 15.6, 22.3, 31.2, 32.1, 55.3, 70.8, 76.6, 78.3, 113.8 (2C), 128.2, 129.3 (2C), 130.2, 134.9, 159.1. IR: 3462 (OH), 2958 (CH), 2929 (CH), 2875 (CH). MS (FAB) *m/z* 301 (MNa^+). HRMS (FAB) calcd for $C_{17}H_{26}NaO_3$ (MNa^+): 301.1780; found: 301.1778. Trifluoroacetic anhydride (0.14 mL, 1.00 mmol) was added to a solution of the allylic alcohol (139 mg, 0.500 mmol) in pyridine (1 mL) with stirring at rt. After 5 min, the reaction was quenched with saturated NH_4Cl at 0 °C and the mixture was extracted with Et_2O. The extract was washed with water and brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel hexane–Et_2O (10:1) to give (*E*)-**12a** (159 mg, 85%) as a colorless oil. $[\alpha]_D^{24}$ +23.8 (*c* 0.82, $CHCl_3$). ^1H NMR δ: 0.89 (t, *J*=7.3 Hz, 3H, CH_2CH_3), 1.14 (d, *J*=6.7 Hz, 3H, PMBOCHCH_3), 1.26–1.39 (m, 4H, $CH_2\times2$), 2.06 (qd, *J*=7.3, 1.5 Hz, 2H, CH=$CHCH_2$), 3.65 (quint, *J*=6.7 Hz, 1H, PMBOCH), 3.80 (s, 3H, OCH_3), 4.47 (d, *J*=11.3 Hz, 1H, CH_2Ar), 4.55 (d, *J*=11.3 Hz, 1H, CH_2Ar), 5.33 (dd, *J*=8.2, 6.7 Hz, 1H, CHOC(O)CF_3), 5.41 (ddt, *J*=15.3, 8.2, 1.5 Hz, 1H, CH=$CHCH_2$), 5.89 (dt, *J*=15.3, 7.3 Hz, 1H, CH=$CHCH_2$), 6.87 (d, *J*=8.5 Hz, 2H, ArH), 7.23 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 13.9, 16.1, 22.2, 30.8, 32.0, 55.3, 71.3, 74.7, 82.4, 113.7 (2C), 114.5 (q, J_{C-F}=285.6 Hz), 122.6, 129.2 (2C), 130.0, 139.4, 156.5 (q, J_{C-F}=41.9 Hz), 159.1. IR: 2960 (CH), 2931 (CH), 2862 (CH), 1782 (C=O). MS (FAB) *m/z* 397 (MNa^+). HRMS (FAB) calcd for $C_{19}H_{25}F_3NaO_4$ (MNa^+): 397.1603; found: 397.1595.

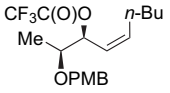
3.1.17. (*E*,3*S*,4*S*)-3-(4-Methoxybenzyloxy)-2-methyl-5-decen-4-yl trifluoroacetate [(*E*)-12b].



Compound **11b** (153 mg, 0.50 mmol) was converted into allylic alcohol (142 mg, 92%) in a similar procedure (reaction time 1 h) as described for (*E*)-**12a**. Colorless oil. $[\alpha]_D^{26}$ +24.7 (*c* 1.15, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.90 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.95 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 1.01 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 1.28–1.41 (m, 4H, $CH_2\times2$), 1.92 (septd, *J*=6.7, 5.5 Hz, 1H, $CH(CH_3)_2$), 2.05 (dt, *J*=6.7, 6.7 Hz, 2H, C=$CHCH_2$), 2.35 (br, 1H, OH), 3.09 (dd, *J*=5.5, 5.5 Hz, 1H, CHOPMB), 3.81 (s, 3H, $ArOCH_3$), 4.07 (dd, *J*=6.7, 6.7 Hz, 1H, CHOH), 4.54 (d, *J*=11.0 Hz, 1H, CH_2Ar), 4.61 (d, *J*=11.0 Hz, 1H, CH_2Ar), 5.46 (dd, *J*=15.6, 6.7 Hz, 1H, CH(OH)CH=C), 5.74 (dt, *J*=15.6, 6.7 Hz, 1H, C=$CHCH_2$), 6.88 (d, *J*=8.5 Hz, 2H, ArH), 7.28 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 17.4, 20.1, 22.2, 29.8, 31.1, 32.0, 55.1, 73.0, 74.8, 87.5, 113.7 (2C), 129.3 (2C), 130.1, 130.6, 133.2, 159.2. IR: 3464 (OH), 2958 (CH), 2871 (CH). MS (FAB) *m/z* 329 (MNa^+). HRMS (FAB) calcd for $C_{19}H_{30}NaO_3$ (MNa^+): 329.2093; found: 329.2078. Trifluoroacetic anhydrous (0.47 mL, 3.4 mmol) was added to a solution of the allylic alcohol (515 mg, 1.7 mmol) and pyridine (0.30 mL, 3.7 mmol) in ether (3.3 mL) with stirring at 0 °C. The stirring was continued at rt for 30 min. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with ether. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (3:1) to give (*E*)-**12b** (670 mg, 99%) as a colorless oil. $[\alpha]_D^{26}$ +10.4 (*c* 1.00, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.81 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.82 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 0.90 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 1.16–1.33 (m, 4H, $CH_2\times2$), 1.75 (qnd, *J*=6.7, 3.6 Hz, 1H, $CH(CH_3)_2$), 1.98 (dt, *J*=6.7, 6.7 Hz, 2H, C=$CHCH_2$), 3.30 (dd, *J*=6.7, 3.6 Hz, 1H, CHOPMB), 3.70 (s, 3H, $ArOCH_3$), 4.40 (d, *J*=11.0 Hz, 1H, CH_2Ar), 4.56 (d, *J*=11.0 Hz, 1H, CH_2Ar), 5.33 (dd, *J*=14.6, 8.5 Hz, 1H, CH(OC(O)CF_3)CH=C),

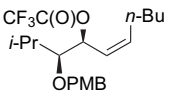
5.38 (dd, J=8.5, 8.5 Hz, 1H, CHOC(O)CF_3), 5.86 (dt, J=14.6, 6.7 Hz, 1H, C=C*H*CH_2), 6.78 (d, J=8.5 Hz, 2H, ArH), 7.15 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 15.9, 20.0, 22.1, 29.5, 30.7, 32.0, 55.2, 74.9, 82.2, 83.9, 113.7 (2C), 114.6 (q, J_{C-F}=286.5 Hz), 123.1, 129.4 (2C), 130.4, 139.5, 156.6 (q, J_{C-F}=41.7 Hz), 159.2. IR: 2960 (CH), 2877 (CH), 1782 (C=O). MS (FAB) m/z 425 (MNa^+). HRMS (FAB) calcd for $C_{21}H_{29}F_3NaO_4$ (MNa^+): 425.1916; found: 425.1941.

3.1.18. (*Z*,2*S*,3*S*)-2-(4-Methoxybenzyloxy)-4-nonen-3-yl trifluoroacetate [(*Z*)-12a].



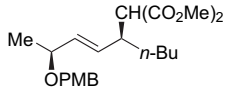
A solution of **11a** (55.3 mg, 0.200 mmol) in MeOH (2 mL) was hydrogenated on Lindlar catalyst (2.8 mg, 5% w/w) with stirring at rt for 21 h. Lindlar catalyst (2.8 mg, 5% w/w) was added to the mixture and the stirring was continued for 9 h, and then filtered off through Celite. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel with hexane–EtOAc (4:1) to give the allylic alcohol (54.5 mg, 98%) as a colorless oil. $[\alpha]_D^{24}$ +45.1 (c 1.33, $CHCl_3$). ^{1}H NMR δ: 0.89 (t, J=7.3 Hz, 3H, CH_2CH_3), 1.14 (d, J=6.1 Hz, 3H, PMBOCHCH_3), 1.28–1.40 (m, 4H, CH_2×2), 2.05–2.20 (m, 2H, CH=CHCH_2), 2.77 (s, 1H, OH), 3.39 (dq, J=8.5, 6.1 Hz, 1H, PMBOC*H*), 3.81 (s, 3H, OCH_3), 4.25 (t, J=8.5 Hz, 1H, C*H*OH), 4.40 (d, J=11.0 Hz, 1H, CH_2Ar), 4.62 (d, J=11.0 Hz, 1H, CH_2Ar), 5.32 (dd, J=11.0, 8.5 Hz, 1H, C*H*=CHCH_2), 5.63 (dt, J=11.0, 7.3 Hz, 1H, CH=C*H*CH_2), 6.89 (d, J=8.5 Hz, 2H, ArH), 7.27 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 14.0, 15.5, 22.4, 27.8, 31.7, 55.2, 70.8, 71.2, 78.5, 113.7 (2C), 127.8, 129.3 (2C), 130.2, 135.0, 159.1. IR: 3458 (OH), 2958 (CH), 2931 (CH), 2871 (CH). MS (FAB) m/z 301 (MNa^+). HRMS (FAB) calcd for $C_{17}H_{26}NaO_3$ (MNa^+): 301.1780; found: 301.1786. Compound (*Z*)-**12a** was obtained as a colorless oil (160 mg, 85%) from **11a** (139 mg, 0.500 mmol) as described for (*E*)-**12a**. $[\alpha]_D^{25}$ −18.4 (c 1.07, $CHCl_3$). ^{1}H NMR δ: 0.89 (t, J=7.0 Hz, 3H, CH_2CH_3), 1.15 (d, J=6.4 Hz, 3H, PMBOCHCH_3), 1.26–1.39 (m, 4H, CH_2×2), 2.12–2.26 (m, 2H, CH=CHCH_2), 3.67 (dq, J=7.6, 6.4 Hz, 1H, PMBOC*H*), 3.80 (s, 3H, OCH_3), 4.47 (d, J=11.6 Hz, 1H, CH_2Ar), 4.55 (d, J=11.6 Hz, 1H, CH_2Ar), 5.33 (ddt, J=11.0, 9.8, 1.8 Hz, 1H, C*H*=CHCH_2), 5.69 (dd, J=9.8, 7.6 Hz, 1H, C*H*OC(O)CF_3), 5.77 (dt, J=11.0, 7.9 Hz, 1H, CH=C*H*CH_2), 6.87 (d, J=8.5 Hz, 2H, ArH), 7.23 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 14.0, 15.9, 22.4, 27.9, 31.4, 55.3, 71.4, 75.0, 77.5, 113.7 (2C), 114.6 (q, J_{C-F}=285.6 Hz), 122.0, 129.2 (2C), 129.9, 138.9, 156.5 (q, J_{C-F}=41.9 Hz), 159.1. IR: 2958 (CH), 2935 (CH), 2870 (CH), 1782 (C=O). MS (FAB) m/z 397 (MNa^+). HRMS (FAB) calcd for $C_{19}H_{25}F_3NaO_4$ (MNa^+): 397.1603; found: 397.1603.

3.1.19. (*Z*,3*S*,4*S*)-3-(4-Methoxybenzyloxy)-2-methyl-5-decen-4-yl trifluoroacetate [(*Z*)-12b].



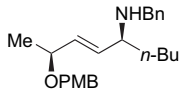
Compound **11b** (208 mg, 0.68 mmol) was converted into allylic alcohol (208 mg, quant.) in a similar procedure (reaction time 22 h) as described for (*Z*)-**12a**. $[\alpha]_D^{25}$ +25.1 (c 2.19, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90 (t, J=6.7 Hz, 3H, CH_2CH_3), 0.96 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 1.03 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 1.25–1.45 (m, 4H, CH_2×2), 1.90 (septd, J=6.7, 4.9 Hz, 1H, C*H*(CH_3)$_2$), 2.01 (m, 2H, C=CHCH_2), 2.45 (br, 1H, OH), 3.11 (dd, J=6.7, 4.9 Hz, 1H, C*H*OPMB), 3.81 (s, 3H, ArOCH_3), 4.42 (dd, J=8.5, 6.7 Hz, 1H, C*H*OH), 4.56 (d, J=11.0 Hz, 1H, CH_2Ar), 4.64 (d, J=11.0 Hz, 1H, CH_2Ar), 5.42 (dd, J=11.0, 8.5 Hz, 1H, CH(OH)C*H*=C), 5.55 (dt, J=11.0, 6.7 Hz, 1H, C=C*H*CH_2), 6.89 (d, J=8.5 Hz, 2H, ArH), 7.29 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 17.4, 20.3, 22.3, 27.6, 29.8, 31.6, 55.2, 68.0, 75.0, 88.0, 113.8 (2C), 129.4 (2C), 129.7, 130.6, 133.6, 159.2. IR: 3473 (OH), 2958 (CH), 2871 (CH). MS (FAB) m/z 329 (MNa^+). HRMS (FAB) calcd for $C_{19}H_{30}NaO_3$ (MNa^+): 329.2093; found: 329.2123. The allylic alcohol (61 mg, 0.20 mmol) was converted into (*Z*)-**12b** (73 mg, 90%) in a similar procedure as described for (*E*)-**12b**. $[\alpha]_D^{26}$ −22.9 (c 0.32, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90 (t, J=6.7 Hz, 3H, CH_2CH_3), 0.90 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 0.98 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 1.20–1.75 (m, 4H, CH_2×2), 1.83 (qnd, J=6.7, 3.1 Hz, 1H, C*H*(CH_3)$_2$), 2.10–2.35 (m, 2H, C=CHCH_2), 3.41 (dd, J=8.5, 3.1 Hz, 1H, C*H*OPMB), 3.80 (s, 3H, ArOCH_3), 4.49 (d, J=11.0 Hz, 1H, CH_2Ar), 4.65 (d, J=11.0 Hz, 1H, CH_2Ar), 5.35 (dd, J=11.0, 11.0 Hz, 1H, CH(OC(O)CF_3)C*H*=C), 5.74 (dt, J=11.0, 6.7 Hz, 1H, C=C*H*CH_2), 5.80 (dd, J=11.0, 8.5 Hz, 1H, CHOC(O)CF_3), 6.86 (d, J=8.5 Hz, 2H, ArH), 7.23 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 16.1, 20.3, 22.4, 28.0, 29.6, 31.4, 55.3, 75.1, 84.1 (2C), 113.7 (2C), 114.6 (q, J_{C-F}=285.8 Hz), 122.4, 129.4 (2C), 130.4, 138.1, 156.6 (q, J_{C-F}=42.2 Hz), 159.2. IR: 2962 (CH), 2875 (CH), 1784 (C=O). MS (FAB) m/z 425 (MNa^+). HRMS (FAB) calcd for $C_{21}H_{29}F_3NaO_4$ (MNa^+): 425.1916; found: 425.1937.

3.1.20. Dimethyl 2-[(*E*,2*S*,5*S*)-2-(4-methoxybenzyloxy)-3-nonen-5-yl]malonate (*syn*-13a).



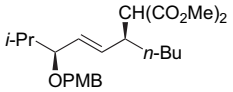
The procedure was described in the communication.[4] $[\alpha]_D^{23}$ −46.9 (c 0.76, $CHCl_3$). ^{1}H NMR δ: 0.87 (t, J=6.7 Hz, 3H, CH_2CH_3), 1.20–1.35 (m, 5H, CH_2×3), 1.23 (d, J=6.7 Hz, 3H, PMBOCHCH_3), 1.42–1.49 (m, 1H, C$H_2CH_2CH_2CH_3$), 2.81 (qd, J=8.9, 3.7 Hz, 1H, C*H*CH(CO_2CH_3)$_2$), 3.42 (d, J=8.9 Hz, 1H, C*H*(CO_2CH_3)$_2$), 3.69 (s, 3H, CO_2CH_3), 3.74 (s, 3H, CO_2CH_3), 3.80 (s, 3H, ArOCH_3), 3.82–3.87 (m, 1H, PMBOC*H*), 4.22 (d, J=11.3 Hz, 1H, CH_2Ar), 4.44 (d, J=11.3 Hz, 1H, CH_2Ar), 5.43–5.50 (m, 2H, C*H*=C*H*), 6.87 (d, J=8.5 Hz, 2H, ArH), 7.26 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 13.9, 21.9, 22.3, 29.1, 32.0, 42.7, 52.3, 52.4, 55.2, 57.0, 69.1, 74.7, 113.6 (2C), 129.3 (2C), 130.7, 131.9, 135.3, 158.9, 168.5, 168.7. IR: 2952 (CH), 2929 (CH), 2861 (CH), 1753 (C=O), 1738 (C=O). MS (FAB) m/z 415 (MNa^+). HRMS (FAB) calcd for $C_{22}H_{32}NaO_6$ (MNa^+): 415.2097; found: 415.2093.

3.1.21. (*E*,2*S*,5*S*)-5-Benzylamino-2-(4-methoxybenzyloxy)-3-nonene (*syn*-14a).



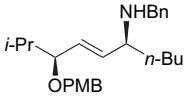
The procedure was described in the communication.[4] $[\alpha]_D^{23}$ −67.1 (*c* 0.57, $CHCl_3$). ^1H NMR δ: 0.88 (t, *J*=7.0 Hz, 3H, CH_2CH_3), 1.25–1.56 (m, 6H, $CH_2\times3$), 1.29 (d, *J*=6.7 Hz, 3H, PMBOCHCH_3), 1.76 (br, 1H, NH), 3.06 (td, *J*=7.9, 5.5 Hz, 1H, C*H*NHBn), 3.69 (d, *J*=13.4 Hz, 1H, NHCH_2Ph), 3.79 (s, 3H, OCH_3), 3.88 (d, *J*=13.4 Hz, 1H, NHCH_2Ph), 3.94 (quint, *J*=6.7 Hz, 1H, PMBOC*H*), 4.35 (d, *J*=11.6 Hz, 1H, CH_2Ar), 4.55 (d, *J*=11.6 Hz, 1H, CH_2Ar), 5.43 (dd, *J*=15.3, 7.9 Hz, 1H, PMBOCHCH=C*H*), 5.51 (dd, *J*=15.3, 6.7 Hz, 1H, PMBOCHC*H*=CH), 6.87 (d, *J*=8.5 Hz, 2H, ArH), 7.24–7.34 (m, 7H, ArH). ^{13}C NMR δ: 14.0, 21.9, 22.6, 28.1, 35.5, 51.4, 55.2, 59.9, 69.5, 75.2, 113.7 (2C), 126.8, 128.1 (2C), 128.4 (2C), 129.2 (2C), 130.7, 134.1, 135.3, 140.3, 158.9. IR: 2954 (CH), 2929 (CH), 2860 (CH). MS (FAB) *m*/*z* 368 (MH^+). HRMS (FAB) calcd for $C_{24}H_{34}NO_2$ (MH^+): 368.2590; found: 368.2584.

3.1.22. Dimethyl 2-[(*E*,3*S*,6*S*)-3-(4-methoxybenzyloxy)-2-methyl-4-dec-6-yl]malonate (*syn*-13b).



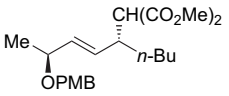
Compound *syn*-**13b** was obtained as a yellow oil (61.7 mg, 98%) from (*E*)-**12b** (60.4 mg, 0.150 mmol) as described for *anti*-**4**. $[\alpha]_D^{25}$ −36.9 (*c* 2.07, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.83 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 0.87 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.91 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 1.22–1.50 (m, 6H, $CH_2\times3$), 1.73 (septd, *J*=6.7, 6.7 Hz, 1H, C*H*(CH_3)$_2$), 2.86 (m, 1H, C*H*CH(CO_2CH_3)$_2$), 3.31 (dd, *J*=6.7, 6.7 Hz, 1H, C*H*OPMB), 3.43 (d, *J*=8.5 Hz, 1H, C*H*(CO_2CH_3)$_2$), 3.69 (s, 3H, CO_2CH_3), 3.74 (s, 3H, CO_2CH_3), 3.80 (s, 3H, ArOCH_3), 4.18 (d, *J*=11.6 Hz, 1H, CH_2Ar), 4.46 (d, *J*=11.6 Hz, 1H, CH_2Ar), 5.43 (m, 2H, CH=CH), 6.86 (d, *J*=8.5 Hz, 2H, ArH), 7.25 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.9, 18.4, 18.9, 22.2, 29.3, 32.1, 32.8, 42.9, 52.3, 52.4, 55.2, 57.2, 69.2, 84.5, 113.6 (2C), 129.2 (2C), 131.0, 132.6, 134.0, 158.9, 168.5, 168.8. IR: 2954 (CH), 2871 (CH), 1759 (C=O), 1738 (C=O). MS (FAB) *m*/*z* 443 (MNa^+). HRMS (FAB) calcd for $C_{24}H_{36}NaO_6$ (MNa^+): 443.2410; found: 443.2427.

3.1.23. (*E*,3*S*,6*S*)-6-Benzylamino-3-(4-methoxybenzyloxy)-2-methyl-4-decene (*syn*-14b).



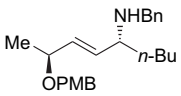
Compound *syn*-**14b** was obtained as a yellow oil (47.6 mg, 80%) from (*E*)-**12b** (60.4 mg, 0.15 mmol) as described for *anti*-**5**. $[\alpha]_D^{24}$ −39.9 (*c* 2.28, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.87 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 0.88 (m, 3H, CH_2CH_3), 0.96 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 1.25–1.60 (m, 6H, $CH_2\times3$), 1.78 (septd, *J*=6.7, 6.7 Hz, 1H, C*H*(CH_3)$_2$), 3.09 (dt, *J*=6.7, 6.7 Hz, 1H, C*H*NHBn), 3.42 (dd, *J*=6.7, 6.7 Hz, 1H, C*H*OPMB), 3.69 (d, *J*=13.4 Hz, 1H, NCH_2Ph), 3.79 (s, 3H, OCH_3), 3.90 (d, *J*=13.4 Hz, 1H, NCH_2Ph), 4.31 (d, *J*=11.6 Hz, 1H, OCH_2Ar), 4.58 (d, *J*=11.6 Hz, 1H, OCH_2Ar), 5.40 (dd, *J*=15.3, 6.7 Hz, 1H, C=C*H*CHNHBn), 5.45 (dd, *J*=15.3, 6.7 Hz, 1H, C=C*H*CHOPMB), 6.86 (d, *J*=8.5 Hz, 2H, ArH), 7.25 (d, *J*=8.5 Hz, 2H, ArH), 7.30–7.40 (m, 5H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 18.5, 19.0, 22.6, 28.2, 32.8, 35.7, 51.4, 55.2, 60.2, 69.7, 84.9, 113.7 (2C), 126.9, 128.1 (2C), 128.4 (2C), 129.2 (2C), 131.0, 131.4, 137.4, 140.5, 158.9. IR: 2956 (CH), 2869 (CH). MS (FAB) *m*/*z* 396 (MH^+). HRMS (FAB) calcd for $C_{26}H_{38}NO_2$ (MH^+): 396.2903; found: 396.2898.

3.1.24. Dimethyl 2-[(*E*,2*S*,5*R*)-2-(4-methoxybenzyloxy)-3-nonen-5-yl]malonate (*anti*-13a).



Compound *anti*-**13a** was obtained as a colorless oil (54.9 mg, 93%) from (*Z*)-**12a** (56.2 mg, 0.150 mmol) as described for *anti*-**4a**. $[\alpha]_D^{24}$ −31.2 (*c* 1.23, $CHCl_3$). ^1H NMR δ: 0.88 (t, *J*=6.1 Hz, 3H, CH_2CH_3), 1.21 (d, *J*=6.1 Hz, 3H, PMBOCHCH_3), 1.24–1.37 (m, 5H, $CH_2\times3$), 1.45–1.50 (m, 1H, C$H_2CH_2CH_2CH_3$), 2.80 (qd, *J*=9.2, 3.7 Hz, 1H, C*H*CH(CO_2CH_3)$_2$), 3.38 (d, *J*=9.2 Hz, 1H, C*H*(CO_2CH_3)$_2$), 3.68 (s, 3H, CO_2CH_3), 3.73 (s, 3H, CO_2CH_3), 3.80 (s, 3H, ArOCH_3), 3.83–3.87 (m, 1H, PMBOC*H*), 4.26 (d, *J*=11.6 Hz, 1H, CH_2Ar), 4.47 (d, *J*=11.6 Hz, 1H, CH_2Ar), 5.42–5.50 (m, 2H, CH=CH), 6.87 (d, *J*=8.5 Hz, 2H, ArH), 7.24 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 14.0, 21.9, 22.3, 29.4, 32.1, 42.8, 52.2, 52.4, 55.2, 57.0, 69.5, 75.1, 113.8 (2C), 129.2 (2C), 130.8, 131.8, 135.4, 159.0, 168.5, 168.7. IR: 2952 (CH), 2929 (CH), 2860 (CH), 1757 (C=O), 1739 (C=O). MS (FAB) *m*/*z* 415 (MNa^+). HRMS (FAB) calcd for $C_{22}H_{32}NaO_6$ (MNa^+): 415.2097; found: 415.2094.

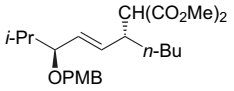
3.1.25. (*E*,2*S*,5*R*)-5-Benzylamino-2-(4-methoxybenzyloxy)-3-nonene (*anti*-14a).



Compound *anti*-**14a** was obtained as a yellow oil (46.7 mg, 85%) from (*Z*)-**12a** (56.2 mg, 0.150 mmol) as described for *anti*-**4**. $[\alpha]_D^{23}$ −25.6 (*c* 0.71, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, *J*=7.0 Hz, 3H, CH_2CH_3), 1.26–1.58 (m, 6H, $CH_2\times3$), 1.30 (d, *J*=6.7 Hz, 3H, PMBOCHCH_3), 1.65 (br, 1H, NH), 3.06 (td, *J*=8.2, 5.5 Hz, 1H, C*H*NHBn), 3.65 (d, *J*=12.8 Hz, 1H, NHCH_2Ph), 3.80 (s, 3H, OCH_3), 3.81 (d, *J*=12.8 Hz, 1H, NHCH_2Ph), 3.94 (quint, *J*=6.7 Hz, 1H, PMBOC*H*), 4.31 (d, *J*=11.3 Hz, 1H, CH_2Ar), 4.50 (d, *J*=11.3 Hz, 1H, CH_2Ar), 5.43 (dd, *J*=15.3, 8.2 Hz, 1H, PMBOCHCH=C*H*), 5.50 (dd, *J*=15.3, 6.7 Hz, 1H,

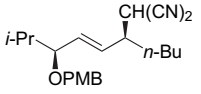
PMBOCHC*H*═CH), 6.87 (d, *J*=9.2 Hz, 2H, ArH), 7.23–7.33 (m, 7H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.2, 22.1, 22.7, 28.4, 35.7, 51.3, 55.3, 59.9, 69.5, 75.1, 113.7 (2C), 126.8, 128.1 (2C), 128.3 (2C), 129.1 (2C), 130.7, 134.1, 135.1, 140.4, 158.9. IR: 2958 (CH), 2929 (CH), 2858 (CH). MS (FAB) *m*/*z* 368 (MH^+). HRMS (FAB) calcd for $C_{24}H_{34}NO_2$ (MH^+): 368.2590; found: 368.2575.

3.1.26. Dimethyl 2-[(*E*,3*S*,6*R*)-3-(4-methoxybenzyloxy)-2-methyl-4-decen-6-yl]malonate (*anti*-13b).



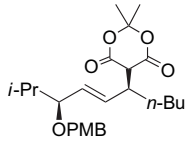
Compound *anti*-**13b** was obtained as a yellow oil (61.0 mg, 97%) from (*Z*)-**12b** (60.4 mg, 0.150 mmol) as described for *anti*-**4**. $[\alpha]_D^{25}$ −29.7 (*c* 2.38, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.82 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 0.88 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.91 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 1.20–1.50 (m, 6H, CH_2×3), 1.73 (septd, *J*=6.7, 6.7 Hz, 1H, C*H*(CH$_3$)$_2$), 2.85 (m, 1H, C*H*CH(CO$_2$CH$_3$)$_2$), 3.34 (dd, *J*=6.7, 6.7 Hz, 1H, C*H*OPMB), 3.40 (d, *J*=8.5 Hz, 1H, C*H*(CO$_2$CH$_3$)$_2$), 3.68 (s, 3H, CO_2CH_3), 3.73 (s, 3H, CO_2CH_3), 3.80 (s, 3H, ArOCH_3), 4.22 (d, *J*=11.6 Hz, 1H, CH_2Ar), 4.49 (d, *J*=11.6 Hz, 1H, CH_2Ar), 5.38–5.48 (m, 2H, CH═CH), 6.86 (d, *J*=8.5 Hz, 2H, ArH), 7.23 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 18.3, 18.7, 22.3, 29.4, 32.2, 32.8, 42.9, 52.3, 52.4, 55.2, 57.0, 69.6, 84.6, 113.6 (2C), 129.1 (2C), 131.0, 132.6, 133.5, 158.9, 168.4, 168.7. IR: 2954 (CH), 2871 (CH), 1759 (C═O), 1739 (C═O). MS (FAB) *m*/*z* 443 (MNa^+). HRMS (FAB) calcd for $C_{24}H_{36}NaO_6$ (MNa^+): 443.2410; found: 443.2441.

3.1.27. 2-[(*E*,3*S*,6*S*)-3-(4-Methoxybenzyloxy)-2-methyl-4-decen-6-yl]malononitrile (15).



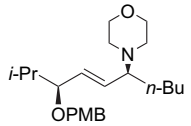
$Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol) was added to a solution of (*E*)-**12b** (60.4 mg, 0.150 mmol) in THF (2 mL) with stirring at rt. After 15 min, a solution of sodiomalononitrile generated from NaH (60% in oil, 12 mg, 0.30 mmol) and $CH_2(CN)_2$ (0.019 mL, 0.30 mmol) in THF (2 mL) was added to the mixture. After 30 min, the reaction was quenched with water. Almost of the solvent was evaporated and the residue was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica with gel with hexane–EtOAc (5:1) to give **15** (50.7 mg, 95%) as a yellow oil. $[\alpha]_D^{25}$ −8.7 (*c* 2.04, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 0.91 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.96 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 1.25–1.70 (m, 6H, CH_2×3), 1.81 (septd, *J*=6.7, 6.7 Hz, 1H, C*H*(CH$_3$)$_2$), 2.71 (ddt, *J*=9.8, 9.8, 4.9 Hz, 1H, C*H*CH(CN)$_2$), 3.45 (dd, *J*=6.7, 6.7 Hz, 1H, C*H*OPMB), 3.68 (d, *J*=4.9 Hz, 1H, CH(CN)$_2$), 3.80 (s, 3H, ArOCH_3), 4.32 (d, *J*=11.6 Hz, 1H, CH_2Ar), 4.56 (d, *J*=11.6 Hz, 1H, CH_2Ar), 5.45 (dd, *J*=15.3, 8.5 Hz, 1H, C═C*H*CHCH(CN)$_2$), 5.70 (dd, *J*=15.3, 8.5 Hz, 1H, C═C*H*CHOPMB), 6.87 (d, *J*=8.5 Hz, 2H, ArH), 7.26 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8, 18.6, 18.7, 22.1, 28.9 (2C), 31.7, 32.7, 44.2, 55.2, 70.1, 84.0, 111.6, 112.1, 113.7 (2C), 129.2, 129.3 (2C), 130.6, 136.9, 159.0. IR: 2958 (CH), 2873 (CH), 2254 (CN). MS (FAB) *m*/*z* 377 (MNa^+). HRMS (FAB) calcd for $C_{22}H_{30}N_2NaO_2$ (MNa^+): 377.2205; found: 377.2206.

3.1.28. 5-[(*E*,3*S*,6*S*)-3-(4-Methoxybenzyloxy)-2-methyl-4-decen-6-yl]-2,2-dimethyl-1,3-dioxane-4,6-dione (16).



$Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol) was added to a solution of (*E*)-**12b** (60.4 mg, 0.150 mmol) and in THF (2 mL) at rt. After stirring for 15 min, a solution of sodium salt of Meldrum's acid generated from NaH (60% in oil, 12 mg, 0.30 mmol) and meldrum's acid (43 μL, 0.30 mmol) in THF (2 mL) was added. After 30 min, the reaction was quenched with water. Almost of the solvent was evaporated and the residue was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (4:1) to give **16** (15.7 mg, 24%) as a yellow oil. $[\alpha]_D^{25}$ −18.2 (*c* 0.51, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.83 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 0.89 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 0.91 (d, *J*=6.7 Hz, 3H, CH(CH_3)$_2$), 1.20–1.60 (m, 6H, CH_2×3), 1.71 (s, 3H, O$_2$C(CH$_3$)$_2$), 1.75 (s, 3H, O$_2$C(CH$_3$)$_2$), 1.81 (m, 1H, C*H*(CH$_3$)$_2$), 3.19 (m, 1H, C*H*n-Bu), 3.34 (dd, *J*=6.7, 6.7 Hz, 1H, C*H*OPMB), 3.50 (d, *J*=3.0 Hz, 1H, C*H*(CO$_2$R)$_2$), 3.79 (s, 3H, ArOCH_3), 4.19 (d, *J*=11.6 Hz, 1H, CH_2Ar), 4.44 (d, *J*=11.6 Hz, 1H, CH_2Ar), 5.50 (dd, *J*=15.6, 8.5 Hz, 1H, C═C*H*CHn-Bu), 5.67 (dd, *J*=15.6, 8.5 Hz, 1H, C═C*H*CHOPMB), 6.85 (d, *J*=8.5 Hz, 2H, ArH), 7.22 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 18.3, 18.9, 22.3, 27.6, 28.2, 30.0, 31.7, 32.6, 42.7, 51.1, 55.2, 69.6, 84.6, 104.8, 113.7 (2C), 129.3 (2C), 130.9, 133.3, 133.4, 158.9, 164.7, 165.1. IR: 2962 (CH), 2873 (CH), 1784 (C═O), 1749 (C═O). MS (FAB) *m*/*z* 455 (MNa^+). HRMS (FAB) calcd for $C_{25}H_{36}NaO_6$ (MNa^+): 455.2410; found: 455.2427.

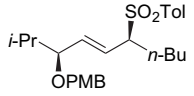
3.1.29. (*E*,3*S*,6*S*)-3-(4-Methoxybenzyloxy)-2-methyl-6-morpholino-4-decene (17).



$Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol) was added to a solution of (*E*)-**12b** (60.4 mg, 0.15 mmol) and in THF (4 mL) with stirring at rt. After 15 min, morpholine (26 μL, 0.30 mmol) was added to the mixture. After 45 min, the reaction was quenched with water. Almost of solvent was evaporated and the residue was extracted with EtOAc. The combined

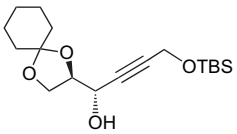
organic layers were washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (1:1) to give **17** (50.6 mg, 90%) as a yellow oil. $[\alpha]_D^{24}$ −19.4 (*c* 2.55, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.84–0.98 (m, 9H, $CH(CH_3)_2$ and CH_2CH_3), 1.20–1.65 (m, 6H, $CH_2\times3$), 1.78 (m, 1H, $CH(CH_3)_2$), 2.50 (m, 2H, NCH_2), 2.63 (m, 2H, NCH_2), 2.79 (m, 1H, NCH), 3.41 (m, 1H, CHOPMB), 3.73 (br, 4H, $O(CH_2)_2$), 3.81 (s, 3H, $ArOCH_3$), 4.29 (dd, *J*=11.6, 3.0 Hz, 1H, CH_2Ar), 4.54 (dd, *J*=11.6, 3.0 Hz, 1H, CH_2Ar), 5.45 (m, 2H, CH=CH), 6.87 (dd, *J*=8.5, 3.0 Hz, 2H, ArH), 7.25 (dd, *J*=8.5, 3.0 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0, 18.5, 18.9, 22.6, 28.5, 31.6, 32.8, 50.3 (2C), 55.2, 67.3, 67.7 (2C), 69.8, 84.9, 113.6 (2C), 129.1 (2C), 130.9, 133.0, 133.2, 158.9. IR: 2956 (CH), 2856 (CH). MS (FAB) *m/z* 376 (MH^+). HRMS (FAB) calcd for $C_{23}H_{38}NO_3$ (MH^+): 376.2852; found: 376.2852.

3.1.30. (*E*,3*S*,6*S*)-3-(4-Methoxybenzyloxy)-2-methyl-6-(*p*-tolylsulfonyl)-4-decene (18).



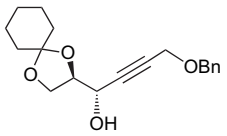
$Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol) was added to a solution of (*E*)-**12b** (60.4 mg, 0.15 mmol) in THF (2 mL) at rt. After 10 min, a solution of sodium sulfinate (0.054 mL, 0.30 mmol) in THF–MeOH (1:1, 2 mL) was added. After 10 min, the reaction was quenched with water. Almost of the solvent was evaporated and the mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was chromatographed on silica gel with hexane–EtOAc (3:1) to give **18** (62.6 mg, 94%) as a yellow oil. $[\alpha]_D^{24}$ −18.8 (*c* 2.87, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.76 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 0.87 (d, *J*=6.7 Hz, 3H, $CH(CH_3)_2$), 0.88 (t, *J*=6.7 Hz, 3H, CH_2CH_3), 1.15–1.50 (m, 4H, $CH_2\times2$), 1.57–1.77 (m, 2H, $CH_2CH_2CH_2CH_3$), 2.14 (m, 1H, $CH(CH_3)_2$), 2.33 (s, 3H, $ArCH_3$), 3.34 (ddd, *J*=5.8, 5.8, 1.8 Hz, 1H, CHOPMB), 3.60 (m, 1H, $CHSO_2Ar$), 3.81 (s, 3H, $ArOCH_3$), 4.00 (d, *J*=11.0 Hz, 1H, CH_2Ar), 4.12 (d, *J*=11.0 Hz, 1H, CH_2Ar), 5.38–5.46 (m, 2H, CH=CH), 6.86 (d, *J*=8.5 Hz, 2H, ArH), 7.15 (d, *J*=8.5 Hz, 2H, ArH), 7.27 (d, *J*=8.5 Hz, 2H, ArH), 7.74 (d, *J*=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.3, 18.9, 19.3, 22.1, 22.6, 27.1, 29.3, 33.3, 55.8, 69.4, 70.4, 84.7, 114.2 (2C), 126.3, 129.3 (2C), 129.8 (2C), 130.2 (2C), 131.1, 135.5, 139.6, 145.1, 159.6. IR: 2956 (CH), 2871 (CH), 1143 (SO_2), 1298 (SO_2). MS (FAB) *m/z* 467 (MNa^+). HRMS (FAB) calcd for $C_{26}H_{36}NaO_4S$ (MNa^+): 467.2232; found: 467.2235.

3.1.31. (2*R*,3*S*)-6-(*tert*-Butyldimethylsilyloxy)-1,2-cyclohexylidenedioxy-4-hexyn-3-ol (20a).



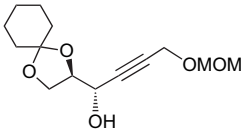
Compound **20a** was obtained as a colorless oil (306 mg, 70%, >97:3 dr) from **19** (560 mg, 3.28 mmol) as described for **11a**. $[\alpha]_D^{26}$ +18.3 (*c* 1.00, $CHCl_3$). ^1H NMR δ: 0.11 (s, 6H, $SiCH_3$), 0.90 (s, 9H, $C(CH_3)_3$), 1.30–1.70 (m, 10H, $CH_2\times5$), 2.23 (d, *J*=4.8 Hz, 1H, OH), 4.05 (dd, *J*=17.3, 8.5 Hz, 1H, H-1), 4.06 (dd, *J*=17.3, 8.5 Hz, 1H, H-1), 4.23 (m, 1H, H-2 or H-3), 4.34 (d, *J*=1.2 Hz, 2H, H-6), 4.52–4.55 (m, 1H, H-2 or H-3). ^{13}C NMR δ: −5.3 (2C), 18.1, 23.6, 23.8, 25.0, 25.6 (3C), 34.5, 35.8, 51.5, 62.4, 64.8, 77.5, 82.1, 84.5, 110.5. IR: 3438 (OH), 2929 (CH), 2860 (CH). MS (FAB) *m/z* 341 (MH^+). HRMS (FAB) calcd for $C_{18}H_{33}O_4Si$ (MH^+): 341.2148; found: 341.2150.

3.1.32. (2*R*,3*S*)-6-Benzyloxy-1,2-cyclohexylidenedioxy-4-hexyn-3-ol (20b).



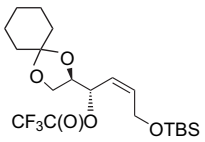
Compound **20b** was obtained as a colorless oil (643 mg, 68%, >97:3 dr) from **19** (538 mg, 3.16 mmol) as described for **11a**. $[\alpha]_D^{26}$ +18.6 (*c* 1.00, $CHCl_3$). ^1H NMR δ: 1.30–1.70 (m, 10H, $CH_2\times5$), 2.25 (d, *J*=4.3 Hz, 1H, OH), 4.05 (dd, *J*=8.5, 6.4 Hz, 1H, H-1), 4.09 (dd, *J*=8.5, 6.4 Hz, 1H, H-1), 4.22 (d, *J*=1.2 Hz, 2H, H-6), 4.23–4.28 (m, 1H, H-2 or H-3), 4.53–4.57 (m, 1H, H-2 or H-3), 4.59 (s, 2H, CH_2Ph), 7.28–7.38 (m, 5H, ArH). ^{13}C NMR δ: 23.6, 23.9, 25.0, 34.5, 35.9, 57.2, 62.6, 64.9, 71.5, 77.4, 82.0, 83.9, 110.6, 127.8, 128.0 (2C), 128.3 (2C), 137.2. IR: 3415 (OH), 2937 (CH), 2857 (CH), 1626 (Ph). MS (FAB) *m/z* 317 (MH^+). HRMS (FAB) calcd for $C_{19}H_{25}O_4Si$ (MH^+): 317.1753; found: 317.1747.

3.1.33. (2*R*,3*S*)-1,2-Cyclohexylidenedioxy-6-methoxymethoxy-4-hexyn-3-ol (20c).



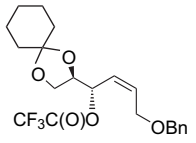
Compound **20c** was obtained as a yellow oil (608 mg, 75%, >97:3 dr) from **19** (556 mg, 3.26 mmol) as described for **11a**. $[\alpha]_D^{25}$ +16.9 (*c* 1.00, $CHCl_3$). ^1H NMR δ: 1.30–1.75 (m, 10H, $CH_2\times5$), 2.41 (br, 1H, OH), 3.38 (s, 3H, OCH_3), 4.05 (m, 2H, H-1), 4.24 (m, 1H, H-2), 4.26 (s, 2H, H-6), 4.53 (dd, *J*=4.0, 1.2 Hz, 1H, H-3), 4.70 (s, 2H, OCH_2O). ^{13}C NMR δ: 23.6, 23.8, 25.0, 34.5, 35.9, 54.2, 55.5, 62.5, 64.9, 77.4, 81.6, 83.6, 94.6, 110.6. IR: 3452 (OH), 2936 (CH), 2864 (CH). MS (FAB) *m/z* 271 (MH^+). HRMS (FAB) calcd for $C_{14}H_{23}O_5$ (MH^+): 271.1546; found: 271.1540.

3.1.34. (*Z*,2*R*,3*S*)-6-(*tert*-Butyldimethylsilyloxy)-1,2-cyclohexylidenedioxy-4-hexen-3-yl trifluoroacetate [(*Z*)-21a].



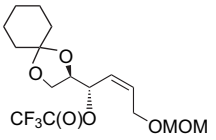
Compound **20a** (682 mg, 2.00 mmol) was converted into the (*Z*)-allylic alcohol (610 mg, 90%) by the procedure that described for compound (*Z*)-**12a**. Yellow oil. $[\alpha]_D^{25}$ +26.9 (*c* 1.00, $CHCl_3$). ^1H NMR δ: 0.08 (s, 6H, $Si(CH_3)_2$), 0.91 (s, 9H, $C(CH_3)_3$), 1.30–1.75 (m, 10H, $CH_2\times5$), 2.58 (br, 1H, OH), 3.92 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.00 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.07 (m, 1H, H-3), 4.13 (dd, *J*=12.8, 5.5 Hz, 1H, H-6), 4.26 (dd, *J*=12.8, 8.5 Hz, 1H, H-6), 4.55 (m, 1H, H-2), 5.48 (ddd, *J*=11.6, 8.5, 1.2 Hz, 1H, H-4), 5.74 (m, 1H, H-5). ^{13}C NMR δ: −5.44, −5.40, 18.1, 23.6, 23.8, 25.0, 25.7 (3C), 34.5, 36.0, 59.7, 64.9, 67.8, 77.7, 109.6, 128.9, 132.9. IR: 3457 (OH), 2937 (CH), 2894 (CH). MS (FAB) *m/z* 343 (MH^+). HRMS (FAB) calcd for $C_{18}H_{35}O_4Si$ (MH^+): 343.2305; found: 343.2310. Compound (*Z*)-**21a** was obtained as a yellow oil (210 mg, 89%) from the (*Z*)-allylic alcohol (186 mg, 054 mmol) as described for (*E*)-**12a**. $[\alpha]_D^{25}$ +4.5 (*c* 1.07, $CHCl_3$). ^1H NMR δ: 0.11 (s, 6H, $Si(CH_3)_2$), 0.92 (s, 9H, $C(CH_3)_3$), 1.35–1.70 (m, 10H, $CH_2\times5$), 3.88 (dd, *J*=7.6, 7.6 Hz, 1H, H-1), 4.07 (dd, *J*=7.6, 7.6 Hz, 1H, H-1), 4.26 (m, 1H, H-2), 4.37 (m, 2H, H-6), 5.37 (m, 1H, H-4), 5.89 (dt, *J*=11.6, 5.5 Hz, 1H, H-5), 5.94 (m, 1H, H-3). ^{13}C NMR δ: −5.5, −5.4, 18.2, 23.7, 23.8, 25.0, 25.8 (3C), 34.8, 35.7, 60.0, 64.5, 73.5, 75.7, 110.7, 114.5 (q, J_{C-F}=285.9 Hz), 121.8, 137.4, 156.5 (q, J_{C-F}=42.4 Hz). IR: 2942 (CH), 2890 (CH), 1786 (C═O). MS (FAB) *m/z* 461 (MNa^+). HRMS (FAB) calcd for $C_{20}H_{33}F_3NaO_5Si$ (MNa^+): 461.1947; found: 461.1927.

3.1.35. (*Z*,2*R*,3*S*)-6-Benzyloxy-1,2-cyclohexylidenedioxy-4-hexen-3-yl trifluoroacetate [(*Z*)-21b].



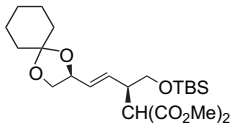
Compound **20b** (682 mg, 2.16 mmol) was converted into the (*Z*)-allylic alcohol (655 mg, 95%) by the procedure that described for compound (*Z*)-**12a**. Yellow oil. $[\alpha]_D^{25}$ +22.3 (*c* 0.87, $CHCl_3$). ^1H NMR δ: 1.36–1.65 (m, 10H, $CH_2\times5$), 2.48 (br, 1H, OH), 3.89 (dd, *J*=8.1, 6.7 Hz, 1H, H-1), 3.98 (dd, *J*=8.1, 6.7 Hz, 1H, H-1), 4.04 (m, 1H, H-2), 4.09 (ddd, *J*=12.8, 6.7, 1.2 Hz, 1H, H-6), 4.16 (ddd, *J*=12.8, 6.7, 1.2 Hz, 1H, H-6), 4.49 (m, 1H, H-3), 4.52 (q, *J*=16.5, 11.6 Hz, 2H, CH_2Ph), 5.57 (dd, *J*=11.6, 7.9 Hz, 1H, H-4), 5.82 (dt, *J*=11.6, 6.7 Hz, 1H, H-5), 7.25–7.37 (m, 5H, ArH). ^{13}C NMR δ: 23.7, 23.9, 25.1, 34.6, 36.1, 64.7, 66.1, 67.9, 72.6, 77.2, 77.7, 109.9, 127.8 (2C), 128.4 (2C), 130.4, 131.0, 137.8. IR: 3446 (OH), 2937 (CH), 2858 (CH). MS (FAB) *m/z* 319 (MH^+). HRMS (FAB) calcd for $C_{19}H_{27}O_4$ (MH^+): 319.1909; found: 319.1919. Compound (*Z*)-**21b** was obtained as a yellow oil (868 mg, quant.) from the (*Z*)-allylic alcohol (637 mg, 2.0 mmol) as described for (*E*)-**12a**. $[\alpha]_D^{25}$ +9.1 (*c* 1.00, $CHCl_3$). ^1H NMR δ: 1.25–1.75 (m, 10H, $CH_2\times5$), 3.85 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.04 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.21 (m, 2H, H-6), 4.22 (m, 1H, H-2), 4.54 (dd, *J*=14.3, 11.6 Hz, 2H, CH_2Ph), 5.48 (dd, *J*=11.6, 8.5 Hz, 1H, H-4), 5.80 (dd, *J*=8.5, 5.5 Hz, 1H, H-3), 5.98 (dt, *J*=11.6, 5.5 Hz, 1H, H-5), 7.20–7.40 (m, 5H, ArH). ^{13}C NMR δ: 23.7, 23.8, 24.9, 34.7, 35.6, 64.6, 66.1, 72.8, 73.6, 75.5, 110.7, 114.4 (q, J_{C-F}=285.9 Hz), 123.9, 127.8 (3C), 128.4 (2C), 134.8, 137.7, 156.5 (q, J_{C-F}=42.4 Hz). IR: 2937 (CH), 2894 (CH), 1786 (C═O). MS (FAB) *m/z* 415 (MH^+). HRMS (FAB) calcd for $C_{21}H_{26}F_3O_5$ (MH^+): 415.1732; found: 415.1729.

3.1.36. (*Z*,2*R*,3*S*)-1,2-Cyclohexylidenedioxy-6-methoxymethoxy-4-hexen-3-yl trifluoroacetate [(*Z*)-21c].



Compound **20c** (500 mg, 1.85 mmol) was converted into the (*Z*)-allylic alcohol (495 mg, 98%) by the procedure that described for compound (*Z*)-**12a**. Yellow oil. $[\alpha]_D^{25}$ +26.9 (*c* 1.00, $CHCl_3$). ^1H NMR δ: 1.30–1.70 (m, 10H, $CH_2\times5$), 3.38 (s, 3H, OCH_3), 3.92 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.00 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.08 (dd, *J*=11.6, 6.7 Hz, 1H, H-3), 4.13 (ddd, *J*=12.8, 5.5, 1.2 Hz, 1H, H-6), 4.26 (ddd, *J*=12.8, 7.9, 1.2 Hz, 1H, H-6), 4.55 (m, 1H, H-2), 4.63 (d, *J*=6.7 Hz, 1H, OCH_2O), 4.67 (d, *J*=6.7 Hz, 1H, OCH_2O), 5.61 (m, 1H, H-4 or H-5), 5.78 (m, 1H, H-4 or H-5). ^{13}C NMR δ: 23.5, 23.7, 24.9, 34.4, 35.8, 55.0, 62.8, 64.9, 67.6, 77.5, 95.2, 109.6, 129.3, 131.5. IR: 3467 (OH), 2937 (CH), 2894 (CH). MS (FAB) *m/z* 273 (MH^+). HRMS (FAB) calcd for $C_{14}H_{25}O_5$ (MH^+): 273.1702; found: 273.1705. Compound (*Z*)-**21c** was obtained as a yellow oil (421 mg, quant.) from the (*Z*)-allylic alcohol (300 mg, 1.1 mmol) as described for (*E*)-**12a**. $[\alpha]_D^{26}$ +0.65 (*c* 1.30, $CHCl_3$). ^1H NMR δ: 1.25–1.60 (m, 10H, $CH_2\times5$), 3.31 (s, 3H, OCH_3), 3.80 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.01 (dd, *J*=8.5, 6.7 Hz, 1H, H-1), 4.14 (ddd, *J*=13.3, 6.7, 1.2 Hz, 1H, H-6), 4.18 (dd, *J*=11.6, 6.7 Hz, 1H, H-2), 4.23 (ddd, *J*=13.3, 6.7, 1.2 Hz, 1H, H-6), 4.58 (s, 2H, OCH_2O), 5.42 (dddd, *J*=11.6, 9.7, 1.2, 1.2 Hz, 1H, H-4), 5.74 (dd, *J*=9.7, 6.7 Hz, 1H, H-3), 5.88 (dt, *J*=11.6, 6.7 Hz, 1H, H-5). ^{13}C NMR δ: 23.7, 23.8, 24.9, 34.7, 35.6, 55.3, 63.1, 64.7, 73.4, 75.5, 95.9, 110.8, 114.4 (q, J_{C-F}=285.9 Hz), 124.0, 134.3, 156.4 (q, J_{C-F}=42.4 Hz). IR: 2942 (CH), 2890 (CH), 1786 (C═O). MS (FAB) *m/z* 369 (MH^+). HRMS (FAB) calcd for $C_{16}H_{24}F_3O_6$ (MH^+): 369.1525; found: 369.1532.

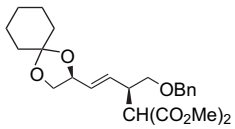
3.1.37. Dimethyl 2-[(*E*,2*S*,5*R*)-6-(*tert*-butyldimethylsilyloxy)-1,2-cyclohexylidenedioxy-3-hexen-5-yl]malonate (*syn*-22a).



Compound *syn*-**22a** was obtained as a yellow oil (70 mg, 77%) from (*Z*)-**21a** (88 mg, 0.20 mmol) as described for *anti*-**4**. $[\alpha]_D^{24}$ +35.9 (*c* 1.12, $CHCl_3$). ^1H NMR δ: 0.10 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 1.30–1.70 (m, 10H, $CH_2\times5$), 2.99 (m, 1H, H-5′), 3.52 (m, 1H, H-2′), 3.62 (dd, *J*=9.8, 6.7 Hz, 1H, CH_2OTBS), 3.69 (m, 1H, CH_2OTBS), 3.70 (s, 3H, OCH_3), 3.71 (d, *J*=8.5 Hz, 1H, $CH(CO_2CH_3)_2$), 3.72 (s, 3H, OCH_3), 4.05 (dd, *J*=13.7, 8.5 Hz, 1H, H-1′),

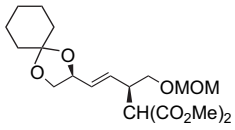
4.44 (dd, J=13.7, 6.7 Hz, 1H, H-1′), 5.56 (dd, J=15.6, 6.7 Hz, 1H, H-4′), 5.81 (dd, J=15.6, 8.5 Hz, 1H, H-3′). ^{13}C NMR δ: −5.4 (2C), 18.5, 24.1, 24.2, 25.4, 26.0 (3C), 35.7, 36.5, 44.9, 52.4, 52.6, 52.6, 63.9, 69.3, 76.4, 110.1, 130.9, 132.0, 169.0, 169.1. IR: 2933 (CH), 2861 (CH), 1755 (C═O), 1739 (C═O). MS (FAB) m/z 479 (MNa^+). HRMS (FAB) calcd for $C_{23}H_{40}NaO_7Si$ (MNa^+): 479.2441; found: 479.2474.

3.1.38. Dimethyl 2-[(*E*,2*S*,5*R*)-6-benzyloxy-1,2-cyclohexylidenedioxy-3-hexen-5-yl]malonate (*syn*-22b).



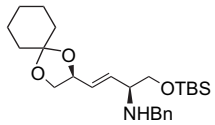
Compound *syn*-**22b** was obtained as a yellow oil (166 mg, 77%) from (*Z*)-**21b** (207 mg, 0.50 mmol) as described for *anti*-**4**. $[\alpha]_D^{25}$ +30.9 (c 1.02, $CHCl_3$). ^{1}H NMR δ: 1.30–1.65 (m, 10H, $CH_2\times5$), 3.16 (dddd, J=8.5, 6.7, 6.7, 5.5 Hz, 1H, H-5′), 3.51 (d, J=8.5 Hz, 1H, CH($CO_2CH_3)_2$), 3.52 (dd, J=8.5, 6.7 Hz, 1H, CH_2OBn), 3.58 (dd, J=8.5, 5.5 Hz, 1H, CH_2OBn), 3.66 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.68 (dd, J=15.6, 6.7 Hz, 1H, H-1′), 4.04 (dd, J=8.5, 6.7 Hz, 1H, H-2′), 4.44 (dd, J=15.6, 6.7 Hz, 1H, H-1′), 4.45 (s, 2H, CH_2Ph), 5.57 (dd, J=15.6, 6.7 Hz, 1H, H-4′), 5.80 (dd, J=15.6, 8.5 Hz, 1H, H-3′), 7.20–7.35 (m, 5H, ArH). ^{13}C NMR δ: 23.8, 23.8, 25.0, 35.3, 36.1, 42.4, 52.2, 52.3, 53.0, 68.9, 70.7, 73.0, 76.0, 110.0, 127.5, 127.7 (2C), 128.2 (2C), 130.2, 131.8, 137.9, 168.4, 168.5. IR: 2941 (CH), 2864 (CH), 1755 (C═O), 1736 (C═O). MS (FAB) m/z 455 (MNa^+). HRMS (FAB) calcd for $C_{24}H_{32}NaO_7$ (MNa^+): 455.2046; found: 455.2048.

3.1.39. Dimethyl 2-[(*E*,2*S*,5*R*)-1,2-cyclohexylidenedioxy-6-methoxymethoxy-3-hexen-5-yl]malonate (*syn*-22c).



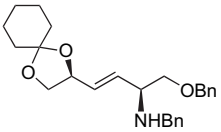
Compound *syn*-**22c** was obtained as a yellow oil (62 mg, 81%) from (*Z*)-**21c** (74 mg, 0.20 mmol) as described for *anti*-**4**. $[\alpha]_D^{25}$ +29.5 (c 2.54, $CHCl_3$). ^{1}H NMR δ: 1.30–1.70 (m, 10H, $CH_2\times5$), 3.12 (ddd, J=9.6, 7.6, 4.8 Hz, 1H, H-5′), 3.33 (s, 3H, CH_2OCH_3), 3.53 (m, 1H, H-2′), 3.57 (dd, J=9.8, 7.6 Hz, 1H, CH_2OMOM), 3.62 (dd, J=9.8, 4.8 Hz, 1H, CH_2OMOM), 3.68 (d, J=7.6 Hz, 1H, CH($CO_2CH_3)_2$), 3.71 (s, 3H, CO_2CH_3), 3.74 (s, 3H, CO_2CH_3), 4.05 (dd, J=14.0, 7.6 Hz, 1H, H-1′), 4.46 (dd, J=14.0, 6.7 Hz, 1H, H-1′), 4.57 (s, 2H, OCH_2O), 5.56 (dd, J=15.6, 7.6 Hz, 1H, H-3′), 5.81 (dd, J=15.6, 9.8 Hz, 1H, H-4′). ^{13}C NMR δ: 23.9, 23.9, 25.1, 35.4, 36.2, 42.4, 52.3, 52.4, 53.0, 55.3, 68.2, 69.0, 76.0, 96.4, 109.9, 130.2, 132.0, 168.4, 168.6. IR: 2937 (CH), 2863 (CH), 1755 (C═O), 1736 (C═O). MS (FAB) m/z 409 (MNa^+). HRMS (FAB) calcd for $C_{19}H_{30}NaO_8$ (MNa^+): 409.1838; found: 409.1833.

3.1.40. (*E*,2*S*,5*S*)-5-Benzylamino-6-(*tert*-butyldimethylsilyloxy)-1,2-cyclohexylidenedioxy-3-hexene (*syn*-23a).



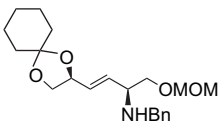
Compound *syn*-**23a** was obtained as a yellow oil (44 mg, 51%) from (*Z*)-**21a** (88 mg, 0.20 mmol) as described for *anti*-**5**. $[\alpha]_D^{24}$ +37.1 (c 2.00, $CHCl_3$). ^{1}H NMR δ: 0.10 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 1.30–1.70 (m, 10H, $CH_2\times5$), 2.05 (br, 1H, NH), 3.25 (ddd, J=6.7, 6.7, 6.7 Hz, 1H, H-5), 3.51 (dd, J=17.4, 6.7 Hz, 1H, H-6), 3.54 (dd, J=17.4, 6.7 Hz, 1H, H-6), 3.57 (dd, J=6.7, 6.7 Hz, 1H, H-2), 3.67 (d, J=13.4 Hz, 1H, CH_2Ph), 3.87 (d, J=13.4 Hz, 1H, CH_2Ph), 4.07 (dd, J=13.7, 6.7 Hz, 1H, H-1), 4.52 (dd, J=13.7, 6.7 Hz, 1H, H-1), 5.60 (dd, J=15.6, 6.7 Hz, 1H, H-4), 5.68 (dd, J=15.6, 6.7 Hz, 1H, H-3), 7.20–7.40 (m, 5H, ArH). ^{13}C NMR δ: −5.4, −5.4, 18.2, 23.9, 23.9, 25.1, 25.8 (3C), 35.5, 36.2, 51.2, 61.1, 66.1, 69.2, 76.3, 109.9, 126.7, 128.0 (2C), 128.3 (2C), 131.4, 133.3, 140.5. IR: 2933 (CH), 2860 (CH). MS (FAB) m/z 432 (MH^+). HRMS (FAB) calcd for $C_{25}H_{42}NO_3Si$ (MH^+): 432.2934; found: 432.2956.

3.1.41. (*E*,2*S*,5*S*)-5-Benzylamino-6-benzyloxy-1,2-cyclohexylidenedioxy-3-hexene (*syn*-23b).



Compound *syn*-**23b** was obtained as a yellow oil (50 mg, 61%) from (*Z*)-**21b** (83 mg, 0.20 mmol) as described for *anti*-**5**. $[\alpha]_D^{25}$ +28.0 (c 1.61, $CHCl_3$). ^{1}H NMR δ: 1.30–1.70 (m, 10H, $CH_2\times5$), 2.06 (br, 1H, NH), 3.43 (m, 1H, H-5 or H-6), 3.45 (m, 2H, H-5 or H-6), 3.55 (dd, J=6.7, 6.7 Hz, 1H, H-2), 3.66 (d, J=13.4 Hz, 1H, CH_2Ph), 3.85 (d, J=13.4 Hz, 1H, CH_2Ph), 4.07 (dd, J=13.7, 6.7 Hz, 1H, H-1), 4.48 (dd, J=15.6, 12.2 Hz, 2H, CH_2Ph), 4.52 (dd, J=13.7, 6.7 Hz, 1H, H-1), 5.65 (dd, J=15.6, 5.5 Hz, 1H, H-4), 5.70 (dd, J=15.6, 6.7 Hz, 1H, H-3), 7.20–7.40 (m, 10H, ArH). ^{13}C NMR δ: 23.8, 23.9, 25.1, 35.5, 36.2, 51.3, 59.1, 69.1, 73.1, 73.2, 76.2, 110.0, 126.8, 127.7 (4C), 128.1, 128.4 (4C), 131.5, 133.0, 138.0, 140.3. MS (FAB) m/z 408 (MH^+). HRMS (FAB) calcd for $C_{26}H_{34}NO_3$ (MH^+): 408.2539; found: 408.2538.

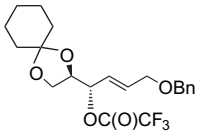
3.1.42. (*E*,2*S*,5*S*)-5-Benzylamino-1,2-cyclohexylidenedioxy-6-methoxymethoxy-3-hexene (*syn*-23c).



Compound *syn*-**23c** was obtained as a yellow oil (53 mg, 74%) from (*Z*)-**21c** (74 mg, 0.20 mmol) as described for *anti*-**5**. $[\alpha]_D^{26}$ +28.8 (c 2.72, $CHCl_3$). ^{1}H NMR δ: 1.30–1.70

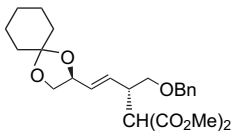
(m, 10H, $CH_2\times5$), 2.05 (br, 1H, NH), 3.33 (s, 3H, OCH_3), 3.40 (m, 1H, H-5), 3.47 (m, 1H, H-6), 3.54 (m, 1H, H-2), 3.56 (m, 1H, H-6), 3.68 (d, J=13.4 Hz, 1H, CH_2Ph), 3.87 (d, J=13.4 Hz, 1H, CH_2Ph), 4.07 (dd, J=13.4, 6.1 Hz, 1H, H-1), 4.53 (dd, J=13.4, 6.1 Hz, 1H, H-1), 4.60 (s, 2H, OCH_2O), 5.66 (dd, J=15.6, 6.1 Hz, 1H, H-4), 5.72 (dd, J=15.6, 6.1 Hz, 1H, H-3), 7.20–7.40 (m, 5H, ArH). ^{13}C NMR δ: 23.8, 23.9, 25.1, 35.4, 36.2, 51.3, 55.3, 59.2, 69.1, 70.7, 76.2, 96.6, 110.0, 126.9, 128.1 (2C), 128.4 (2C), 131.6, 132.9, 140.2. IR: 2933 (CH), 2864 (CH). MS (FAB) m/z 362 (MH^+). HRMS (FAB) calcd for $C_{21}H_{32}NO_4$ (MH^+): 362.2331; found: 362.2325.

3.1.43. (*E*,2*R*,3*S*)-6-Benzyloxy-1,2-cyclohexylidenedioxy-4-hexen-3-yl trifluoroacetate [(*E*)-21b].



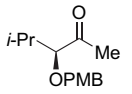
Red-Al® (65% in toluene, 1.20 mL, 4.0 mmol) was added to a solution of **20b** (633 mg, 2.0 mmol) with stirring at 0 °C. The stirring was continued for 1 h at this temperature. The reaction was quenched with saturated aqueous Rochelle salt and extracted with Et_2O. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (3:1) to give (*E*)-allylic alcohol (547 mg, 86%) as a yellow oil. $[\alpha]_D^{21}$ +4.20 (*c* 1.00, $CHCl_3$). ^{1}H NMR δ: 1.25–1.65 (m, 10H, $CH_2\times5$), 2.15 (br, 1H, OH), 3.92 (m, 2H, H-1), 4.05 (dd, J=5.5, 1.2 Hz, 2H, H-6), 4.11 (ddd, J=6.7, 6.7, 4.3 Hz, 1H, H-2), 4.35 (dd, J=5.5, 4.3 Hz, 1H, H-3), 4.52 (s, 2H, CH_2Ph), 5.72 (ddt, J=15.6, 5.5, 1.2 Hz, 1H, H-4), 5.94 (dtd, J=15.6, 5.5, 1.2 Hz, 1H, H-5), 7.20–7.40 (m, 5H, ArH). ^{13}C NMR δ: 23.7, 23.9, 25.1, 34.6, 36.1, 64.3, 69.9, 71.1, 72.2, 77.7, 109.9, 127.6, 127.7 (2C), 128.4 (2C), 129.2, 130.2, 138.1. IR: 3444 (OH), 2931 (CH), 2860 (CH). MS (FAB) m/z 319 (MH^+). HRMS (FAB) calcd for $C_{19}H_{27}O_4$ (MH^+): 319.1909; found: 319.1916. Compound (*E*)-**21b** was obtained as a yellow oil (240 mg, quant.) from the (*E*)-allylic alcohol (191 mg, 0.6 mmol) as described for (*E*)-**12a**. $[\alpha]_D^{25}$ +23.6 (*c* 1.00, $CHCl_3$). ^{1}H NMR δ: 1.25–1.75 (m, 10H, $CH_2\times5$), 3.85 (dd, J=8.5, 6.7 Hz, 1H, H-2), 4.05 (m, 1H, H-1), 4.06 (m, 2H, H-6), 4.26 (dd, J=11.6, 6.7 Hz, 1H, H-1), 4.52 (s, 2H, CH_2Ph), 5.57 (dd, J=8.5, 6.7 Hz, 1H, H-3), 5.74 (dd, J=15.6, 6.7 Hz, 1H, H-4), 5.98 (dt, J=15.6, 5.5 Hz, 1H, H-5), 7.25–7.37 (m, 5H, ArH). ^{13}C NMR δ: 23.8, 23.8, 25.1, 34.8, 35.8, 64.7, 69.2, 72.6, 75.7, 77.1, 110.9, 114.5 (q, J_{C-F}= 285.9 Hz), 123.8, 127.8 (2C), 127.8, 128.5 (2C), 133.9, 137.8, 156.5 (q, J_{C-F}=42.4 Hz). IR: 2937 (CH), 2859 (CH), 1788 (C=O). MS (FAB) m/z 421 (MLi^+). HRMS (FAB) calcd for $C_{21}H_{25}F_3LiO_5$ (MLi^+): 421.1814; found: 421.1818.

3.1.44. Dimethyl 2-[(*E*,2*S*,5*S*)-6-benzyloxy-1,2-cyclohexylidenedioxy-3-hexen-5-yl]malonate (*anti*-22b).



Compound *anti*-**22b** was obtained as a yellow oil (65 mg, 77%) from (*E*)-**24b** (83 mg, 0.20 mmol) as described for *anti*-**4**. $[\alpha]_D^{25}$ −14.4 (*c* 1.69, $CHCl_3$). ^{1}H NMR δ: 1.30–1.70 (m, 10H, $CH_2\times5$), 3.15 (ddd, J=8.5, 6.7, 4.9 Hz, 1H, H-1′), 3.50 (dd, J=8.5, 8.5 Hz, 1H, CH(CO_2CH_3)$_2$), 3.52 (dd, J=8.5, 6.7 Hz, 1H, CH_2OBn), 3.58 (dd, J=8.5, 4.9 Hz, 1H, CH_2OBn), 3.65 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.71 (dd, J=14.6, 8.5 Hz, 1H, H-5′), 4.02 (td, J=8.5, 6.7 Hz, 1H, H-4′), 4.44 (dd, J=14.6, 6.7 Hz, 1H, H-5′), 4.45 (s, 2H, CH_2Ph), 5.58 (dd, J=15.6, 8.5 Hz, 1H, H-2′), 5.81 (dd, J=15.6, 8.5 Hz, 1H, H-3′), 7.27–7.35 (m, 5H, ArH). ^{13}C NMR δ: 23.8, 23.9, 25.1, 35.4, 36.2, 42.7, 52.2, 52.3, 53.0, 68.9, 70.6, 73.1, 76.3, 110.0, 127.6, 127.6 (2C), 128.3 (2C), 130.8, 131.9, 137.9, 168.4, 168.6. IR: 2942 (CH), 2937 (CH), 2863 (CH), 1754 (C=O), 1737 (C=O). MS (FAB) m/z 455 (MNa^+). HRMS (FAB) calcd for $C_{24}H_{32}NaO_7$ (MNa^+): 455.2046; found: 455.2045.

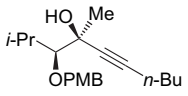
3.1.45. (*S*)-3-(4-Methoxybenzyloxy)-4-methylpentan-2-one (24).



A solution of (*S*)-(+)-2-hydroxy-3-methylbutyric acid (650 mg, 5.50 mmol), *N*,*O*-dimethylhydroxyamine (488 mg, 5.00 mmol), 1-hydroxybenzotriazole (676 mg, 5.00 mmol) and *i*Pr_2NEt (0.86 mL, 5.00 mmol) in CH_2Cl_2 (20 mL) was stirred at rt for 30 min. A solution of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.05 g, 5.50 mmol) in CH_2Cl_2 (10 mL) was added, and the mixture was stirred at 0 °C for 2 h and then at rt for 3 h. The reaction mixture was extracted with EtOAc and acid after evaporation. The combined organic layers were washed with saturated $NaHCO_3$ and brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (1:1) to give 2-hydroxy-*N*-methoxy-3,*N*-dimethylbutyramide (746 mg, 93%) as a pale oil. $[\alpha]_D^{24}$ −14.8 (*c* 1.19, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.79 (d, J=6.7 Hz, 3H, CCH_3), 1.01 (d, J=6.7 Hz, 3H, CCH_3), 2.01 (br, 1H, CH(CH_3)$_2$), 3.08 (br, 1H, OH), 3.22 (s, 3H, NCH_3), 3.68 (br, 3H, OCH_3), 4.24 (br, 1H, CHOH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 15.1 (2C), 19.3, 31.1, 60.9, 72.5, 174.4. IR: (KBr) cm^{-1}: 3462 (OH), 2966 (CH), 1655 (C=O). MS (FAB) m/z 162 (MH^+). HRMS (FAB) calcd for $C_7H_{16}NO_3$ (MH^+): 162.1130; found: 162.1125. *p*-Methoxybenzyl chloride (0.150 mL, 1.10 mmol) and tetrabutylammonium iodide (19.0 mg, 0.05 mmol) were added to the alkoxide generated from the alcohol (161 mg, 1.00 mmol) and NaH (60% in oil, 44.0 mg, 1.10 mmol) in THF (4 mL) at rt and stirred for 8 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (2:1) to give *N*-methoxy-2-(4-methoxybenzyloxy)-3,*N*-dimethylbutyramide (199 mg, 71%) as a brown oil. $[\alpha]_D^{26}$ −51.0 (*c* 1.04, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.91 (d, J=6.7 Hz, 3H, CCH_3), 1.01 (d, J=6.7 Hz, 3H, CCH_3), 2.09 (m, 1H, CH(CH_3)$_2$), 3.22 (s, 3H, NCH_3), 3.59 (s, 3H, $NOCH_3$), 3.80 (s, 3H, ArOCH_3),

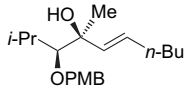
4.01 (br, 1H, CHCO), 4.30 (d, J=11.6 Hz, 1H, CH_2Ar), 4.62 (d, J=11.6 Hz, 1H, CH_2Ar), 6.86 (d, J=8.5 Hz, 2H, ArH), 7.28 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.1, 18.9, 30.8, 32.2, 55.1, 61.0, 71.1, 80.0, 113.5 (2C), 129.3 (2C), 130.0, 159.1, 173.2. IR: (KBr) cm^{-1} 2962 (CH), 1670 (C=O). MS (FAB) m/z 282 (MH^+). HRMS (FAB) calcd for $C_{15}H_{24}NO_4$ (MH^+): 282.1705; found: 282.1690. MeMgI (0.84 M in ether, 16.6 mL, 14.0 mmol) was added to a solution of the PMB ether (1.12 g, 4.0 mmol) in ether (20 mL) at 0 °C and stirred for 2 h after warming to rt. The reaction was quenched with saturated aqueous NH_4Cl solution. The reaction mixture was extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (2:1) to give **24** (727 mg, 77%) as a colorless oil. $[\alpha]_D^{26}$ −81.0 (c 1.10, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (d, J=6.7 Hz, 3H, CCH_3), 0.94 (d, J=6.7 Hz, 3H, CCH_3), 1.94 (hept, J=6.7 Hz, 1H, CH(CH_3)$_2$), 2.12 (s, 3H, $COCH_3$), 3.37 (d, J=6.7 Hz, 1H, CHOPMB), 3.78 (s, 3H, ArOCH_3), 4.29 (d, J=11.6 Hz, 1H, CH_2Ar), 4.48 (d, J=11.6 Hz, 1H, CH_2Ar), 6.85 (d, J=8.5 Hz, 2H, ArH), 7.23 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.2, 18.6, 25.8, 30.8, 55.2, 72.5, 90.2, 113.8 (2C), 129.5 (2C), 129.6, 159.3, 211.9. IR: (KBr) cm^{-1} 2964 (CH), 1712 (C=O). MS (FAB) m/z 259 (MNa^+). HRMS (FAB) calcd for $C_{14}H_{20}NaO_3$ (MNa^+): 259.1310; found: 259.1318.

3.1.46. (3*S*,4*S*)-3-(4-Methoxybenzyloxy)-2,4-dimethyl-5-decyn-4-ol (25).



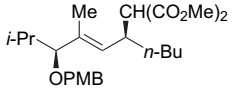
EtMgBr (1 M in THF, 4.5 mL, 4.51 mmol) was added to a solution of 1-hexyne (0.49 mL, 4.27 mmol) in THF (4 mL) at rt and stirred under reflux for 1 h. The magnesium acetylide was added to a solution of **24** (288 mg, 1.22 mmol) in THF (10 mL) at 45 °C and stirred for 2 h at the same temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (9:1) to give **25** (379 mg, 97%, >97:3 dr) as a brown oil. $[\alpha]_D^{21}$ +31.2 (c 1.64, $CHCl_3$). ^{1}H NMR δ: 0.82 (t, J=7.3 Hz, 3H, H-10), 0.92 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 0.97 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 1.25–1.48 (m, 4H, H-8 and H-9), 1.35 (s, 3H, 4-CH_3), 1.96 (heptd, J=6.7, 3.1 Hz, 1H, H-2), 2.13 (t, J=7.3 Hz, 2H, H-7), 2.68 (br, 1H, OH), 3.27 (d, J=3.1 Hz, 1H, H-3), 3.73 (s, 3H, ArOCH_3), 4.57 (d, J=11.0 Hz, 1H, CH_2Ar), 4.75 (d, J=11.0 Hz, 1H, CH_2Ar), 6.81 (d, J=8.5 Hz, 2H, ArH), 7.24 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 13.6, 17.3, 18.4, 22.0, 22.6, 25.5, 29.7, 30.7, 55.2, 70.4, 75.7, 83.7, 84.3, 89.0, 113.7 (2C), 129.3 (2C), 130.8, 159.2. IR: 3479 (OH), 2956 (CH). MS (FAB) m/z 341 (MNa^+). HRMS (FAB) calcd for $C_{20}H_{30}NaO_3$ (MNa^+), 341.2093; found: 341.2111.

3.1.47. (*E*,3*S*,4*S*)-3-(4-Methoxybenzyloxy)-2,4-dimethyl-5-decen-4-ol (26).



$LiAlH_4$ (25 mg, 0.65 mmol) was added to a solution of **25** (104 mg, 0.33 mmol) in THF (3 mL) at 0 °C and stirred under reflux for 1 h. The reaction mixture was quenched with saturated aqueous Rochelle salt and extracted with ether. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (9:1) to give **26** (92.1 mg, 88%) as a colorless oil. $[\alpha]_D^{27}$ +31.5 (c 0.90, $CHCl_3$). ^{1}H NMR δ: 0.89 (t, J=6.7 Hz, 3H, H-10), 1.00 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 1.02 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 1.23 (s, 3H, 4-CH_3), 1.28–1.40 (m, 4H, H-8 and H-9), 1.95 (septd, J=6.7, 3.1 Hz, 1H, H-2), 2.06 (dt, J=6.7, 6.7 Hz, 2H, H-7), 2.33 (br, 1H, OH), 3.12 (d, J=3.1 Hz, 1H, H-3), 3.81 (s, 3H, ArOCH_3), 4.56 (d, J=12.8 Hz, 1H, CH_2Ar), 4.58 (d, J=12.8 Hz, 1H, CH_2Ar), 5.56 (d, J=15.9 Hz, 1H, H-5), 5.70 (dt, J=15.9, 6.7 Hz, 1H, H-6), 6.88 (d, J=8.5 Hz, 2H, ArH), 7.26 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 13.9, 17.3, 22.2, 23.0, 23.7, 29.4, 31.4, 32.0, 55.2, 75.2, 75.5, 89.4, 113.6, 113.7, 128.9, 129.1, 129.3, 130.9, 136.0, 159.1. IR: 3548 (OH), 2958 (CH). MS (FAB) m/z 343 (MNa^+). HRMS (FAB) calcd for $C_{20}H_{32}NaO_3$ (MNa^+), 343.2249; found: 343.2234.

3.1.48. Dimethyl 2-[(*E*,3*S*,6*S*)-3-(4-methoxybenzyloxy)-2,4-dimethyl-4-decen-6-yl]malonate (27).



Trifluoroacetic anhydrate (0.30 mL, 2.2 mmol) was added to a solution of **26** (342 mg, 1.1 mmol) and pyridine (0.19 mL, 2.4 mmol) in ether (5.0 mL) at 0 °C. After the reaction mixture was warmed to rt, stirred for 30 min. The reaction was quenched with saturated aqueous NH_4Cl. The reaction mixture was extracted with ether. The combined organic layers were washed with brine prior to drying and solvent evaporation. The crude was used in next step. $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) was added to a solution of the crude (62 mg) in THF (1.5 mL) at rt and the stirring was continued for 15 min. Dimethyl malonate (0.072 mL, 0.63 mmol) was added to a suspension of NaH (60% in oil, 24 mg, 0.60 mmol) in THF (2 mL) with stirring at 0 °C. After stirring for 30 min, the solution was added to the abovementioned mixture and the whole was refluxed for 3 h. The reaction was quenched with water. The reaction mixture was concentrated before extracted with EtOAc. The combined organic layers were washed with brine prior to drying and solvent evaporation. The residue was purified by column chromatography on silica gel with hexane–EtOAc (9:1) to give **27** (31 mg, 48%) as a pale oil. $[\alpha]_D^{25}$ −23.7 (c 1.56, $CHCl_3$). ^{1}H NMR δ: 0.71 (d, J=6.7 Hz, 3H, CH(CH_3)$_2$), 0.85 (t, J=6.7 Hz, 3H, CH_2CH_3), 0.97 (d, J=6.7 Hz, 3H,

$CH(CH_3)_2$), 1.15–1.50 (m, 6H, $CH_2\times3$), 1.63 (s, 3H, $C{=}CCH_3$), 1.78 (dsept, J=9.1, 6.7 Hz, 1H, $CH(CH_3)_2$), 3.10 (d, J=9.1 Hz, 1H, CHOPMB), 3.20 (dtd, J=10.4, 9.1, 3.0 Hz, 1H, H-6′), 3.40 (d, J=9.1 Hz, 1H, $CH(CO_2CH_3)_2$), 3.68 (s, 3H, CO_2CH_3), 3.74 (s, 3H, $ArOCH_3$), 3.80 (s, 3H, CO_2CH_3), 4.04 (d, J=11.6 Hz, 1H, CH_2Ar), 4.33 (d, J=11.6 Hz, 1H, CH_2Ar), 5.10 (d, J=10.4 Hz, 1H, C=CH), 6.86 (d, J=8.5 Hz, 2H, ArH), 7.24 (d, J=8.5 Hz, 2H, ArH). ^{13}C NMR δ: 11.2, 13.9, 19.2, 19.8, 22.4, 29.2, 30.2, 32.9, 38.2, 52.3, 52.4, 55.2, 57.3, 69.0, 90.8, 113.6 (2C), 129.4 (2C), 130.0, 131.0, 137.3, 158.9, 168.7, 168.9. IR: 2954 (CH), 1759 (C=O), 1736 (C=O). MS (FAB) *m/z* 457 (MNa^+). HRMS (FAB) calcd for $C_{25}H_{38}NaO_6$ (MNa^+): 457.2566: found: 457.2588.

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Synthesis of 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives by annulation of *N*,*N*-dialkyl[2-(pyrrol-1-yl)benzylidene]ammonium salts in the presence of an isocyanide

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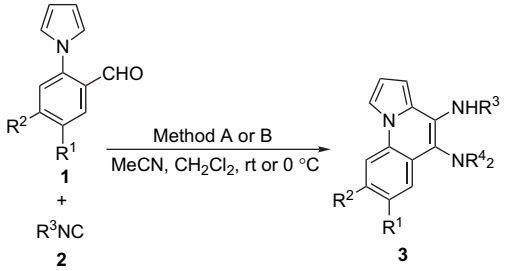
Abstract—A facile synthetic method for 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives has been developed. Treatment of 2-(pyrrol-1-yl)-benzaldehydes with secondary amine hydrochloride/NaI/TMSCl/Et_3N in the presence of an isocyano compound leads to the formation of 4-alkyl(or aryl)amino-5-dialkylaminopyrrolo[1,2-*a*]quinolines.

1. Introduction

We previously described the synthesis of 9-dialkylamino-9*H*-pyrrolo[1,2-*a*]indole derivatives by annulation of iminium salts generated from 2-(pyrrol-1-yl)benzaldehydes and secondary amine hydrochlorides in the presence of NaI/TMSCl/Et_3N.[1] In this paper we wish to report that the successful use of isocyanides in this annulation provides 4-alkyl(or aryl)amino-5-dialkylaminopyrrolo[1,2-*a*]quinolines **3**. Although a number of useful methods for the preparation of pyrrolo[1,2-*a*]quinoline derivatives have already been reported[2] due to their both practical and theoretical utilities as benzo analogues of indolizines,[3] this is the first report on the synthesis of 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives.

2. Results and discussion

The procedure we have developed for the synthesis of **3** is outlined in Scheme 1. First, using the following procedure (Method A), the preparation of 5-dimethyl(or diethyl)aminopyrrolo[1,2-*a*]quinoline derivatives **3a**, **3b**, **3d**, **3e**, and **3i–k** has been achieved. Sodium iodide (2.2 mmol), the secondary amine hydrochloride (1.0 mmol), triethylamine (2.1 mmol), and chlorotrimethylsilane (2.3 mmol) were mixed in acetonitrile (2.2 mL) at room temperature.[4] To this mixture were added solutions of the isocyanides **2** (1.0 mmol) and the 2-(pyrrol-1-yl)benzaldehydes **1** in dichloromethane under stirring, and stirring was continued for 4 h. After usual workup followed by separation using column chromatography on silica gel the desired products were obtained in the yields summarized in Table 1 (Entries 1, 2, 4, 5, and 9–11). Compounds **1a** and **1c** gave fair to good yields of the desired products in general. An aliphatic isocyanide, such as *tert*-butyl isocyanide (**1c**), proved to be usable in the present procedure, but the yield of the expected product **3i** was lower (Entry 9). The reaction using **1b** was carried out at lower temperature (0 °C) for a longer reaction time (overnight) by considering the lability of the methoxy substituents toward iodotrimethylsilane generated in situ (Entry 10).



Method A: $R^4{}_2N^+H_2Cl^-$, NaI, Me_3SiCl, Et_3N
Method B: $R^4{}_2NH$, NaI, Me_3SiCl, Et_3N, $Et_3N^+HCl^-$

Scheme 1.

Keywords: Iminium salt; Isocyanide; Pyrrole; Pyrroloquinoline; Secondary amine.
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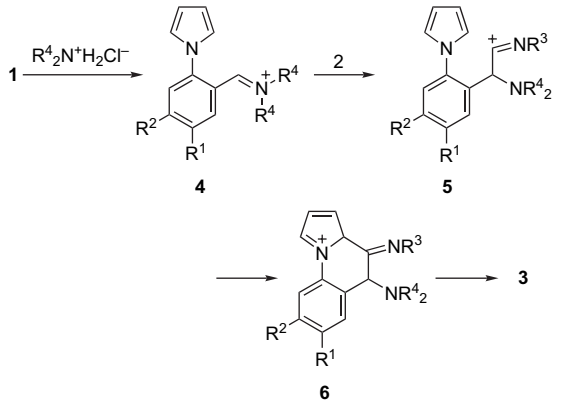
Table 1. Preparation of 4-alkyl(or aryl)amino-5-dialkylaminopyrrolo[1,2-*a*]quinolines **3**[a]

Entry	Starting material **1**	Isocyanide **2**	Amine	Method	**3** (Yield/%)[b]
1	**1a** (R^1=R^2=H)	**2a** (R^3=*o*-Tol)	$HNMe_2$	A	**3a** (57)
2	**1a**	**2a**	$HNEt_2$	A	**3b** (57)
3	**1a**	**2a**	Piperidine	B	**3c** (59)
4	**1a**	**2b** (R^3=Ph)	$HNMe_2$	A	**3d** (70)
5	**1a**	**2b**	$HNEt_2$	A	**3e** (54)
6	**1a**	**2b**	Pyrrolidine	B	**3f** (60)
7	**1a**	**2b**	Piperidine	B	**3g** (56)
8	**1a**	**2b**	Morpholine	B	**3h** (48)
9	**1a**	**2c** (R^3=*t*-Bu)	$HNMe_2$	A	**3i** (32)
10	**1b** (R^1=R^2=OMe)	**2b**	$HNMe_2$	A	**3j** (58)[c]
11	**1c** (R^1=Cl, R^2=H)	**2b**	$HNMe_2$	A	**3k** (65)

[a] Reactions were conducted at room temperature for 4 h.
[b] Isolated yields by column chromatography on silica gel.
[c] Reaction was conducted at 0 °C overnight.

Subsequently, in order to investigate the generality of the present procedure, other secondary amines were used. The reactions using free secondary amines instead of secondary amine hydrochlorides under the same reaction conditions described above resulted in the formation of intractable mixtures of products including the starting material **1a**. However, in the presence of triethylamine hydrochloride (1.1 equiv), the reactions proceeded more cleanly to give moderate-to-fair yields of the desired 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives **3c** and **3f–h** (Method B; Entries 3 and 6–8). It indicates that somewhat acidic media are essential for the generation of the iminium salts **4**. Mechanistically, this annulation process appears to proceed as shown in Scheme 2. Thus, the isocyano carbon of an isocyanide attacks at the imino carbon of the iminium salts **4**, derived from **1** and secondary amine hydrochlorides in the presence of NaI/TMSCl/Et_3N, to generate the intermediate **5**. An intramolecular combination of the resulting cation center of **5** and the 2-carbon of the pyrrole ring affords **6**, which gives rise to **3** through tautomerization.



Scheme 2.

The results reported above demonstrate that 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives can be conveniently synthesized from 2-(pyrrol-1-yl)benzaldehydes, isocyanides, and secondary amines. The ready availability of the starting materials and the ease of operations make the present method attractive.

3. Experimental

3.1. General

The melting points were determined on a Laboratory Devices MEL-TEMP II melting-point apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer 1600 Series FT IR spectrometer. The ^1H NMR spectra were determined using $SiMe_4$ as an internal reference with a JEOL JNM-GX270 FT NMR spectrometer operating at 270 MHz in $CDCl_3$. The ^{13}C NMR spectrum was determined using $SiMe_4$ as an internal reference with a JEOL ECP500 FT NMR spectrometer operating at 125 MHz in $CDCl_3$. Low resolution mass spectra were recorded on a JEOL AUTO-MASS 20 spectrometer (Center for Joint Research and Development, this University). High-resolution MS analyses were performed a JEOL JMS-AX505 HA spectrometer (Faculty of Agriculture, this University). Thin-layer chromatography (TLC) was carried out on Merck Kieselgel 60 PF_{254}. All of the solvents used were dried over appropriate drying agents and distilled under argon prior to use.

3.2. Starting materials

2-(Pyrrol-1-yl)benzaldehydes **1a–c** were prepared as described in our recent report.[1] Isocyanides **2a** and **2b** were prepared by a modification[5a] of Ugi's method.[5b] All other chemicals used in this study were commercially available.

3.3. Typical procedure for the preparation of 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives 3 under Method A

3.3.1. 5-Dimethylamino-4-[(2-methylphenyl)amino]pyrrolo[1,2-*a*]quinoline (3a). To a stirred mixture of NaI (0.34 g, 2.2 mmol), dimethylamine hydrochloride (83 mg, 1.0 mmol), Et_3N (0.21 g, 2.1 mmol), and Me_3SiCl (0.25 g, 2.3 mmol) in acetonitrile (2.2 mL) (Risch's conditions)[4] at room temperature was added a solution of *o*-tolyl isocyanide (**2a**) (0.12 g, 1.0 mmol) and 2-(pyrrol-1-yl)benzaldehyde (**1a**) (0.15 g, 0.90 mmol) in CH_2Cl_2 (5 mL). After stirring for 4 h at the same temperature, the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with saturated aqueous $NaHCO_3$ and then brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a residue, which was

purified by column chromatography on silica gel to give the title compound **3a** (0.16 g, 57%); a pale-yellow oil; R_f 0.28 (1:3 CH_2Cl_2/hexane); IR (neat) 3306, 1601 cm^{-1}; ^1H NMR δ 2.41 (3H, s), 2.98 (6H, s), 5.99 (1H, dd, J=3.6, 1.3 Hz), 6.30 (1H, br s), 6.60 (1H, dd, J=3.6, 3.0 Hz), 6.73 (1H, d, J=7.9 Hz), 6.90 (1H, td, J=7.9, 1.0 Hz), 7.01 (1H, dd, J=7.9, 7.3 Hz), 7.20 (1H, d, J=7.3 Hz), 7.25–7.45 (2H, m), 7.77 (1H, dd, J=3.0, 1.3 Hz), 7.8–7.95 (2H, m); MS m/z 315 (M^+, 100). Calcd for $C_{21}H_{21}N_3$: C, 79.97; H, 6.71; N, 13.32. Found: C, 79.92; H, 6.62; N, 13.36.

3.3.2. 5-Diethylamino-4-[(2-methylphenyl)amino]pyrrolo[1,2-*a*]quinoline (3b). A pale-yellow oil; R_f 0.28 (1:1 CH_2Cl_2/hexane); IR (neat) 3288, 1602 cm^{-1}; ^1H NMR δ 1.05 (6H, t, J=7.3 Hz), 2.42 (3H, s), 3.2–3.4 (4H, m), 5.98 (1H, dd, J=4.0, 1.6 Hz), 6.61 (1H, dd, J=4.0, 2.6 Hz), 6.74 (1H, d, J=7.9 Hz), 6.85–7.05 (3H, m), 7.21 (1H, d, J=6.9 Hz), 7.25–7.4 (2H, m), 7.74 (1H, dd, J=7.9, 1.6 Hz), 7.79 (1H, dd, J=2.6, 1.6 Hz), 7.90 (1H, dd, J=7.9, 1.3 Hz); MS m/z 343 (M^+, 100). HRMS Calcd for $C_{23}H_{25}N_3$: M, 343.2048. Found: m/z 343.2052.

3.3.3. 5-Dimethylamino-4-(phenylamino)pyrrolo[1,2-*a*]quinoline (3d). A pale-yellow oil; R_f 0.62 (1:1 CH_2Cl_2/hexane); IR (neat) 3296, 1601 cm^{-1}; ^1H NMR δ 2.97 (6H, s), 6.11 (1H, dd, J=4.0, 1.6 Hz), 6.53 (1H, br s), 6.63 (1H, dd, J=4.0, 2.6 Hz), 6.85–6.95 (3H, m), 7.21 (2H, t, J=7.9 Hz), 7.3–7.45 (2H, m), 7.77 (1H, dd, J=2.6, 1.6 Hz), 7.86 (1H, dd, J=7.6, 1.6 Hz), 7.89 (1H, dd, J=7.9, 1.3 Hz); MS m/z 301 (M^+, 100). Calcd for $C_{20}H_{19}N_3$: C, 79.70; H, 6.35; N, 13.94. Found: C, 79.59; H, 6.49; N, 13.59.

3.3.4. 5-Diethylamino-4-(phenylamino)pyrrolo[1,2-*a*]quinoline (3e). A pale-yellow oil; R_f 0.54 (2:3 CH_2Cl_2/hexane); IR (neat) 3315, 1602 cm^{-1}; ^1H NMR δ 1.03 (6H, t, J=7.3 Hz), 3.15–3.4 (4H, m), 6.08 (1H, dd, J=4.0, 1.6 Hz), 6.64 (1H, dd, J=4.0, 2.6 Hz), 6.9–7.0 (3H, m), 7.15–7.45 (5H, m), 7.77 (1H, dd, J=7.9, 1.3 Hz), 7.80 (1H, dd, J=2.6, 1.6 Hz), 7.91 (1H, dd, J=8.2, 1.3 Hz); MS m/z 329 (M^+, 100). Calcd for $C_{22}H_{23}N_3$: C, 80.21; H, 7.04; N, 12.76. Found: C, 80.14; H, 7.23; N, 12.54.

3.3.5. 5-Dimethylamino-4-(1,1-dimethylethylamino)pyrrolo[1,2-*a*]quinoline (3i). A pale-yellow oil; R_f 0.54 (1:5 AcOEt/hexane); IR (neat) 3293, 1602 cm^{-1}; ^1H NMR δ 1.37 (9H, s), 2.99 (6H, s), 6.35 (1H, br s), 6.70 (1H, dd, J=4.0, 3.0 Hz), 6.77 (1H, dd, J=4.0, 1.6 Hz), 7.2–7.4 (2H, m), 7.66 (1H, dd, J=7.9, 1.3 Hz), 7.75 (1H, dd, J=3.0, 1.6 Hz), 7.85 (1H, dd, J=7.9, 1.3 Hz); MS m/z 281 (M^+, 94), 183 (100). Calcd for $C_{18}H_{23}N_3$: C, 76.83; H, 8.24; N, 14.93. Found: C, 76.70; H, 8.24; N, 14.94.

3.3.6. 7,8-Dimethoxy-5-dimethylamino-4-(phenylamino)-pyrrolo[1,2-*a*]quinoline (3j). Preparation of this compound was carried out at 0 °C overnight. A pale-yellow solid; mp 183 °C (hexane/CH_2Cl_2); IR (KBr disk) 3380, 1620, 1600 cm^{-1}; ^1H NMR δ 2.96 (6H, s), 4.00 (3H, s), 4.04 (3H, s), 6.03 (1H, br s), 6.13 (1H, dd, J=4.0, 1.3 Hz), 6.64 (1H, dd, J=4.0, 3.0 Hz), 6.8–6.9 (3H, m), 7.19 (2H, dd, J=8.3, 7.6 Hz), 7.31 (1H, s), 7.40 (1H, s), 7.63 (1H, dd, J=3.0, 1.3 Hz); MS m/z 361 (M^+, 100). Calcd for $C_{22}H_{23}N_3O_2$: C, 73.11; H, 6.41; N, 11.63. Found: C, 73.08; H, 6.57; N, 11.39.

3.3.7. 7-Chloro-5-dimethylamino-4-phenylaminopyrrolo[1,2-*a*]quinoline (3k). A pale-yellow oil; R_f 0.36 (1:3 AcOEt/hexane); IR (neat) 3388, 1602 cm^{-1}; ^1H NMR δ 2.96 (6H, s), 6.10 (1H, dd, J=3.6, 1.3 Hz), 6.53 (1H, br s), 6.62 (1H, dd, J=3.6, 3.0 Hz), 6.9–7.0 (3H, m), 7.23 (2H, dd, J=8.3, 7.6 Hz), 7.34 (1H, dd, J=8.9, 2.3 Hz), 7.72 (1H, dd, J=3.0, 1.3 Hz), 7.75–7.85 (2H, m); MS m/z 335 (M^+, 100). Calcd for $C_{20}H_{18}ClN_3$: C, 71.53; H, 5.40; N, 12.51. Found: C, 71.52; H, 5.52; N, 12.50.

3.4. Typical procedure for the preparation of 4,5-diaminopyrrolo[1,2-*a*]quinoline derivatives 3 under Method B

3.4.1. 4-[(2-Methylphenyl)amino]-5-piperidinopyrrolo-[1,2-*a*]quinoline (3c). To a stirred mixture of NaI (0.34 g, 2.3 mmol), triethylamine hydrochloride (0.15 g, 1.1 mmol), pyrrolidine (72 mg, 1.0 mmol), Et_3N (0.22 g, 2.2 mmol), and Me_3SiCl (0.26 g, 2.4 mmol) in acetonitrile (2.2 mL) at room temperature was added a solution of *o*-tolyl isocyanide (**2a**) (0.12 g, 1.0 mmol) and 2-(pyrrol-1-yl)benzaldehyde (**1a**) (0.16 g, 1.0 mmol) in CH_2Cl_2 (5 mL). After stirring for 4 h at the same temperature, the reaction mixture was worked up in a manner similar to that described for the preparation of **3a**. Chromatographic separation of the crude product gave the title compound **3c** (0.21 g, 59%); a pale-yellow oil; R_f 0.63 (1:1 CH_2Cl_2/hexane); IR (neat) 3288, 1605 cm^{-1}; ^1H NMR δ 1.55–1.75 (6H, m), 2.43 (3H, s), 3.0–3.1 (2H, m), 3.3–3.4 (2H, m), 6.05 (1H, dd, J=4.0, 1.6 Hz), 6.40 (1H, br s), 6.61 (1H, dd, J=4.0, 2.6 Hz), 6.68 (1H, d, J=7.9 Hz), 6.86 (1H, t, J=8.6 Hz), 6.97 (1H, t, J=8.6 Hz), 7.19 (1H, d, J=7.9 Hz), 7.25–7.45 (2H, m), 7.76 (1H, dd, J=2.6, 1.6 Hz), 7.87 (1H, dd, J=7.9, 1.3 Hz), 7.98 (1H, dd, J=7.9, 1.3 Hz); MS m/z 355 (M^+, 86), 298 (100). Calcd for $C_{24}H_{25}N_3$: C, 81.09; H, 7.09; N, 11.82. Found: C, 80.95; H, 7.11; N, 11.82.

3.4.2. 4-Phenylamino-5-(pyrrolidin-1-yl)pyrrolo[1,2-*a*]quinoline (3f). A pale-yellow solid; mp 130–132 °C (hexane); IR (KBr disk) 3405, 1600 cm^{-1}; ^1H NMR δ 2.05–2.15 (4H, m), 3.25–3.35 (4H, m), 6.09 (1H, dd, J=4.0, 1.6 Hz), 6.63 (1H, dd, J=4.0, 3.0 Hz), 6.66 (1H, br s), 6.9–7.0 (3H, m), 7.21 (2H, d, J=8.2 Hz), 7.31 (1H, td, J=7.9, 1.3 Hz), 7.40 (1H, td, J=7.9, 1.3 Hz), 7.66 (1H, dd, J=7.9, 1.3 Hz), 7.79 (1H, dd, J=3.0, 1.6 Hz), 7.91 (1H, dd, J=7.9, 1.3 Hz); ^{13}C NMR δ 26.51, 51.09, 103.54, 111.92, 112.30, 114.75, 119.65 (two overlapped C's), 121.18, 122.83, 123.17, 123.98, 125.29, 127.44, 128.69, 131.87, 132.06, 144.77; MS m/z 327 (M^+, 100). Calcd for $C_{22}H_{21}N_3$: C, 80.70; H, 6.46; N, 12.83. Found: C, 80.69; H, 6.44; N, 12.54.

3.4.3. 4-Phenylamino-5-piperidinopyrrolo[1,2-*a*]quinoline (3g). A pale-yellow oil; R_f 0.59 (2:3 CH_2Cl_2/hexane); IR (neat) 3360, 1601 cm^{-1}; ^1H NMR δ 1.6–1.8 (6H, m), 3.0–3.2 (2H, m), 3.3–3.5 (2H, m), 6.14 (1H, dd, J=4.0, 1.3 Hz), 6.40 (1H, br s), 6.63 (1H, dd, J=4.0, 3.0 Hz), 6.85–6.95 (3H, m), 7.20 (2H, t, J=8.2 Hz), 7.33 (1H, td, J=8.2, 1.3 Hz), 7.43 (1H, td, J=8.2, 1.3 Hz), 7.77 (1H, dd, J=3.0, 1.3 Hz), 7.88 (1H, d, J=8.2 Hz), 8.02 (1H, dd, J=8.2, 1.3 Hz); MS m/z 341 (M^+, 68), 284 (100). HRMS Calcd for $C_{23}H_{23}N_3$: M, 341.1892. Found: m/z 341.1901.

3.4.4. 5-Morpholino-4-(phenylamino)pyrrolo[1,2-*a*]-quinoline (3h). A pale-yellow solid; mp 155–159 °C (hexane/Et_2O); IR (KBr disk) 3348, 1602 cm^{-1}; ^1H NMR δ 3.1–3.5 (4H, m), 3.7–4.0 (4H, m), 6.17 (1H, dd, J=4.0, 1.5 Hz), 6.23 (1H, br s), 6.64 (1H, dd, J=4.0, 2.9 Hz), 6.8–6.95 (3H, m), 7.20 (2H, t, J=8.2 Hz), 7.35 (1H, td, J=8.1, 1.1 Hz), 7.46 (1H, td, J=8.1, 1.1 Hz), 7.79 (1H, dd, J=2.9, 1.5 Hz), 7.89 (1H, dd, J=8.1, 1.1 Hz), 8.08 (1H, dd, J=8.1, 1.1 Hz); MS m/z 343 (M^+, 100). Calcd for $C_{22}H_{21}N_3O$: C, 76.94; H, 6.16; N, 12.24. Found: C, 76.85; H, 6.08; N, 12.06.

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Synthesis of 1-*C*-alkyl-α-D-glucopyranosides by Lewis acid- or Brønsted acid-catalyzed O-glycosidation

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Abstract—We prepared several kinds of 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose derivatives containing methyl, ethyl, *n*-butyl, and benzyl groups as the alkyl groups at their anomeric positions. The Lewis acid- or Brønsted acid-catalyzed O-glycosidations using them as the glycosyl donors to synthesize 1-*C*-alkyl-D-glucopyranosides were investigated. Using 10 mol % of triphenylmethyl perchlorate efficiently catalyzed the glycosidation of 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl dimethylphosphinothioate. The glycosidation using the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl acetates smoothly proceeded in the presence of only 5 mol % of scandium(III) trifluoromethanesulfonate. The dehydration–condensation type glycosidation using the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoses was significantly promoted using 5 mol % of bis(trifluoromethane)sulfonimide. These glycosidations successfully afforded various 1-*C*-alkyl-α-D-glucopyranosides in good yields with high α-stereoselectivities.

1. Introduction

The 1-*C*-alkyl-sugars, which have alkyl groups at their anomeric carbon centers, are considered to be a novel class of artificial ketoses, which replace naturally occurring aldoses. Their glycosylated compounds, i.e., 1-*C*-alkyl-glycosides, are expected to show biological functions different from those of natural compounds.[1] Therefore, considerable attention has been paid to developing glycosidation methods for synthesizing the 1-*C*-alkyl-*O*-glycosides.[2]

The reported methods for synthesizing the 1-*C*-alkyl-*O*-hexopyranosides involve glycosidations using the *exo*-glycal or 1-*C*-alkyl-sugar derivatives as the glycosyl donors. Although a few *exo*-glycals are conveniently utilized as the glycosyl donors in both enzymatic and chemical glycosidations,[3] there appears to be limitations in the use of the *exo*-glycals due to their synthetic difficulty. On the other hand, various kinds of 1-*C*-alkyl-sugar derivatives could be readily prepared by the reactions of the corresponding glycono-1,5-lactones with organometallic reagents such as organolithium reagents or Grignard reagents.[4] To the best of our knowledge, however, only a few O-glycosidations using 1-*C*-alkyl-hexopyranose derivatives as the glycosyl donors have ever been reported.[5]

We previously studied the glycosidations using 1-*O*-dimethylphosphinothioyl sugars[6] and 1-*O*-acetyl sugars[7] as the glycosyl donors. The former study showed that the glycosidations using the dimethylphosphinothioyloxy function as the leaving group were activated by a catalytic amount of triphenylmethyl perchlorate ($TrtClO_4$). In the latter study, ytterbium(III) triflate ($Yb(OTf)_3$) was found to be an effective activator for the glycosidations using the acetoxy function as the leaving group.

Our recent interest in the formation reaction of 1-*C*-alkyl-hexopyranosidic linkages by the O-glycosidation prompted us to start an investigation of the catalytic synthesis of 1-*C*-alkyl-hexopyranosides using our newly developed glycosidation methods. Part of the study was reported in a preliminary letter about the synthesis of 1-*C*-alkyl-glucopyranosides by the glycosidation of the 1-*C*-alkyl-D-glucopyranosyl donors having the acetoxy function as a leaving group. The reaction was activated by a catalytic amount of

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scandium(III) triflate ($Sc(OTf)_3$).[8] The following letter describes the Brønsted acid-catalyzed glycosidation used to synthesize 1-*C*-alkyl-glucopyranosides. During the glycosidation, the anomeric hydroxyl function of the glycosyl donors, the 1-*C*-alkyl-D-glucopyranoses, was conveniently utilized as the leaving group.[9] In these letters were mentioned some of the interesting glycosidation properties based on these 1-*C*-alkyl-D-glucopyranosyl donor's reactivities. However, we considered that the 1-*C*-alkyl-hexopyranosyl donors would have some unknown reactivities and their elucidation would be important for their utilization in synthetic carbohydrate chemistry.

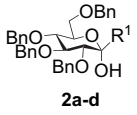
In order to develop efficient synthetic methods for producing 1-*C*-alkyl-hexopyranosides, in this full paper, we summarize the glycosidations using the glycosyl donors of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl dimethylphosphinothioates, 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl acetates, and 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranoses in the presence of catalytic amounts of Lewis acids or Brønsted acids as the activators.

2. Results and discussion

2.1. Glycosidation of the 1-*C*-alkyl-D-glucopyranosyl dimethylphosphinothioate derivatives

We describe the preparation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl dimethylphosphinothioates and their catalytic O-glycosidation to synthesize the 1-*C*-alkyl-D-glucopyranosides.

2.1.1. Preparation of 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoses. According to the reported method, several 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoses (R^1=alkyl; methyl: **a**, ethyl: **b**, *n*-butyl: **c**, benzyl: **d**) were prepared in high yields by the addition of the RLi or RMgX reagent to 2,3,4,6-tetra-*O*-benzyl-D-glucono-1,5-lactone (**1**)[10] (Scheme 1; Table 1).



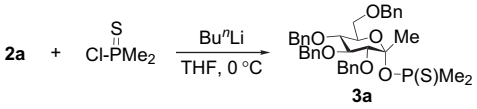
2a-d

Scheme 1.

Table 1. 1-*C*-Alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose

Entry	R^1	1-*C*-Alkyl-D-glucopyranose derivatives
1	Me	**2a**
2	Et	**2b**
3	Bu^n	**2c**
4	CH_2Ph	**2d**

2.1.2. Preparation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl dimethylphosphinothioates. We examined the preparation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl dimethylphosphinothioates from **2a–d**, according to our previously reported method for preparing the aldopyranosyl dimethylphosphinothioates.[6] The reaction of **2a** with dimethylphosphinothioyl (Mpt) chloride (1.2 equiv) using Bu^nLi (1.2 equiv) in THF at 0 °C produced 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl dimethylphosphinothioate (**3a**) in 55% yield (Scheme 2). However, similar reaction conditions using **2b** and **2c** gave the corresponding dimethylphosphinothioates in very poor yields. It is possible that the steric hindrances on the anomeric centers of **2b** and **2c** and the bulkiness of Mpt-Cl prevented introduction of the Mpt group into **2b** and **2c**. Compound **3a** was not as stable as the aldopyranosyl dimethylphosphinothioates that we had previously prepared. Therefore, **3a** was used in the glycosidation reaction immediately after the purification using thin-layer chromatography.

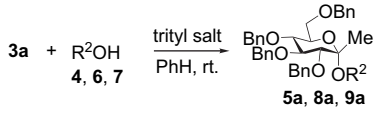


3a

Scheme 2.

2.1.3. Glycosidation of 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl dimethylphosphinothioate (3a).[11] The reactivity of **3a** as a glycosyl donor was expected to be high because the electron donating effect of the methyl group in **3a** would stabilize the glycosyl cation intermediate generated from **3a** in spite of its tertiary anomeric carbon center's steric hindrance.

Since our former research showed that the trityl salts were efficient activators for the highly reactive glycosyl donors such as the 2-deoxy-glycopyranosyl dimethylphosphinothioates, we used the trityl salts as the activators of **3a** in this glycosidation study. When **3a** was glycosylated with 3β-cholestanol (**4**) using 10 mol % of $TrtClO_4$ in benzene at room temperature (Scheme 3), the corresponding 1-*C*-methyl-D-glucopyranoside derivative (**5a**) was successfully obtained in high yield of 88% with an α-stereoselectivity. Even the reaction using only 5 mol % of $TrtClO_4$ could afford **5a** in 71% yield. When other trityl salts were used, the reactions using 10 mol % of triphenylmethyl hexachloroantimonate ($TrtSbCl_6$), triphenylmethyl tetrafluoroborate ($TrtBF_4$), and triphenylmethyl pentachlorotin ($TrtSnCl_5$) afforded **5a** in moderate yields from 34 to 43%. The maximum yield was attained from the reaction using 10 mol % of $TrtClO_4$. Although the combined use of I_2 and 10 mol % of $TrtClO_4$ was found to be an effective activating system for the glycosidation of several aldopyranosyl dimethylphosphinothioates, the reaction between **3a** and **4** using this activating system did not produce **5a** at all, but gave an unknown product.



5a, **8a**, **9a**

Scheme 3.

Next, the glycosidation of **3a** with *n*-octanol (**6**) or 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**7**) as the

Table 2. The glycosidation of **3a** with alcohols (**4**, **6**, and **7**) in the presence of trityl salts[a]

Entry	Trityl salt (mol %)	Alcohol	Product	Yield (%)
1	$TrtClO_4$ (10)	**4**	**5a**	88
2	$TrtClO_4$ (5)	**4**	**5a**	71
3	$TrtSbCl_6$ (10)	**4**	**5a**	43
4	$TrtBF_4$ (10)	**4**	**5a**	34
5	$TrtPF_6$ (10)	**4**	**5a**	No reaction
6	$TrtSnCl_5$ (10)	**4**	**5a**	43
7	$TrtClO_4$ (10)	**6**	**8a**	82
8	$TrtClO_4$ (10)	**7**	**9a**	82[b]

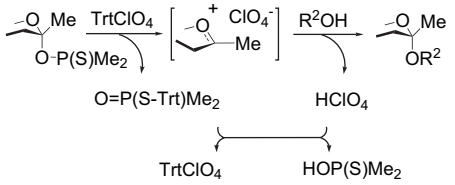
[a] Reaction conditions: molar ratio, **3a**:alcohol=1:1; reaction time, 1 h; reaction temperature, rt.
[b] The α/β ratio of glycoside was 70/30.

glycosyl acceptor was performed under similar reaction conditions using 10 mol % of $TrtClO_4$ in benzene. The corresponding *n*-octyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (**8a**) was obtained in 82% yield with an α-stereoselectivity, and 6-*O*-(2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**9a**) was also obtained in 82% yield with an α/β ratio=70/30. The β-form of **9a** would be partially formed by the S_N2-like reaction mechanism. Judging from the glycosidation yields, **3a** expectedly worked as a highly reactive glycosyl donor in spite of its sterically hindered tertiary anomeric carbon center. These results are summarized in Table 2 (Fig. 1). The expected reaction mechanism is indicated in Scheme 4.

We found that the glycosidation of **3a** with several alcohols in the presence of 10 mol % of $TrtClO_4$ in benzene at room temperature smoothly proceeded to afford the 1-*C*-methyl-D-glucopyarnosides in good yields with high α-stereoselectivities.

2.2. Glycosidation of the 1-*C*-alkyl-D-glucopyranosyl acetate derivatives

In order to establish the method for producing the 1-*C*-alkyl-D-glucopyranosides having various kinds of alkyl groups, we



Scheme 4.

investigated the glycosidation of the 1-*C*-alkyl-D-glucopyranosyl donors having an acetoxy function as a leaving group using Lewis acids as activators. As the acetyl group was less bulky than the Mpt group, the acetyl group was expected to be smoothly introduced into **2a–d** in order to prepare the corresponding 1-*C*-alkyl-D-glucopyranosyl acetates. We describe the preparation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl acetates and the synthesis of various 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosides by the Lewis acid-catalyzed O-glycosidation using them as the glycosyl donors.

2.2.1. Preparation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl acetates. We investigated the preparation of 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-D-glucopyranosyl acetate (**10a**)[12] by the acetylation of **2a** (Scheme 5). Although the ordinary acetylation of **2a** using Ac_2O/pyridine could not give **10a** at all, the use of Bu^{n}Li as a strong base in lieu of pyridine was effective. Compound **10a** was obtained in 69% yield by the reaction of **2a** with Bu^{n}Li followed by the addition of Ac_2O in THF at −78 °C. In the next experiment, the reaction temperature was gradually raised as follows. After the reaction of **2a** with Bu^{n}Li at −78 °C, Ac_2O was added to the reaction mixture at −30 °C, and the reaction was quenched at 0 °C. The yield of **10a** increased to 80%.[13]

As another convenient synthetic route, the one-pot synthesis of **10a** from **1** was examined (Scheme 5). When the reaction

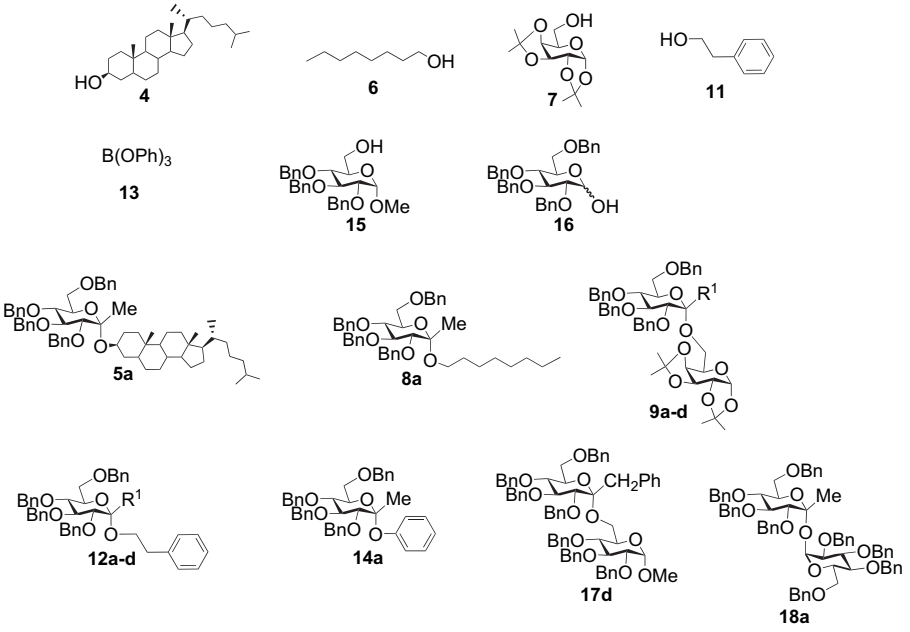
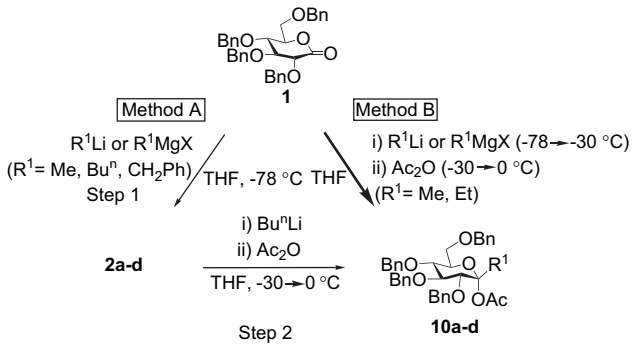


Figure 1. The acceptors utilized in the glycosidations and glycosides synthesized.



Scheme 5.

of **1** with MeLi was carried out at −78 °C in THF and Ac_2O was added at −30 °C, followed by quenching the reaction at 0 °C, **10a** was obtained in 73% yield.

Furthermore, we examined the preparation of the 2,3,4,6-tetra-*O*-benzyl-1-*C*-ethyl-α-D-glucopyranosyl acetate (**10b**), 2,3,4,6-tetra-*O*-benzyl-1-*C*-*n*-butyl-α-D-glucopyranosyl acetate (**10c**), and 1-*C*-benzyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl acetate (**10d**). The acetate **10b–d** were prepared in good yields with α-stereoselectivities by the acetylation of **2c** and **2d** using Bu^{n}Li and Ac_2O or by the one-pot synthesis from **1** using EtMgBr and Ac_2O (Scheme 5). These results are summarized in Table 3.

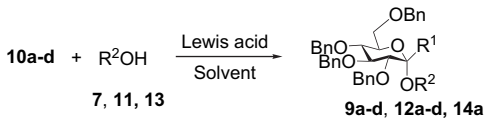
Table 3. Preparation of **10a–d**

Entry	Starting sugar derivative	Method[a]	Product	Yield (%)
1	**2a**	A	**10a**	80 (69)[b]
2	**1**	B	**10a**	73
3	**1**	B	**10b**	86
4	**2c**	A	**10c**	88
5	**2d**	A	**10d**	81

[a] Method A: after the reaction of **2a** (**2c** or **2d**) with Bu^{n}Li (1.1 equiv) was carried out in THF at −78 °C, Ac_2O (6 equiv) was added to the reaction mixture at −30 °C and the reaction was quenched by satd $NaHCO_3$ solution at 0 °C; Method B: after the reaction of **1** with MeLi or EtMgBr (1.2 equiv) was carried out in THF at −78 °C, Ac_2O (6 equiv) was added to the reaction mixture at −30 °C and the reaction was quenched by satd $NaHCO_3$ solution at 0 °C.

[b] The reaction was performed at −78 °C.

2.2.2. Glycosidation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl acetates (10a–d). We examined in detail the glycosidation of **10a** with phenethyl alcohol (**11**) in the presence of various Lewis acids (Scheme 6). When 5 mol % of a Lewis acid such as $Yb(OTf)_3$, $Sc(OTf)_3$, TMSOTf, $TrtClO_4$, and $BF_3\cdot OEt_2$ was used as the activator in dichloromethane at 0 °C, each Lewis acid could activate the glycosidation to give the corresponding glycoside **12a** in 67–80% yields with an α-stereoselectivity. $Yb(OTf)_3$ and $Sc(OTf)_3$ were especially effective for the activation of



Scheme 6.

10a. Even the reaction using only 1 mol % of $Sc(OTf)_3$ could afford **12a** in the yield of 73%, and only this reaction produced a small amount of the β-isomer (α/β=81/19). The effect of the solvents was also examined using CH_2Cl_2, $PhCH_3$, and CH_3CN. The reaction using $PhCH_3$ increased the yield of **12a** up to 89%, however, these solvents did not influence the glycosidation stereoselectivities at all.

Furthermore, we examined the glycosidation of the 1-*C*-alkyl-D-glucopyranosyl acetates (**10a–d**) with **11** or **7** under similar reaction conditions. The reactions of **10a–d** with **11** or **7** in the presence of 5 mol % of $Sc(OTf)_3$ in $PhCH_3$ stereoselectively afforded the corresponding phenethyl 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosides (**12b–d**) or 6-*O*-(1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoses (**9a–d**) in the yields from 73 to 89%, respectively.

Our recent study indicated that triaryloxyboranes worked as the reactive glycosyl acceptors of glycosyl acetates in the presence of a catalytic amount of $Yb(OTf)_3$ to afford the corresponding aryl O-glycosides.[7c] Therefore, the synthesis of phenyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (**14a**) was attempted by the reaction of **10a** with triphenoxyborane (**13**) using 5 mol % of $Yb(OTf)_3$ in CH_2Cl_2 at −78 °C. The desired **14a** was obtained in 80% yield with an α-stereoselectivity. These results are shown in Table 4 (Fig. 1). The proposed reaction mechanism is shown in Scheme 7.

Table 4. Synthesis of various 1-*C*-alkyl-α-D-glucopyranosides by the glycosidation of **10a–d** with acceptors (**7**, **11**, and **13**)[a]

Entry	Acetate	Alcohol	Activator	Solvent	Product	Yield (%)[b]
1	**10a**	**11**	$Yb(OTf)_3$	CH_2Cl_2	**12a**	76
2	**10a**	**11**	$Sc(OTf)_3$	CH_2Cl_2	**12a**	80
3	**10a**	**11**	TMSOTf	CH_2Cl_2	**12a**	69
4	**10a**	**11**	$TrtClO_4$	CH_2Cl_2	**12a**	74
5	**10a**	**11**	$BF_3\cdot OEt_2$	CH_2Cl_2	**12a**	67
6[c]	**10a**	**11**	$Sc(OTf)_3$	CH_2Cl_2	**12a**	73[d]
7	**10a**	**11**	$Sc(OTf)_3$	$PhCH_3$	**12a**	89
8	**10a**	**11**	$Sc(OTf)_3$	CH_3CN	**12a**	63
9	**10b**	**11**	$Sc(OTf)_3$	$PhCH_3$	**12b**	86
10	**10c**	**11**	$Sc(OTf)_3$	$PhCH_3$	**12c**	75
11	**10d**	**11**	$Sc(OTf)_3$	$PhCH_3$	**12d**	77
12	**10a**	**7**	$Sc(OTf)_3$	$PhCH_3$	**9a**	82
13[c]	**10a**	**7**	$Sc(OTf)_3$	$PhCH_3$	**9a**	87[e]
14	**10b**	**7**	$Sc(OTf)_3$	$PhCH_3$	**9b**	77
15	**10c**	**7**	$Sc(OTf)_3$	$PhCH_3$	**9c**	73
16	**10d**	**7**	$Sc(OTf)_3$	$PhCH_3$	**9d**	74
17[f]	**10a**	**13**	$Yb(OTf)_3$	CH_2Cl_2	**14a**	80

[a] Reaction conditions: molar ratio, **10a–d**:**11**:activator=1:1:0.05; **10a–d**:**7**:activator=1.5:1:0.075; reaction time, 1–3 h; reaction temperature, 0 °C.

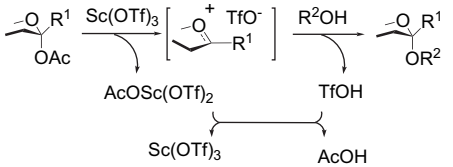
[b] Only the α-glycoside was obtained.

[c] $Sc(OTf)_3$ (1 mol %) was used.

[d] The α/β ratio of glycoside was 81/19.

[e] The α/β ratio of glycoside was 85/15.

[f] Reaction conditions: molar ratio, **10a**:**13**:activator=1:0.5:0.05; reaction time, 3 h; reaction temperature, −78 °C.



Scheme 7.

Inanaga et al. reported that lanthanide triflates could not activate at all the glycosidation using 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl acetate as the glycosyl donor.[14] This is quite different from our observations that any glycosidation using **10a–d** smoothly proceeded in the presence of only 5 mol % of $Sc(OTf)_3$. These results showed that **10a–d** indicated a much higher reactivity than the 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl acetate as the glycosyl donor and the existence of the alkyl groups on **10a–d** remarkably increased their glycosyl donor's reactivities. Moreover, the species of the alkyl groups at the anomeric carbon centers of **10a–d** had almost no influence on the reactivities and stereoselectivities of the glycosidation. The reactivities of **10a–d** were similar to those of **3a**.

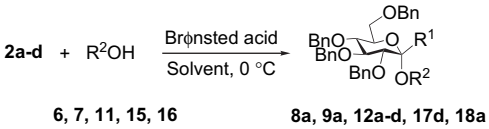
The high α-stereoselectivity of the glycosidation could be explained by the anomeric effect and the disadvantageous formation of β-glycosides due to the 1,3-diaxial interaction. It was also possible that the anomerization might occur and 1-*C*-alkyl-β-glucopyranosides might be converted to the corresponding α-glucopyranosides in the reaction system. In order to examine this point, the β-glycoside of **9a** (Table 4, entry 13) was added to the reaction in the presence of 10 mol % of $Sc(OTf)_3$ in toluene at room temperature overnight. No anomerization was observed in the reaction.

We found that **10a–d** worked as good glycosyl donors in the presence of only 5 mol % of $Sc(OTf)_3$ in toluene at 0 °C to afford various 1-*C*-alkyl-D-glucopyranosides in good yields with high α-stereoselectivities.

2.3. Glycosidation of the 1-*C*-alkyl-α-D-glucopyranose derivatives

In order to develop a more convenient synthetic method for producing 1-*C*-alkyl-D-glucopyranosides, the direct glycosidation of the 1-*C*-alkyl-D-glucopyranoses **2a–d** without introducing any other leaving group was examined. We describe the dehydration–condensation type glycosidation of **2a–d** with alcohols using a catalytic amount of Brønsted acids as the activators.

2.3.1. Glycosidation of the 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoses (2a–d). We investigated the dehydration–condensation type glycosidation using **2a** as a glycosyl donor and **11** as a glycoyl acceptor (Scheme 8). Brønsted acids were used as the activators for this glycosidation because they were expected to be potentially resistant to water. As the Brønsted acids, 5 mol % of camphorsulfonic acid, trifluoroacetic acid (CF_3CO_2H), and TfOH were used in dichloromethane at 0 °C in the presence of a drying agent, Drierite (anhydrous $CaSO_4$). Only TfOH could effectively activate the glycosidation to stereoselectively give the corresponding **12a** in 59% yield. The use of acetonitrile as the solvent slightly increased the yield of **12a** up to 73% with an α-stereoselectivity. Heptadecafluorooctanesulfonic acid



Scheme 8.

($C_8F_{17}SO_3H$) and bis(trifluoromethane)sulfonimide (Tf_2NH) as the Brønsted acid analogs of TfOH and tetrafluoroboric acid (HBF_4) were similarly effective for the glycosidation of **2a** under similar reaction conditions. Particularly, Tf_2NH gave **12a** in the maximum yield of 77%. Although 10 mol % of Tf_2NH hardly increased the yield of **12a**, the reaction even using 1 mol % of Tf_2NH gave **12a** in 56% yield.

We also investigated the glycosidation of **2b–d** with **11** in acetonitrile at 0 °C using 5 mol % of Tf_2NH in order to investigate how the difference in the alkyl groups at the anomeric carbon centers would influence the reactivity and stereoselectivity of the glycosidation. The desired **12b–d** were then obtained in yields ranging from 56 to 64% with high α-stereoselectivities. The difference in the alkyl groups at the anomeric carbon centers of **2a–d** had almost no influence on the glycosidation reactivity and stereoselectivity. This is similar to the above-mentioned glycosidation properties using **10a–d**.

Furthermore, we examined the glycosidation of **2a** (or **2d**) with **6**, **7**, and methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**15**) in acetonitrile at 0 °C using 5 mol % of Tf_2NH or TfOH. The desired **8a**, **9a**, and methyl 6-*O*-(1-*C*-benzyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**17d**) were stereoselectively obtained in good yields of 78, 55, and 66%, respectively. Interestingly, even when the anomeric mixture of **16** was used as the glycosyl acceptor, the trehalose analog (**18a**), the product by the reaction of **2a** with **16** was obtained as a single isomer based on its NMR spectrum. In the ^1H NMR spectrum of **18a**, the anomeric proton of the glucopyranosyl residue was observed at 5.34 ppm with a doublet peak (J=3.4 Hz), and the value of the coupling constant indicated α. This suggested that the glycosidation strictly recognized the anomeric stereochemistry of the acceptor **16** and only the α-isomer of **16** was utilized as the glycosyl acceptor. These results are summarized in Table 5 (Fig. 1). The proposed glycosidation mechanism is indicated in Scheme 9.

We have successfully developed the direct dehydrative glycosidation using **2a–d** without introducing any other leaving group into them, and found that the glycosidation using **2a–d** was efficiently catalyzed using only 5 mol % of Tf_2NH or TfOH as the activators to afford various 1-*C*-alkyl-α-D-glucopyranosides in good yields.

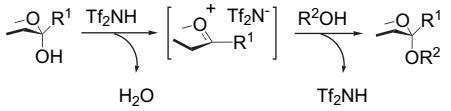
3. Determination of the anomeric configurations of 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl donors and 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-D-glucopyranosides

The anomeric configurations of all the 1-*C*-alkyl-D-glucopyranosyl donors (**2a–d**, **3a**, and **10a–d**) and 1-*C*-alkyl-D-glucopyranosides (**5a**, **8a**, **9a–d**, **12a–d**, **14a**, **17d**, and **18a**) were determined by their NMR spectra. The α-forms of them were determined by the measurement of the NOE interactions between H-2 and H-1′ of the alkyl groups of the 1-*C*-alkyl-D-glucopyranosyl rings as shown in Figure 2. The β-forms of **9a** and **12a** were determined by the measurements of the NOE interactions between H-3 (or H-5) and H-1′ of the methyl groups. Furthermore, the anomeric

Table 5. The glycosidation of **2a–d** with various alcohols (**6**, **7**, **11**, **15**, and **16**) in the presence of Brønsted acid[a]

Entry	1-*C*-Alkyl-glucopyranose	Alcohol	Brønsted acid (mol %)	Solvent	Product	Yield (%)
1	**2a**	**11**	Camphorsulfonic acid (5)	CH_2Cl_2	**12a**	No reaction
2	**2a**	**11**	CF_3CO_2H (5)	CH_2Cl_2	**12a**	No reaction
3	**2a**	**11**	TfOH (5)	CH_2Cl_2	**12a**	59
4	**2a**	**11**	TfOH (5)	CH_3CN	**12a**	73
5	**2a**	**11**	$C_8F_{17}SO_3H$ (5)	CH_3CN	**12a**	56
6	**2a**	**11**	Tf_2NH (5)	CH_3CN	**12a**	77
7	**2a**	**11**	HBF_4 (5)	CH_3CN	**12a**	73
8	**2a**	**11**	Tf_2NH (10)	CH_3CN	**12a**	69
9	**2a**	**11**	Tf_2NH (3)	CH_3CN	**12a**	65
10	**2a**	**11**	Tf_2NH (1)	CH_3CN	**12a**	56
11	**2b**	**11**	Tf_2NH (5)	CH_3CN	**12b**	57
12	**2c**	**11**	Tf_2NH (5)	CH_3CN	**12c**	64
13	**2d**	**11**	Tf_2NH (5)	CH_3CN	**12d**	56
14	**2a**	**6**	Tf_2NH (5)	CH_3CN	**8a**	78
15[b]	**2a**	**7**	Tf_2NH (5)	CH_3CN	**9a**	55
16[b]	**2d**	**15**	TfOH (5)	CH_3CN	**17d**	66
17[b]	**2a**	**16**	Tf_2NH (5)	CH_3CN	**18a**	47

[a] Reaction conditions: molar ratio, **2a–d**:alcohol (**6** or **11**):Brønsted acid=1:1:0.05; reaction time, 2 h; reaction temperature, 0 °C.
[b] Reaction conditions: molar ratio, **2a**,**d**:alcohol (**7**, **15** or **16**):Brønsted acid=1.5:1:0.075; reaction time, 3 h; reaction temperature, 0 °C.



Scheme 9.

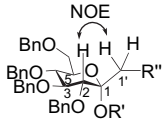


Figure 2. The determination of anomeric configurations of **3a**, **10a–d**, and glycosides.

configuration of **12a** could be determined by the NMR spectrum of the three bond coupling constants between the carbon of the 1′-*C*-methyl and the H-2 ($^3J_{C1',H2}$=2.5 Hz was α; $^3J_{C1',H2}$=1.5 Hz was β), according to the observations of Schlesselmann et al.[3e]

4. Conclusions

We have successfully developed the catalytic synthesis of various kinds of 1-*C*-alkyl-α-D-glucopyranosides carrying the methyl, ethyl, *n*-butyl, and benzyl groups as the alkyl groups at their anomeric positions by glycosidation using several 1-*C*-alkyl-D-glucopyranosyl donors with the dimethylphosphinothioyloxy, acetoxy, and hydroxyl functions as the leaving groups in the presence of $TrtClO_4$, $Sc(OTf)_3$, and Tf_2NH as the activators, respectively. We believe that this study can provide novel methods to synthesize biologically important neoglycoconjugates having 1-*C*-alkyl-sugar units.

5. Experimental

5.1. General

The NMR spectra were measured using an ECA-600 (JEOL) spectrometer at 600 MHz (^{1}H) and 150 MHz (^{13}C). The ^{1}H NMR chemical shifts are referenced to the internal standard TMS (δ_H=0.00). The ^{13}C NMR chemical shifts are referenced to the solvent signal (δ_C=77.0 for the central line of $CDCl_3$). The ESI-MS spectra were recorded on a Mariner (Applied Biosystems) spectrometer. Optical rotations were recorded using a JASCO DIP-360 digital polarimeter. All reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated plates (0.25 mm).

5.2. Preparation of 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl dimethylphosphinothioate (3a)

To a solution of **2a** (300 mg, 0.54 mmol) in dry THF (3 mL) at 0 °C was added a hexane solution of Bu^{n}Li (0.65 mmol) under an Ar atmosphere and stirred for 30 min. Mpt-Cl (88 mg, 0.69 mmol) was added to the solution, and stirred for 1 h at 0 °C. The reaction was then quenched by the addition of water. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and a satd NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica gel TLC (CH_2Cl_2/ethyl acetate/hexane=1/1/4) to give **3a** as a colorless oil (191 mg, 55%). $[\alpha]_D^{23}$ +48.8 (*c* 1.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.87 (3H, d, J_{HCCOP}=0.6 Hz, CH_3), 1.90 (3H, d, J_{HCP}=6.2 Hz, PCH_3), 1.92 (3H, d, J_{HCP}=6.2 Hz, PCH_3), 3.19 (1H, dd, *J*=9.6 Hz, J_{HCCOC}=4.1 Hz, H-2′), 3.92–3.80 (2H, m, H-6), 3.74 (1H, t, *J*=9.6 Hz, H-4), 3.85 (1H, t, *J*=9.6 Hz, H-3), 3.87–3.90 (1H, m, H-5); ^{13}C NMR ($CDCl_3$): δ 24.3 (J_{CCOP}=4.3 Hz), 25.5 (J_{CP}=73.7 Hz), 26.0 (J_{CP}=70.8 Hz), 68.3, 73.0, 73.5, 75.2, 75.5, 75.7, 78.8, 82.8, 84.3 (J_{CCOP}=5.8 Hz), 105.8 (J_{COP}=10.1 Hz); HRMS (ESI) *m*/*z* calcd for $C_{37}H_{43}O_6PS\cdot Na^+$: 669.2410; found: 669.2443.

5.3. Preparation of 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl acetates

5.3.1. Typical preparation by the acetylation of 1-*C*-alkyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoses. To a solution of **2a** (123 mg, 0.2 mmol) in dry THF (1 mL) at −78 °C was added a hexane solution of Bu^{n}Li (0.24 mmol) under an Ar atmosphere and the reaction temperature was raised to −30 °C for 1 h while stirring. Then, acetic

anhydride (0.3 mL) was added to the solution and the reaction temperature was gradually raised to 0 °C for 1 h. The reaction was then quenched by the addition of a satd $NaHCO_3$ solution (5 mL). The reaction mixture was extracted with $CHCl_3$, and the organic layer was washed with water and a satd NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica gel TLC (ethyl acetate/hexane=1/4) to give **10a** as a colorless oil (111 mg, 85%).

5.3.2. Typical preparation from 2,3,4,6-tetra-*O*-benzyl-D-glucono-1,5-lactone. To a solution of **1** (288.8 mg, 0.54 mmol) in dry THF (3 mL) at −78 °C was added a diethyl ether solution of MeLi (0.64 mmol) under an Ar atmosphere and the reaction temperature was raised to −30 °C for 1 h while stirring. Acetic anhydride (0.6 mL) was then added to the solution, and the reaction temperature was gradually raised to 0 °C for 1 h. The reaction was then quenched by the addition of a satd $NaHCO_3$ solution (5 mL). The reaction mixture was extracted with $CHCl_3$, and the organic layer was washed with water and a satd NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica gel TLC (ethyl acetate/hexane=1/4) to give **10a** as a colorless oil (233.7 mg, 73%).

5.3.3. 2,3,4,6-Tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl acetate (10a). $[\alpha]_D^{23}$ +45.2 (*c* 1.97, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.76 (3H, s, CH_3), 2.07 (3H, s, $C(O)CH_3$), 3.29 (1H, d, *J*=9.5 Hz, H-2), 3.55–3.58 (1H, m, H-5), 3.67 (1H, dd, *J*=3.2 Hz, *J*=11.1 Hz, H_a-6), 3.80 (1H, dd, *J*=3.2 Hz, *J*=11.1 Hz, H_b-6), 3.82 (1H, t, *J*=10.0 Hz, H-4), 4.02 (1H, t, *J*=9.3 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 22.1, 22.3, 68.1, 73.2, 73.5, 75.2, 75.5, 75.9, 77.6, 82.8, 84.0, 104.3, 168.7; HRMS (ESI) *m/z* calcd for $C_{37}H_{40}O_7\cdot Na^+$: 619.2672; found: 619.2602.

5.3.4. 2,3,4,6-Tetra-*O*-benzyl-1-*C*-ethyl-α-D-glucopyranosyl acetate (10b). Colorless oil; $[\alpha]_D^{23}$ +47.7 (*c* 2.45, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, *J*=1.6 Hz, CH_2CH_3), 2.05 (1H, m, $CH_aH_bCH_3$), 2.08 (3H, s, $C(O)CH_3$), 2.58 (1H, m, $CH_aH_bCH_3$), 3.52 (1H, d, *J*=8.9 Hz, H-2), 3.65 (1H, m, H-5), 3.70 (1H, dd, *J*=1.4 Hz, *J*=10.0 Hz, H_a-6), 3.80 (2H, m, H-4, H_b-6), 4.00 (1H, t, *J*=8.9 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 8.1, 22.3, 26.6, 68.4, 73.4, 73.5, 75.2, 75.3, 75.5, 77.8, 80.0, 83.1, 107.0, 168.7; HRMS (ESI) *m/z* calcd for $C_{38}H_{42}O_7\cdot Na^+$: 633.2823; found 633.2786.

5.3.5. 2,3,4,6-Tetra-*O*-benzyl-1-*C*-*n*-butyl-α-D-glucopyranosyl acetate (10c). Colorless oil; $[\alpha]_D^{23}$ +35.1 (*c* 7.16, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.83 (3H, t, *J*=7.6 Hz, $CH_2CH_2CH_2CH_3$), 1.15 (1H, m, $CH_2CH_aH_bCH_2CH_3$), 1.17 (1H, m, $CH_2CH_2CH_aH_bCH_3$), 1.25 (1H, m, $CH_2CH_2CH_aH_bCH_3$), 1.47 (1H, m, $CH_2CH_aH_bCH_2CH_3$), 2.02 (1H, m, $CH_aH_bCH_2CH_2CH_3$), 2.07 (3H, s, $C(O)CH_3$), 2.51 (1H, m, $CH_aH_bCH_2CH_2CH_3$), 3.51 (1H, d, *J*=8.9 Hz, H-2), 3.58 (1H, m, H-5), 3.68 (1H, dd, *J*=1.4 Hz, *J*=11.0 Hz, H_a-6), 3.77–3.82 (2H, m, H-4, H_b-6), 4.00 (1H, t, *J*=9.0 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 14.0, 22.3, 22.8, 25.9, 33.4, 68.4, 73.4, 73.4, 75.2, 75.3, 75.5, 77.8, 80.3, 83.2, 106.7, 168.7; HRMS (ESI) *m/z* calcd for $C_{40}H_{46}O_7\cdot Na^+$: 661.3136; found 661.3157.

5.3.6. 1-*C*-Benzyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl acetate (10d). Colorless oil; $[\alpha]_D^{23}$ +46.1 (*c* 1.37, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.14 (3H, s, CH_3), 3.25 (1H, d, *J*=13.7 Hz, CCH_aH_bPh), 3.30 (1H, d, *J*=8.9 Hz, H-2), 3.67–3.70 (1H, m, H-5), 3.73 (1H, t, *J*=9.0 Hz, H-4), 3.80 (1H, dd, *J*=2.0 Hz, *J*=11.0 Hz, H_a-6), 3.87 (1H, dd, *J*=2.8 Hz, *J*=11.0 Hz, H_b-6), 4.03 (1H, t, *J*=8.9 Hz, H-3), 4.20 (1H, d, *J*=13.8 Hz, CCH_aH_bPh); ^{13}C NMR ($CDCl_3$): δ 22.4, 39.9, 68.5, 73.3, 73.6, 74.5, 75.2, 75.5, 77.6, 79.5, 83.3, 106.8, 168.9; HRMS (ESI) *m/z* calcd for $C_{43}H_{44}O_7\cdot Na^+$: 695.2985; found: 695.2944.

5.4. Glycosidation

5.4.1. Typical glycosidation procedure using 3a as the glycosyl donor. To a stirred solution of $TrtClO_4$ (4 mg, 0.015 mmol) and 3β-cholestanol (58 mg, 0.15 mmol) in benzene (2 mL) at room temperature was added **3a** (96 mg, 0.15 mmol) in the presence of powdered 4 Å molecular sieves (ca. 100 mg). The resulting mixture was stirred for 1 h. The reaction was then quenched by the addition of a satd $NaHCO_3$ solution (5 mL), and the reaction mixture was filtered. The reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water and a satd NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica gel TLC (ethyl acetate/hexane=1/4) to give **5a** as a white solid (112 mg, 80%).

5.4.2. Typical glycosidation procedure using 10a–d as the glycosyl donors. To a stirred solution of $Sc(OTf)_3$ (4.4 mg, 0.0089 mmol) and phenethyl alcohol (20.7 mg, 0.17 mmol) in toluene was added **10a** (101.2 mg, 0.17 mmol) at 0 °C in the presence of Drierite (ca. 100 mg). The resulting mixture was stirred for 1 h. The reaction was then quenched by the addition of a satd $NaHCO_3$ solution (5 mL). The reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water and a satd NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica gel TLC (ethyl acetate/hexane=1/4) to give **12a** as a colorless oil (99.2 mg, 89%).

5.4.3. Typical glycosidation procedure using 2a–d as the glycosyl donors. To a stirred solution of Tf_2NH (2.8 mg, 0.01 mmol) and **11** (24 mg, 0.2 mmol) in acetonitrile was added **2a** (111 mg, 0.2 mmol) at 0 °C in the presence of Drierite (ca. 100 mg). The resulting mixture was stirred for 2 h. The reaction was then quenched by the addition of a satd $NaHCO_3$ solution (5 mL). The reaction mixture was extracted with ethyl acetate, and the organic layer was washed with water and a satd NaCl solution. After the organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure. The crude product was purified by preparative silica gel TLC (ethyl acetate/hexane=1/4) to give **12a** as a colorless oil (102 mg, 77%).

5.4.4. 3β-Cholestanyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (5a). $[\alpha]_D^{23}$ +39.0 (*c* 2.6, $CHCl_3$); mp: 159–159.5 °C; ^1H NMR ($CDCl_3$): δ 0.89–1.97 (47H, m, 3β-cholestanyl), 1.34 (3H, s, CH_3), 3.27 (1H, d, *J*=9.7 Hz, H-2), 3.58 (1H, t, *J*=9.5 Hz, H-4), 3.63–3.73

(2H, m, H-6), 3.92–3.93 (1H, m, H-5), 4.06 (1H, t, J=9.2 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 12.1, 12.4, 18.7, 21.2, 22.6, 22.9, 23.9, 24.3, 28.1, 28.3, 28.8, 30.3, 32.1, 35.5, 35.5, 35.8, 36.2, 36.4, 37.3, 39.5, 40.1, 42.6, 45.4, 54.4, 56.3, 56.5, 69.0, 71.2, 71.5, 75.4, 75.4, 73.3, 78.9, 83.1, 84.8, 100.8; Anal. Calcd for $C_{62}H_{84}O_6$: C, 80.48; H, 9.15. Found: C, 80.38; H, 9.39.

5.4.5. *n*-Octyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (8a). Colorless oil; $[\alpha]_D^{23}$ +26.8 (c 1.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, t, J=6.9 Hz, $CH_2(CH_2)_6CH_3$), 1.27–1.30 (13H, m, CH_3, $CH_2CH_2(CH_2)_5CH_3$), 1.55–1.63 (2H, m, $CH_2CH_2(CH_2)_5CH_3$), 3.32 (1H, d, J=9.6 Hz, H-2), 3.41 (2H, dd, J=6.9 Hz, J=7.6 Hz, $CH_2(CH_2)_6CH_3$), 3.62 (1H, dd, J=8.6 Hz, J=9.6 Hz, H-4), 3.63–3.72 (3H, m, H-5, H-6), 4.08 (1H, dd, J=8.9 Hz, J=9.6 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 14.1, 21.0, 22.7, 26.3, 29.3, 29.4, 29.7, 31.8, 60.8, 68.9, 71.4, 78.8, 83.2, 84.1, 100.2; HRMS (ESI) m/z calcd for $C_{43}H_{54}O_6\cdot Na^+$: 689.3813; found: 689.3828.

5.4.6. 6-*O*-(2,3,4,6-Tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (9a). Colorless oil; $[\alpha]_D^{23}$ −10.4 (c 3.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.31 (3H, s, $C(CH_3)_2$), 1.32 (3H, s, $C(CH_3)_2$), 1.37 (3H, s, CH_3), 1.42 (3H, s, $C(CH_3)_2$), 1.51 (3H, s, $C(CH_3)_2$), 3.34 (1H, d, J=9.6 Hz, H-2′), 3.60–3.75 (4H, m, H-6′, H-6), 3.68 (1H, t, J=9.0 Hz, H-4′), 3.94 (1H, m, H-5′), 4.01 (1H, t, J=5.5 Hz, H-5), 4.08 (1H, t, J=9.6 Hz, H-3′), 4.27 (1H, m, H-2), 4.30 (1H, dd, J=2.0 Hz, J=8.2 Hz, H-4), 4.57 (1H, t, J=11.0 Hz, H-3), 5.50 (1H, d, J=5.5 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 20.9, 24.4, 25.0, 26.0, 26.2, 60.4, 67.3, 68.7, 70.7, 70.8, 71.2, 71.2, 73.3, 74.2, 75.1, 75.3, 78.6, 82.7, 84.2, 96.2, 100.6, 108.5, 109.1; HRMS (ESI) m/z calcd for $C_{47}H_{56}O_{11}\cdot Na^+$: 819.3715; found: 819.3764. Its β-form: colorless oil; $[\alpha]_D^{23}$ +23.5 (c 0.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, s, $C(CH_3)_2$), 1.31 (3H, s, $C(CH_3)_2$), 1.42 (3H, s, $C(CH_3)_2$), 1.48 (3H, s, CH_3), 1.57 (3H, s, $C(CH_3)_2$), 3.53 (1H, m, H-5′), 3.58 (1H, d, J=8.8 Hz, H-2′), 3.62 (1H, dd, J=8.9 Hz, J=9.6 Hz, H-4′), 3.65–3.69 (3H, m, H-3′, H-6′), 3.84 (2H, m, H-6), 4.00 (1H, m, H-5), 4.23 (1H, dd, J=1.8 Hz, J=7.9 Hz, H-4), 4.29 (1H, dd, J=2.4 Hz, J=5.0 Hz, H-2), 4.53–4.58 (3H, m, H-3, OCH_2Ph), 5.45 (1H, d, J=5.0 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 17.0, 24.3, 25.0, 26.0, 26.1, 60.7, 67.5, 69.4, 70.7, 70.7, 71.3, 73.4, 78.2, 83.2, 83.9, 96.4, 102.3, 108.5, 109.1; HRMS (ESI) m/z calcd for $C_{47}H_{56}O_{11}\cdot Na^+$: 819.3715; found: 819.3739.

5.4.7. 6-*O*-(2,3,4,6-Tetra-*O*-benzyl-1-*C*-ethyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (9b). Colorless oil; $[\alpha]_D^{23}$ +11.3 (c 1.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.81 (3H, t, J=7.6 Hz, CH_2CH_3), 1.31 (3H, s, $C(CH_3)_2$), 1.32 (3H, s, $C(CH_3)_2$), 1.42 (3H, s, $C(CH_3)_2$), 1.52 (3H, s, $C(CH_3)_2$), 1.79 (1H, m, $CH_aH_bCH_3$), 1.88 (1H, m, $CH_aH_bCH_3$), 3.52 (1H, d, J=9.6 Hz, H-2′), 3.61–3.67 (3H, m, H-4′, H_a-6′, H_a-6), 3.75–3.79 (2H, m, H_b-6′, H_b-6), 3.96 (1H, m, H-5′), 4.01 (1H, t, J=5.5 Hz, H-5), 4.15 (1H, t, J=9.6 Hz, H-3′), 4.27 (1H, dd, J=2.0 Hz, J=4.8 Hz, H-2), 4.31 (1H, dd, J=2.0 Hz, J=7.6 Hz, H-4), 4.57 (1H, dd, J=2.1 Hz, J=7.6 Hz, H-3), 5.50 (1H, d, J=5.5 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 8.1, 24.4, 25.0, 25.5, 26.0, 26.2, 60.0, 67.4, 68.9, 70.7, 70.8, 71.2, 71.5, 73.1, 74.2, 74.4, 75.3, 78.7, 79.8, 83.0, 96.2, 102.2, 108.5, 109.1; HRMS (ESI) m/z calcd for $C_{48}H_{58}O_{11}\cdot Na^+$: 833.3877; found: 833.3872.

5.4.8. 6-*O*-(2,3,4,6-Tetra-*O*-benzyl-1-*C*-*n*-butyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (9c). Colorless oil; $[\alpha]_D^{23}$ −4.6 (c 6.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.80 (3H, t, J=7.6 Hz, $CH_2CH_2CH_2CH_3$), 1.05 (1H, m, $CH_2CH_aH_bCH_2CH_3$), 1.13 (1H, m, $CH_2CH_2CH_aH_bCH_3$), 1.21–1.26 (2H, m, $CH_2CH_aH_bCH_aH_bCH_3$), 1.32 (3H, s, $C(CH_3)_2$), 1.33 (3H, s, $C(CH_3)_2$), 1.43 (3H, s, $C(CH_3)_2$), 1.52 (3H, s, $C(CH_3)_2$), 1.73–1.80 (2H, m, $CH_2CH_2CH_2CH_3$), 3.51 (1H, d, J=8.9 Hz, H-2′), 3.61–3.66 (3H, m, H-4′, H_a-6′, H_a-6), 3.75–3.78 (2H, m, H_b-6′, H_b-6), 3.96 (1H, dd, J=2.1 Hz, J=9.6 Hz, H-5), 4.02 (1H, t, J=5.5 Hz, H-5′), 4.14 (1H, t, J=9.6 Hz, H-3′), 4.27 (1H, dd, J=2.1 Hz, J=4.9 Hz, H-2), 4.31 (1H, dd, J=2.1 Hz, J=7.6 Hz, H-4), 4.58 (1H, dd, J=2.0 Hz, J=7.5 Hz, H-3), 5.51 (1H, d, J=4.9 Hz, H-1): ^{13}C NMR ($CDCl_3$): δ 14.0, 23.0, 24.3, 25.0, 25.9, 26.0, 26.2, 32.6, 60.0, 67.5, 68.9, 70.7, 70.8, 71.2, 71.5, 73.0, 74.2, 74.5, 75.2, 78.7, 80.3, 83.1, 96.2, 102.0, 108.5, 109.1; HRMS (ESI) m/z calcd for $C_{50}H_{62}O_{11}\cdot Na^+$: 861.4184; found: 861.4200.

5.4.9. 6-*O*-(1-*C*-Benzyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (9d). Colorless oil; $[\alpha]_{465}^{23}$ +30.4 (c 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.34 (3H, s, $C(CH_3)_2$), 1.37 (3H, s, $C(CH_3)_2$), 1.46 (3H, s, $C(CH_3)_2$), 1.55 (3H, s, $C(CH_3)_2$), 3.09 (1H, d, J=13.8 Hz, CCH_aH_bPh), 3.25 (1H, d, J=9.1 Hz, H-2′), 3.27 (1H, d, J=14.4 Hz, CCH_aH_bPh), 3.57 (1H, t, J=9.6 Hz, H-4′), 3.75–3.79 (2H, m, H_a-6′, H_a-6), 3.84 (1H, dd, J=3.4 Hz, J=9.8 Hz, H_b-6′), 3.92–3.96 (2H, m, H-5′, H_b-6), 4.06 (1H, m, H-5), 4.13 (1H, t, J=9.6 Hz, H-3′), 4.31 (1H, dd, J=2.7 Hz, J=5.5 Hz, H-2), 4.40 (1H, dd, J=2.1 Hz, J=8.7 Hz, H-4), 4.59–4.64 (3H, m, H-3, OCH_2Ph), 5.55 (1H, d, J=5.5 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 24.4, 25.0, 26.0, 26.2, 39.5, 59.8, 67.2, 69.0, 70.7, 70.9, 71.1, 71.9, 73.1, 73.7, 74.5, 75.3, 78.6, 79.8, 83.2, 96.3, 102.5, 108.6, 109.1; HRMS (ESI) m/z calcd for $C_{53}H_{60}O_{11}\cdot Na^+$: 895.4028; found: 895.3995.

5.4.10. Phenethyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (12a). Colorless oil; $[\alpha]_D^{23}$ +45.5 (c 2.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, s, CH_3), 2.93 (2H, m, OCH_2CH_2Ph), 3.31 (1H, d, J=9.5 Hz, H-2), 3.36 (1H, m, H-5), 3.52–3.59 (3H, m, H-6, H-4), 3.61–3.66 (2H, m, OCH_2CH_2Ph), 4.07 (1H, t, J=9.3 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 21.0, 36.3, 62.0, 68.8, 71.4, 73.3, 74.7, 75.4, 75.5, 78.6, 83.0, 83.9, 100.3; HRMS (ESI) m/z calcd for $C_{43}H_{46}O_6\cdot Na^+$: 681.3192; found: 681.3197. Its β-form: colorless oil; $[\alpha]_D^{23}$ +21.4 (c 0.69, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.43 (3H, s, CH_3), 2.91 (2H, t, J=6.9 Hz, OCH_2CH_2Ph), 3.52–3.54 (1H, m, H-5), 3.55 (1H, d, J=8.9 Hz, H-2), 3.62 (1H, t, J=8.9 Hz, H-4), 3.65 (1H, t, J=8.9 Hz, H-3), 3.69 (2H, d, J=2.7 Hz, H-6), 3.86 (2H, m, OCH_2CH_2Ph); ^{13}C NMR ($CDCl_3$): δ 17.0, 36.7, 62.1, 69.4, 73.4, 73.4, 74.2, 75.0, 75.5, 78.2, 83.3, 83.9, 102.0; HRMS (ESI) m/z calcd for $C_{43}H_{46}O_6\cdot Na^+$: 681.3192; found: 681.3175.

5.4.11. Phenethyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-ethyl-α-D-glucopyranoside (12b). Colorless oil; $[\alpha]_D^{23}$ +54.0 (c 3.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, t, J=7.6 Hz,

CH_2CH_3), 1.75 (1H, m, $CH_aH_bCH_3$), 1.81 (1H, m, $CH_aH_bCH_3$), 2.92 (2H, m, OCH_2CH_2Ph), 3.41 (1H, dd, J=2.8 Hz, J=10.3 Hz, H-5), 3.50 (1H, d, J=9.7 Hz, H-2), 3.51–3.70 (4H, m, H-6, OCH_2CH_2Ph), 3.54 (1H, t, J=9.7 Hz, H-4), 4.12 (1H, t, J=9.6 Hz, H-3): ^{13}C NMR ($CDCl_3$): δ 8.1, 25.5, 36.5, 61.5, 68.9, 71.7, 73.2, 74.7, 74.7, 75.4, 78.8, 79.6, 83.3, 102.0; HRMS (ESI) m/z calcd for $C_{44}H_{48}O_6\cdot Na^+$: 695.3343; found: 695.3346.

5.4.12. Phenethyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-*n*-butyl-α-D-glucopyranoside (12c). Colorless oil; $[\alpha]_D^{23}$ +38.2 (c 1.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, t, J=6.8 Hz, $CH_2CH_2CH_2CH_3$), 0.91 (1H, m, $CH_2CH_aH_bCH_2CH_3$), 1.05 (1H, m, $CH_2CH_2CH_aH_bCH_3$), 1.15 (1H, m, $CH_2CH_2CH_aH_bCH_3$), 1.26 (1H, m, $CH_2CH_aH_bCH_2CH_3$), 1.69 (2H, t, J=8.2 Hz, $CH_2CH_2CH_aH_bCH_3$), 2.94 (2H, m, OCH_2CH_2Ph), 3.41 (1H, dd, J=3.4 Hz, J=10.3 Hz, H-5), 3.48 (1H, d, J=9.6 Hz, H-2), 3.53 (1H, t, J=9.6 Hz, H-4), 3.56 (1H, m, H_a-6), 3.60–3.63 (2H, m, H_b-6, $OCH_aH_bCH_2Ph$), 3.67–3.70 (1H, m, $OCH_aH_bCH_2Ph$), 4.11 (1H, t, J=9.6 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 14.0, 23.0, 25.9, 32.7, 36.5, 61.5, 69.0, 71.7, 73.2, 74.8, 74.8, 75.4, 78.8, 80.0, 83.5, 101.8; HRMS (ESI) m/z calcd for $C_{46}H_{52}O_6\cdot Na^+$: 723.3656; found: 723.3648.

5.4.13. Phenethyl 1-*C*-benzyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (12d). Colorless oil; $[\alpha]_D^{23}$ +48.6 (c 1.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.96 (1H, m, $OCH_2CH_aH_bPh$), 3.01 (1H, m, $OCH_2CH_aH_bPh$), 3.03 (1H, d, J=3.7 Hz, CCH_aH_bPh), 3.23 (1H, d, J=9.6 Hz, H-2), 3.27 (1H, d, J=3.7 Hz, CCH_aH_bPh), 3.47 (1H, t, J=9.6 Hz, H-4), 3.49 (1H, m, H-5), 3.66 (1H, d, J=11.0 Hz, H_a-6), 3.69 (1H, dd, J=3.4 Hz, J=10.3 Hz, H_b-6), 3.82–3.86 (2H, m, OCH_2CH_2Ph), 4.11 (1H, t, J=9.7 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 36.6, 39.7, 61.7, 69.1, 71.9, 73.2, 74.0, 74.8, 75.8, 78.6, 79.9, 83.4, 102.4; HRMS (ESI) m/z calcd for $C_{49}H_{50}O_6\cdot Na^+$: 757.3500; found: 757.3549.

5.4.14. Phenyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (14a). Colorless oil; $[\alpha]_D^{23}$ +70.0 (c 4.90, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, s, CH_3), 3.36 (1H, d, J=9.6 Hz, H-2), 3.63 (1H, t, J=9.6 Hz, H-4), 3.66–3.70 (2H, m, H-6), 4.02–4.04 (1H, m, H-5), 4.16 (1H, t, J= 9.0 Hz, H-3); ^{13}C NMR ($CDCl_3$): δ 21.1, 69.1, 71.9, 73.4, 74.8, 75.6, 75.6, 78.6, 83.1, 84.2, 103.3; HRMS (ESI) m/z calcd for $C_{41}H_{42}O_6\cdot K^+$: 669.2613; found: 669.2661.

5.4.15. Methyl 6-*O*-(1-*C*-benzyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (17d). Colorless oil; $[\alpha]_D^{23}$ +50.0 (c 3.84, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.95 (1H, d, J=14.4 Hz, CCH_aH_bPh), 3.14 (1H, d, J=13.7 Hz, CCH_aH_bPh), 3.19 (1H, d, J=9.6 Hz, H-2′), 3.33 (1H, dd, J=8.9 Hz, J=9.6 Hz, H-3), 3.36 (3H, s, OMe), 3.47 (1H, t, J=9.6 Hz, H-4′), 3.46–3.52 (2H, m, H-2, H_a-6), 3.63 (1H, d, J=11.0 Hz, H_a-6′), 3.69 (1H, dd, J=3.4 Hz, J=11.0 Hz, H_b-6′), 3.86–3.90 (3H, m, H-5, H-5′, H_b-6), 3.98 (1H, t, J=8.9 Hz, H-4), 4.05 (1H, dd, J=8.9 Hz, J=9.6 Hz, H-3′), 4.56–4.59 (2H, m, H-1, OCH_aH_bPh); ^{13}C NMR ($CDCl_3$): δ 39.7, 55.0, 60.6, 69.1, 70.3, 71.8, 78.6, 78.7, 79.8, 80.2, 82.3, 83.4, 97.6, 102.4; HRMS (ESI) m/z calcd for $C_{69}H_{72}O_{11}\cdot Na^+$: 1099.4967; found: 1099.5003.

5.4.16. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl 2,3,4,6-tetra-*O*-benzyl-1-*C*-methyl-α-D-glucopyranoside (18a). Colorless oil; $[\alpha]_D^{23}$ +70.0 (c 2.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.49 (3H, s, CH_3), 3.29 (1H, d, J=9.6 Hz, H-2′), 3.33–3.39 (3H, m, H_a-6, H_b-6, H_a-6′), 3.55–3.58 (1H, m, H_b-6′), 3.56 (1H, dd, J=3.4 Hz, J=10.3 Hz, H-2′), 3.64 (1H, dd, J=9.6 Hz, J=10.3 Hz, H-4), 3.68 (1H, t, J=9.6 Hz, H-4′), 4.03 (1H, t, J=10.3 Hz, H-3), 4.05 (1H, t, J=9.6 Hz, H-3′), 4.17–4.19 (1H, m, H-5′), 4.27–4.28 (1H, m, H-5), 5.34 (1H, d, J=3.4 Hz, H-1′); ^{13}C NMR ($CDCl_3$): δ 22.7, 68.3, 68.5, 70.0, 71.0, 78.0, 78.6, 80.1, 81.8, 82.7, 85.1, 90.2, 101.0; HRMS (ESI) m/z calcd for $C_{69}H_{72}O_{11}\cdot Na^+$: 1099.4967; found: 1099.5006.

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Stereochemical challenges in characterizing nitrogenous spiro-axane sesquiterpenes from the Indo-Pacific sponges *Amorphinopsis* and *Axinyssa*

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Abstract—An investigation was conducted to identify the structures and bioactive properties of five compounds isolated from the Halichondrida sponges *Amorphinopsis foetida* and *Axinyssa aplysinoides*. All compounds possessed the spiro-axane sesquiterpene core and all were substituted at C-2 with nitrogen containing functionality. The stereochemistry of one known compound has been revised to (2*R*,5*R*,10*S*)-2-formamido-6-axene (**3**). It exhibited mild selective solid tumor and mild antibacterial activity and was found from *Axinyssa*. A second known substance whose stereochemistry has also been revised, (2*R*,5*R*,10*S*)-2-isothiocyanato-6-axene (**4**) plus its undescribed diastereomer (**5**) were isolated from *Amorphinopsis*. Both sponges were the source of two new *N*-phenethyl-2-formamido-6-axene diastereomeric compounds **6** and **7**. No solid tumor or antibacterial activity was found for **4**–**7**.

1. Introduction

The strategy of using an in vitro cell-based assay to identify solid tumor selectivity[1] continues to be a robust tool in our quest to investigate unusual constituents of Indo-Pacific sponges.[2] An initial stimulus for this work was the observation of large inhibition zones (at 10 μg/mL per disk) against murine colon-38 cells versus much reduced zones against murine leukemia (L1210) cells for extracts obtained from two related Indo-Pacific sponges. The taxa responsible for this data belonged to different genera but were in the same family consisting of the Papua New Guinea *Amorphinopsis foetida*[3] (order Halichondrida, family Halichondriidae) and the Vanuatu *Axinyssa aplysinoides*.[4] This parallel bioactivity pattern suggested that similar metabolites might be present in both sponges because nitrogen containing terpenes, which often possess many structural variants, are commonly isolated from sponges of the order Halichondrida. The most unique terpenoids of this set are compounds containing isocyanide, isothiocyanate, thiocyanate, and formamide moieties.[5]

Our pursuit of the major constituents from *A. foetida* and *Axinyssa aplysinoides* became of priority when mass spectrometry data indicated that nitrogenous spiro-axane sesquiterpene metabolites were to be isolated. The current literature shows that there are 15 sponges and/or nudibranch-derived spiro-axane compounds of general structure **A** (all shown in Table S1, Supplementary data) divided between 14-substituted compounds with *N*-functionality and one compound with an OH group.[5] Use of diagnostic ^{13}C NMR shifts simplifies the dereplication of a spiro-axane skeleton and facilitates the defining of the sites for heteroatom attachment at C-1, C-2, or C-6. Alternatively, establishing the configuration at each of the possible chiral centers (C-1, C-2, C-5, C-7, and C-10) can be more difficult. However, there are four sponge-derived compounds that provide important stereostructural templates for the members of this series and these are (+)-axisonitrile-3 (**1a**),[6] (+)-axenol (**1b**)[7] [*syn* (+)-gleenol], (−)-10-*epi*-axisonitrile-3 (**2a**),[8] and (+)-exiguamide (**2b**),[8b,9] whose absolute configurations at C-5, C-6, C-7, and C-10 were established after extensive experimentation including total synthesis. Relevant to the stereochemical challenges we encounter was that each of the preceding compounds possessed the 10*S* configuration, whereas the configuration varied from 5*R* in **1a** and **1b** to 5*S* in **2a** and **2b**. The biosynthetic assembly of the

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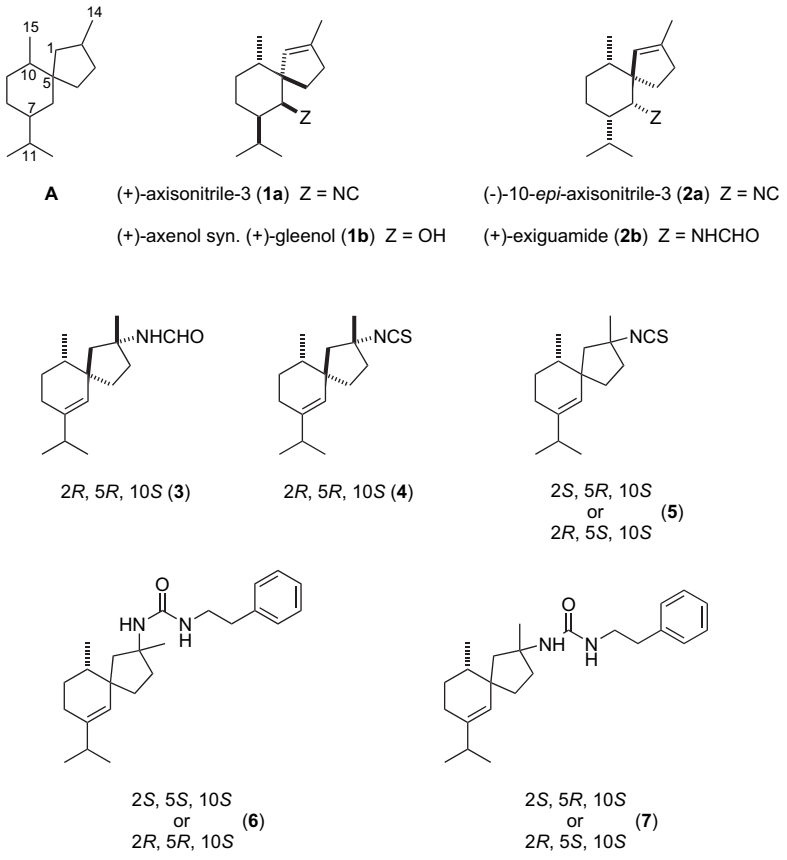
sponge-derived spirobicyclic ring system **A** produces an invariant absolute configuration at C-10, which is consistent with the 10*S* or 10*S** stereochemistry deduced for nine additional metabolites[10] (see Table S1, Supplementary data) and contrasts with the 10*R** stereochemistry previously assigned for **3** and **4**.[11] In contrast, a 10*R* stereochemistry has been found for spiro-axenes isolated from plants including pine,[12] juniper,[13] and cryptmeria trees[14] and a marine algae.[15] Thus, the challenges we faced were to determine the location of the nitrogen substituent and then set the configurations for each of the compounds isolated. In view of the above precedents, all the natural products isolated herein from sponges were assigned with 10*S* chirality.

Described below are properties of five compounds obtained including revision of the configurations for the known substances 2-formamido-6-axene (**3**) and 2-isothiocyanato-6-axene (**4**),[11] a new 2-isothiocyanato-6-axene diastereomer **5**, and a set of new diastereomeric compounds, *N*-phenethyl-2-formamido-6-axenes **6** and **7**. Also discussed below is that our data were consistent with two stereoisomeric possibilities for **5**–**7** with each possessing the 10*S* configuration.



A

(+)-axisonitrile-3 (**1a**) Z = NC

(-)-10-*epi*-axisonitrile-3 (**2a**) Z = NC

(+)-axenol syn. (+)-gleenol (**1b**) Z = OH

(+)-exiguamide (**2b**) Z = NHCHO

2*R*, 5*R*, 10*S* (**3**)

2*R*, 5*R*, 10*S* (**4**)

2*S*, 5*R*, 10*S* or 2*R*, 5*S*, 10*S* (**5**)

2*S*, 5*S*, 10*S* or 2*R*, 5*R*, 10*S* (**6**)

2*S*, 5*R*, 10*S* or 2*R*, 5*S*, 10*S* (**7**)

2. Results and discussion

Investigated first were two semi-pure extract fractions of *Axinyssa aplysinoides* (coll. no. 03411, Vanuatu), which included the hexane soluble sample (coded as XFH) and the dichloromethane soluble material (coded as XFD). Reversed-phase HPLC on both (see Fig. S1, Supplementary data) afforded known **3**,[11] previously isolated from *Amorphinopsis* (syn. *Trachyopsis*) *aplysinoides* ($[\alpha]_D^{25}$ obsd/lit.=+17.4/+14.8) whose ^{1}H NMR ($CDCl_3$) spectra contained doubled peaks ascribed to a mixture of amide rotamers in ratio of 6:4. Further fractionation on the XFD guided by LCMS screening revealed a subfraction containing two isomeric compounds exhibiting a *m*/*z* of 369.3 $[M+H]^+$. Additional HPLC purification afforded small quantities of **6** and **7** (~0.5 mg each) presumed to have a spiro-axane core but, by NMR, without multiple rotameric forms. Their structures were elucidated after re-isolation of more material as described below.

The sponge *A. foetida* (coll. no. 00381, Papua New Guinea) was examined next and the focus was on the butanol semi-pure extract fraction (coded as WB, Fig. S2, Supplementary data). Zone of inhibition data from the disk diffusion solid tumor whole cell assay indicated that this fraction was active against colon-38 cells. HPLC purification afforded 15 fractions (coded as H1–H15), which were then re-assayed. Three HPLC fractions (coded as H4–H6) exhibited the largest inhibition zones. Further LC screening of all the fractions by evaporative light scattering (ELSD), UV, and ESIMS detection was used to identify possible spiro-axane containing samples. Attention was given to two fractions (coded as H7 and H12) with *m*/*z* peaks of 369.3 and 264.2 amu, respectively. Repeated HPLC of the latter fraction yielded the previously reported compound 2-isothiocyanato-6-axene (**4**),[11] previously isolated from *Amorphinopsis* (syn. *Trachyopsis*) *aplysinoides* ($[\alpha]_D^{25}$ obsd/lit.=−22.4/−13.0) and its undescribed diastereomer 2-isothiocyanato-6-axene (**5**)

($[\alpha]_D^{25}$=+44.1). Similarly, HPLC of the former fraction yielded additional samples of **6** (5 mg) and **7** (6 mg).

The structures of **3** (HRESIMS *m/z* 250.2093 $[M+H]^+$ requiring molecular formula $C_{16}H_{27}NO$) and **4** (HRESIMS *m/z* 264.1706 $[M+H]^+$ requiring molecular formula $C_{16}H_{25}NS$) were confirmed as being known by comparing their properties to those in the literature.[11] On re-examining the argument for the previous assignment of the 2*R**,5*R**,10*R** configuration proposed for **3** and **4**,[11] based primarily on NOE data, it was clear that other plausible arrangements had not been ruled out. Based on the biosynthetic analysis presented above, the four possibilities we envisioned consisted of 2*S*,5*S*,10*S*; 2*R*,5*R*,10*S*; 2*R*,5*S*,10*S*; or 2*S*,5*R*,10*S*. An NOE correlation we observed for **4** from H-6 to H_3-14, shown in Figure 1, was used to rule out the latter two. Consistent with the literature,[11] we observed other key NOE enhancements (Fig. 1) from H-6 (δ 5.14) to H-1_b (δ 1.68), H-3_b (δ 1.81), H-4_b (δ 1.43),[16] and H_3-14 (δ 1.51) indicating that these atoms were on the same side of the molecule, but this did not differentiate between the remaining two possibilities. The interpretation of these results was further complicated by the presence of two conformers for the cyclohexene ring of **4** in which H_3-15 was pseudo-axial or pseudo-equatorial. Modeling experiments for all four conformers (Fig. 1) provided two sets of predicted *J* values: (a) H_3-15(eq) $^3J_{9-10}$=7.2 and 5.7 Hz versus (b) H_3-15(ax) $^3J_{9-10}$=2.5 and 2.1 Hz. Comparison of these data to that observed experimentally, $^3J_{9-10}$=6.8 and 2.9 Hz, indicated that significant populations were present for both conformations. Thus, either possibility of 2*S*,5*S*,10*S* or 2*R*,5*R*,10*S* was consistent with the NOE and *J* data.

The results of semi-synthesis provided additional information to resolve this uncertainty. This process began with the LAH reduction of the isothiocyanate **4**, yielding **8**, which was then hydrogenated to afford **9**. The ^{1}H NMR data collected supported the cis arrangement of the equatorial isopropyl at C-7 (based on the diagnostic $\delta_{C\text{-}11}$ 33.0)[17] and the axial methyl at C-10 in **9**. A complex ^{1}H NMR multiplet was observed for H-10 (δ 1.69) with a *J* value sum of 27.1 Hz (see, Fig. S3, Supplementary data). Simulation of the ddq patterns expected for H-10 as a function of variation in its geometry gave a *J* value sum of 33.1 Hz for axial (^3J=9.4, 2.4, 7.1 Hz) versus a *J* value sum of 26.5 Hz for equatorial (^3J=3.7, 1.5, 7.1 Hz). The next step was to obtain and interpret NOE data for **9**. The key result consisted of a strong enhancement from H-4_a to H_3-15 (axial), which required C-4 to be equatorial. Finally, these data indicated the configuration of **9** as 2*R*,5*R*,7*R*,10*S*, which translated into reversal of assignments at C-10 versus that previously reported for **4**.[11] These data also placed in question the previous assignments for **3**. In summary, the stereochemistry of **3** and **4** is now revised to 2*R*,5*R*,10*S* as both compounds were previously determined to have the same relative stereochemistry after they were co-isolated from the same sponge.[11] The logical next step of conducting a side-by-side analysis of **3** and **4** isolated here versus those from the Faulkner repository was considered. However, a search of the UC San Diego compound bank indicated that these compounds were missing.

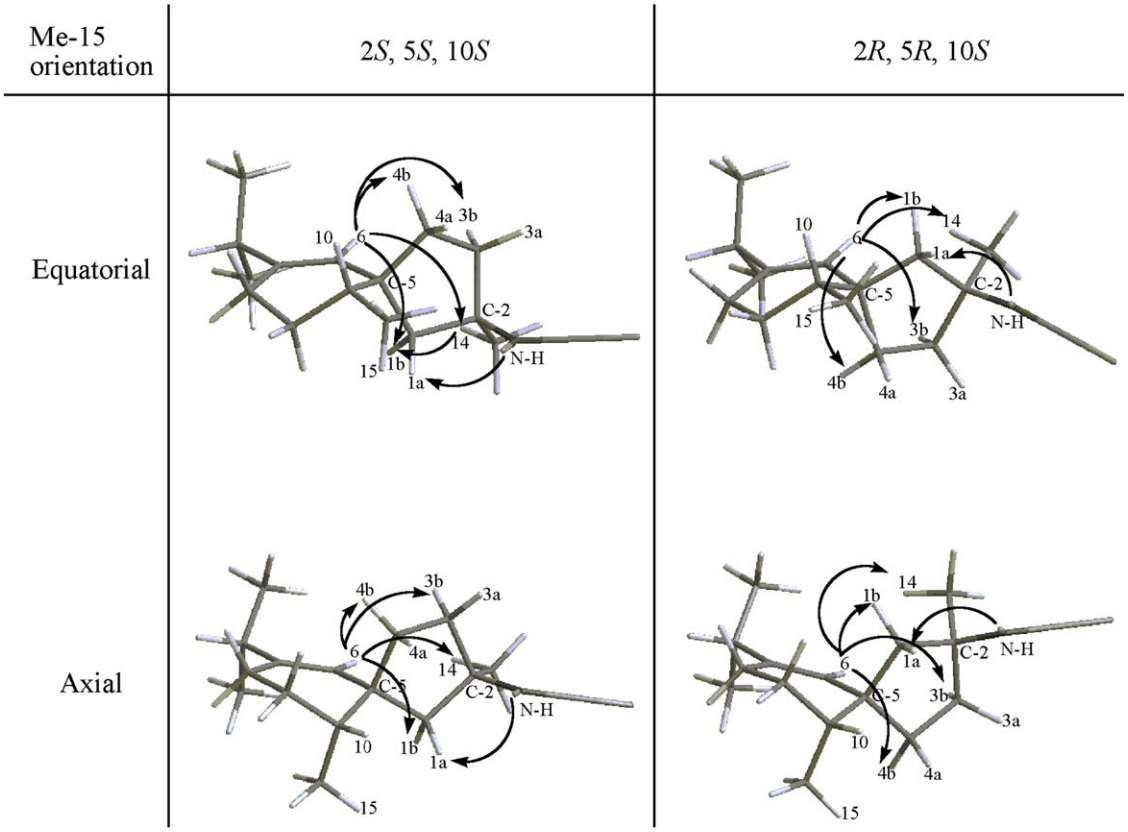
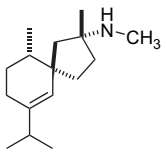
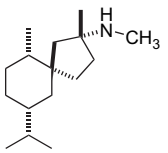


Figure 1. NOE correlations for candidate structures of **4**.



2*R*, 5*R*, 10*S* (**8**)



2*R*, 5*R*, 10*S* (**9**)

The NMR data and stereochemical conclusions discussed above provided an important reference to evaluate the new compounds that were isolated. Compounds **4** and **5** possessed identical molecular formulas and their ^{1}H and ^{13}C NMR data, shown in Table 1, are also extremely similar. Two-dimensional NMR data for **5** including gCOSY, gHMQC, and gHMBC (Table 1) indicated that it had the same atom connectivity as in **4**, however, their optical rotations were of different overall sign and magnitude, **4** $[\alpha]_D^{25}=-22.4$ versus **5** $[\alpha]_D^{25}=+44.1$, indicating that one or more stereocenters were different between this pair.

With the stereochemistry of **4** defined above, there were now three possibilities to be considered for **5**: 2*S*,5*S*,10*S*; 2*S*,5*R*,10*S*; or 2*R*,5*S*,10*S*. The important NOE correlation observed in **4** from H-6 to H_3-14 was not in the data set for **5**, which allowed elimination of the first possibility listed above. In parallel to the situation with **4**, all of the additional NOE data collected for **5** were consistent with the two remaining possibilities. These data included NOE enhancements from H-6 to H-1_a (δ 1.98) and H-4_b (δ 1.63), indicating that these atoms were on the same molecular face. Also important to note is that in **4** the NOE from H-6 was to H-1_b (δ 1.68), indicating that the isothiocyanate and methyl groups at C-2 are on opposite sides of the five-membered ring than that determined for **4**. Subsequently, the changes in the relative shifts of the diastereotopic protons at C-1 and the differences in their NOE correlations to H-6 in **4** versus **5** gave indications to which side of the molecule these atoms were on, however, they were not of real diagnostic value in determining *R* or *S* stereochemistry for the C-2/C-5 positions. Identical synthetic modifications performed on **4** were carried out on **5**, however, overlapping signals and low yields precluded obtaining the NOE data.

The structures of **6** and **7** were deduced as outlined below. A molecular formula of $C_{24}H_{36}N_2O$ (HRESIMS *m/z* 369.2881 $[M+H]^+$), requiring seven degrees of unsaturation, was established for **6**. Dereplication using this formula as a search seed gave 200 compounds as hits, but only one compound *N*-phenethyl-*N*′-2-trachyopsanylurea (**10**)[18] was a natural product and this nitrogenous sesquiterpene was also isolated from an Indo-Pacific *Axinyssa aplysinoides*. Comparison of the ^{1}H and ^{13}C NMR data between **6** (shown in Table 2) and **10** indicated that the phenethyl urea side chain was also present in the former. The remaining NMR signals were also consistent with the presence of a spiro-axane sesquiterpene residue with the nitrogenous group attached at C-2, as seen in **3**–**5**. The elements of the gross structure shown were further confirmed from gCOSY and gHMBC correlations. The formula of $C_{24}H_{36}N_2O$ (HRESIMS *m/z* 369.2879 $[M+H]^+$) was established for **7**, which is identical to that of **6**. The NMR properties of **7** including 2D correlations and ^{13}C shifts were identical to those observed for **6** (Table 2). Minor but significant differences were observed for the ^{1}H NMR shifts of **7** versus **6** for H-3, H-4, H-8, and H-9. Thus, it was clear that **6** and **7** were diastereomers probably differing in the configuration at C-2 and/or C-5. The results of 1D NOE experiments were used to rule out two of the four stereoisomer possibilities. For example, an NOE enhancement at H_3-14 was found when irradiating the vinylic H-6 of **6**, indicating that H_3-14 and H-6 were on the same side of the molecule. This was consistent with a 2*S*,5*S*,10*S* or

Table 1. NMR data[a] for compounds **4** and **5** in $CDCl_3$

Position	**4**			**5**			
	δ_C	δ_H (*J* in Hz)	NOE	δ_C	δ_H (*J* in Hz)	gHMBC	NOE
1	55.5	2.09 dd (1.5, 14.7) 1.68 d (14.7)	 1_a, 6, 14	54.2	1.98 dd (14.1, 2.0) 1.74 d (14.1)	2, 5, 14	1_b, 6 1_a, 3_b, 10, 14, 15
2	68.0			68.5			
3	41.2	2.01 dddd (1.6, 4.2, 6.4, 12.7) 1.81 m		42.2	2.05 m 1.60 m	1, 2, 4, 5	3_b, 4_a, 6
4	35.1	1.46 m 1.43 m	6, 15 6, 15	34.0	1.77 m 1.63 m	1, 2, 3, 10	15
5	46.4			46.5			
6	127.3	5.14 s	1_b, 3_b, 4_b, 11, 12, 13, 14	128.6	5.45 s	1, 5, 8, 10, 11	1_a, 4_b, 11, 12, 13
7	140.5			140.1			
8	22.5	1.92 m 1.82 m		24.4	1.90 ddd (1.5, 5.9, 7.2)	9, 10	9_a, 9_b, 10, 11, 12, 13, 15
9	27.8	1.93 m 1.63 m		28.6	1.58 m 1.36 dddd (7.2, 7.2, 9.3, 13.3)	5, 7, 8, 15	 4_a, 15
10	36.9	1.70 ddq (2.9, 6.8, 6.8)		37.4	1.45 ddq (2.4, 9.3, 6.8)	4, 5, 9	1_b, 8, 15
11	35.0	2.11 sept (6.8)		34.9	2.15 sept (6.8)	6, 7, 8, 12, 13	4_a, 6, 8, 12, 13
12	21.7	0.96 d (6.8)	6, 8_a, 8_b, 11	21.7	0.99 d (6.8)	7, 11	6, 8, 11
13	21.5	0.96 d (6.8)	6, 8_a, 8_b, 11	21.6	0.99 d (6.8)	7, 11	6, 8, 11
14	28.5	1.51 s		27.8	1.49 s	1, 2, 3	1_b
15	15.2	0.90 d (6.8)	1_a, 4_a, 8_a, 10	15.8	0.85 d (6.8)	5, 9, 10	1_b, 4_a, 9_a, 9_b, 10
16	129.2			129.3			

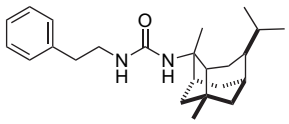
[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C). H_a=downfield proton, H_b=upfield proton.

Table 2. NMR data[a] for compounds **6** and **7** in MeOH-d_4

Position	6						7	
	δ_C	δ_H (J in Hz)	gCOSY	gHMBC	TOCSY	NOE	δ_C	δ_H (J in Hz)
1	53.9	2.04 d (14.2)				1_b	52.4	2.05 dd (1.0, 14.2)
		1.58 d (14.2)		2, 5, 14		1_a		1.60 d (14.2)
2	59.7						60.2	
3	39.7	1.84 m		1, 2, 4	4		40.4	1.92 m
		1.71 m						1.56 ddd (7.3, 8.7, 12.7)
4	34.0	1.76 m		2, 3, 5, 10	3		33.3	1.70 ddd (5.3, 7.4, 12.9)
		1.3 m						1.42 m
5	45.8						45.8	
6	128.5	5.26 s		1, 5, 8, 10, 11		14, 12, 13, 11	129.7	5.40 s
7	139.2						138.4	
8	22.4	1.92 m		9, 10			23.3	1.89 m
		1.85 m						
9	27.5	1.66 m		5, 7, 8, 15			28.1	1.62 m
		1.42 m						
10	36.8	1.6 m	15	4, 5, 9	15		37.3	1.46 m
11	34.7	2.12 sept (6.5)	12, 13	6, 7, 8, 12, 13	12, 13	6	34.7	2.11 sept (6.6)
12	20.5	0.98 d (7.0)	11	7, 11	11	6	20.5	0.95 d (7.0)
13	20.4	0.98 d (7.0)	11	7, 11	11	6	20.4	0.95 d (7.0)
14	26.7	1.38 s		1, 2, 3		6	25.9	1.36 s
15	14.3	0.87 d (7.0)	10	5, 9, 10	10		14.5	0.87 d (7.0)
16	158.9						159.0	
17	40.7	3.30 m	18	16, 18, 19			40.8	3.30 m
18	36.1	2.73 t (7.2)	17	17, 19			36.2	2.75 dt (2.5, 7.2)
19	139.4						139.5	
20	128.0	7.26 m		19, 21, 22	21, 22		128.0	7.26 m
21	128.4	7.19 m		19, 20, 22	20, 22		128.4	7.20 m
22	125.7	7.17 m		20, 21	20, 21		125.7	7.17 m

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C).

2*R*,5*R*,10*S* configuration. Conversely, irradiation of H-6 on **7** showed no enhancement to H_3-14, which, by analogy to the arguments for **5**, would be consistent with a configuration of 2*S*,5*R*,10*S* or 2*R*,5*S*,10*S*.



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3. Conclusion

The combination of bioactivity and LCMS screening was the key factor that allowed us to interrelate the parallel chemistry from two distantly related Indo-Pacific sponges. This came through the isolation of sesquiterpenes **3**, **6**, and **7** from *Axinyssa aplysinoides* versus **4–7** obtained from *A. foetida*. The observation of diastereomeric pairs (**4/5** and **6/7**) adds another element of distinctiveness to this study. We believe that the three new spiro-axane structures reported here along with the 15 others previously described in the literature represent challenging scaffolds for total synthesis because to date there are only a few such examples in the literature, the preparation of (−)-axisonitrile-3 (**1**),[19] (+)-axenol,[20] and (−)-axenol[21] [*syn* (−)-gleenol[22,23]]. The scant availability of the natural products reported above warrants further synthetic studies to: (a) further affirm the absolute stereochemistry of **3–5**, and (b) fully determine the absolute stereochemistry of **5–7**.

Others have noted the difficulties we encountered in defining the compound(s) responsible for the cytotoxicity of the initial extract, which eventually yielded nitrogenous sesquiterpenes. This was the circumstance in the attempt to isolate bioactive constituents from *Axinyssa aplysinoides* active in an assay against DNA-repair deficient yeast mutants,[18] and was also problematic in the study of antimitotic compounds from another collection of this same species.[24] Our search for the potent cytotoxins from these sponges is continuing, and it is clear that **3** was only mildly solid tumor selective while it also exhibited mild antibacterial activity against *Staphylococcus epidermidis* (ATTC no. 12228) and *Enterococcus durans* (ATTC no. 11576) with MIC's of 0.1 and 0.1 mg/mL, respectively. Finally, no antibacterial activity was found for **4–7**.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. UV measurements were recorded on a diode array spectrometer. The NMR spectra were recorded on a 500 spectrometer, operating at 499.9 and 125.7 MHz for ^{1}H and ^{13}C, respectively. High-resolution mass measurements were obtained on a bench-top ESI-TOF mass spectrometer. HPLC was performed at ambient temperature with Alltech Alltima columns of 5 μm ODS (250 mm×10 mm). Some extraction work was performed using an accelerated solvent extractor (ASE) system, which uses high pressure (~1700 psi) and elevated temperature (~100 °C) to assist in compound extraction.

4.2. Animal material

The sponge *A. foetida* (coll. no. 00381)[3] was collected in December 2000 using SCUBA at a depth of 30 ft in the

Madang region of Papua New Guinea. Voucher samples have been deposited at the Zoological Museum of Amsterdam (ZMA POR. 17553). An abovewater photograph of the sponge is available from the Crews laboratory. The sponge *Axinyssa aplysinoides* (coll. no. 03411)[4] was collected in November 2003 by hand using SCUBA at depths of 30–60 ft in the Mele Bay region of Vanuatu. Voucher samples have been deposited at the Zoological Museum of Amsterdam (ZMA POR. 17767). Abovewater and underwater photographs of the sponge are available from the Crews laboratory.

4.3. Extraction and isolation

Both sponges were preserved by soaking in 1:1 ethanol/seawater for 24 h, decanting, and vacuum sealing while transporting back to the laboratory at ambient temperature. The Vanuatu sponge (coll. no. 03411, 1.5 kg wet wt) was extracted using the ASE to give three fractions. The procedure for extraction with the ASE is as follows. The preserved sponge was allowed to air dry (~48 h). After this, the sponge was dissected into small pieces and partitioned using the ASE by first extracting with hexanes (3×), then with CH_2Cl_2 (3×), and finally with MeOH (3×). The CH_2Cl_2 fraction (XFD) (780 mg) was subjected to preparative reversed-phase HPLC (49:50:1 acetonitrile/water/isopropanol to 99:1 acetonitrile/isopropanol) over 50 min to yield 11 fractions. Fraction 6 yielded compound **3** (78 mg). Fraction 8 (32 mg) was further purified using reversed-phase semi-preparative HPLC (isocratic 75:35 acetonitrile/water both with 0.1% formic acid) to yield 20 fractions. H7 contained compound **3** (24 mg), H10 contained **6** (0.6 mg), and H11 contained **7** (1.0 mg).

The Papua New Guinean sponge (coll. no. 00381, 1.5 kg wet wt) was extracted using a Kupchan style solvent partition method. The field preserved sponge was first extracted with MeOH (3×). The resulting oil was then partitioned between water and CH_2Cl_2. The aqueous layer was extracted with butanol and the butanol layer was evaporated in vacuo to yield a brown gum. The gum was subjected to reversed-phase HPLC using a gradient solvent system of 10:90 methanol/water to 100% methanol over 60 min to afford 15 fractions (H1–H15). Fraction H7 (24.7 mg) was run on HPLC using a gradient solvent system of 40:60 methanol/water to 60:40 methanol/water over 50 min resulting in five fractions. The fourth fraction (H7H4) contained a mixture of both **6** and **7**. Fraction H7H4 was subjected to a shallow gradient HPLC run using a solvent system of 45:55 methanol/water to 50:50 methanol/water over 50 min resulting in **6** (5.2 mg) and **7** (6.3 mg) in pure form. Fraction H12 from the crude WB was run on HPLC to yield **4** (7.0 mg) and **5** (5.5 mg).

4.4. Antibacterial assay

Three different bacterial strains were employed including *Escherichia coli*, *S. epidermidis* (ATTC no. 12228), and *E. durans* (ATTC no. 11576). Minimum inhibitory concentrations (MIC) against these three bacteria were measured using a micro broth dilution test in 96-well microtiter plates with 0.2 mL per well. The maximum concentration of **3** used was 400 μg/mL, and this was serially diluted down to 6.25 μg/mL. The microtiter plates were inoculated with 0.1 mL of overnight cultures that were diluted and adjusted to give concentrations of 10^5–10^6 CFU/mL (per well) and a final volume of 0.2 mL. The 96-well microtiter plates were then incubated at 37 °C overnight for 24 h. A growth control was included to demonstrate the viability of the inoculum in each assay plate. Penicillin G and vancomycin were included as positive controls and DMSO was used as a negative control. The MIC values were determined by visual inspection as the minimum concentration of compound gives 100% inhibition of bacterial growth.

4.4.1. (2*R*,5*R*,10*S*)-2-Formamido-6-axene (3). Colorless solid, $[\alpha]_D^{25}$ +17.4 (*c* 1.5, $CHCl_3$); NMR data were in accordance with literature values; HRESIMS *m/z* 250.2089 $[M+H]^+$ (calcd for $C_{16}H_{27}NO$+H: 250.2093).[11]

4.4.2. (2*R*,5*R*,10*S*)-2-Isothiocyanato-6-axene (4). Colorless oil, $[\alpha]_D^{25}$ −22.4 (*c* 1.0, $CHCl_3$); for ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 264.1706 $[M+H]^+$ (calcd for $C_{16}H_{25}NS$+H: 264.1702).

4.4.3. 2-Isothiocyanato-6-axene (5). Colorless oil, $[\alpha]_D^{25}$ +44.1 (*c* 1.0, $CHCl_3$); for ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 264.1706 $[M+H]^+$ (calcd for $C_{16}H_{25}NS$+H: 264.1702).

4.4.4. *N*-Phenethyl-2-formamido-6-axene (6). Colorless solid, $[\alpha]_D^{25}$ −18.6 (*c* 0.1, MeOH); for ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 369.2881 $[M+H]^+$ (calcd for $C_{24}H_{36}N_2O$+H: 369.2900).

4.4.5. *N*-Phenethyl-2-formamido-6-axene (7). Colorless solid, $[\alpha]_D^{25}$ +38.6 (*c* 0.1, MeOH); for ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 369.2879 $[M+H]^+$ (calcd for $C_{24}H_{36}N_2O$+H: 369.2900).

4.4.6. (2*R*,5*R*,10*S*)-2-*N*-Methyl-6-axene (8). To a suspension of 10 mg LAH in 20 mL dry ether was added dropwise **4** (3.0 mg) in 250 μL dry ether. This was then allowed to reflux overnight. Workup consisted of cooling to 0 °C then adding 0.25 mL 10% NaOH followed by 0.5 mL H_2O. Vacuum filtration followed by HPLC resulted in **8** (1.0 mg). Colorless oil; ^1H NMR ($CDCl_3$) δ 9.20 (N-H), 5.31 (H-6), 2.62 (H-16), 2.15 (H-11), 1.78 (H-1_b), 1.43 (H-14), 0.98 (H-12, H-13), 0.92 (H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.8 (C-7), 126.9 (C-6), 65.7, 50.9, 46.3, 37.3, 36.5, 35.0, 33.7, 27.9, 27.6, 23.7, 23.4, 21.6 (C-12 or C-13), 21.5 (C-12 or C-13), 15.2 (C-15); HRESIMS *m/z* 236.2369 $[M+H]^+$ (calcd for $C_{16}H_{29}N$+H: 236.2373).

4.4.7. (2*R*,5*R*,10*S*)-2-*N*-Methyl axane (9). A solution of **8** (1.0 mg) in MeOH (0.5 mL) along with 2.0 mg of 10 wt % Pd/C was placed under 3 atm of H_2 and shaken overnight to yield **9** (0.5 mg). Colorless oil; ^1H NMR ($CDCl_3$) δ 9.19 (N-H), 2.57 (H-16), 2.08 (H-3_a), 1.98 (H-1_a), 1.92 (H-4_a), 1.79 (H-1_b), 1.78 (H-3_b), 1.69 (H-10), 1.40 (H-4_b, H-8_a, H-11), 1.27 (H-6_a, H-9_a), 1.13 (H-6_b, H-8_b, H-9_b), 0.89 (H-15), 0.86 (H-12, H-13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 66.3 (C-2), 48.2 (C-1), 46.6 (C-5), 40.4 (C-7), 38.0 (C-10), 37.4 (C-4), 35.3 (C-3), 34.6 (C-6), 33.0 (C-11), 30.0 (C-9), 27.4 (C-16), 25.0 (C-14), 23.0 (C-8), 20.0 (C-12 or C-13), 19.8 (C-12 or C-13), 15.0 (C-15); HRESIMS *m/z* 238.2533 $[M+H]^+$ (calcd for $C_{16}H_{31}N$+H: 238.2529).

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Supplementary data

A complete list of sponge/nudibranch-derived spiro-axane compounds, compound isolation diagrams, comparison of simulated and experimental H-10 multiplets for **9**, and proton NMR spectra of **4–7**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.08.070.

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A direct efficient diastereoselective synthesis of enantiopure 3-substituted-isobenzofuranones

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Abstract—Condensation of (−)-8-benzylaminomenthol with *o*-phthaldehyde lead to the chiral perhydro-1,3-benzoxazine **2** as single diastereoisomer. That compound reacted with different organometallics leading to **3** in excellent de. Hydrolysis of carbinols, followed by oxidation of the intermediates allowed for the synthesis of enantiopure phthalides.

1. Introduction

Phthalides (1(3*H*)-isobenzofuranones) are valuable synthetic intermediates[1] and a class of compounds possessing significant biological properties.[2] For example, some 3-alkyl substituted phthalides exhibit pharmacological applications,[3] and some others have been used as starting materials for the synthesis of carbo- and heterocycles.[4] These facts led to an increased interest in the synthesis of these compounds.[5] Specially interesting are the methodologies leading to optically active 3-alkyl substituted phthalides and, in this way, asymmetric hydrogenations[6] or alkylations,[7] enantioselective additions of alkylzinc,[8] asymmetric reductions of ketoesters,[9] and the use of chiral templates[10] have been successfully developed.

Herein we report on a versatile diastereoselective synthesis of chiral 3-substituted phthalides in excellent enantiomeric excess (ee) by using a chiral perhydro-1,3-benzoxazine derived from (−)-8-benzylaminomenthol.[11] This template has been successfully used in diastereoselective synthesis of aza-heterocycles by intramolecular cyclizations,[12] and now we envisaged that it can be used in a different diastereoselective approach. In this way, the perhydro-1,3-benzoxazine nucleus is now used as a chiral inductor in diastereoselective additions of different organometallics to a carbonyl group and a masked formyl substituent. The hydrolysis of the template, with recovering of the starting aminoalcohol, after the creation of a novel stereocenter and further elaboration of the intermediate allowed for the synthesis of a great variety of enantioenriched 3-substituted phthalides.

The versatility of the method is based on the possibility to introduce different substituents at C-3 in the final phthalides starting from a single compound by simply changing the nature of the nucleophile.

2. Results and discussion

The starting perhydro-1,3-benzoxazine **2**[13] was prepared as previously described by condensation of (−)-8-benzylaminomenthol and *o*-phthaldehyde in excellent yield and total diastereoselectivity, and transformed into the corresponding alcohols **3a**–**k** by reaction with different organometallics (Scheme 1). To this end, a solution of **2** in an ethereal solvent was reacted with excess of Grignard, organolithium, organozinc, and organocerium reagents[14] to afford alcohols **3a**–**k** as single diastereomers or with good diastereomeric excesses except for allylmagnesium or allylzinc derivatives (Table 1).

The chemical yields are excellent except for the reactions with ethyl-, isopropyl-, and *n*-butylmagnesium bromides. In these cases significant amount of the reduction derivative **4** was isolated (entries 3–6), but this problem was circumvented by using organollithium reagents, which lead to alkylation products, and no reduction derivatives were formed (compare entries 1 and 6, vs 2 and 7, respectively). The organocerium derivatives[15] also provided better yields than those obtained with Grignard or lithium reagents maintaining the degree of diastereoselection (compare entry 11 vs 9). Trifluromethylated alcohol **3i** was obtained in very good yield by addition

Keywords: Asymmetric synthesis; (−)-8-Benzylaminomenthol; Chiral templates; Isobenzofuranones; Phthalides.
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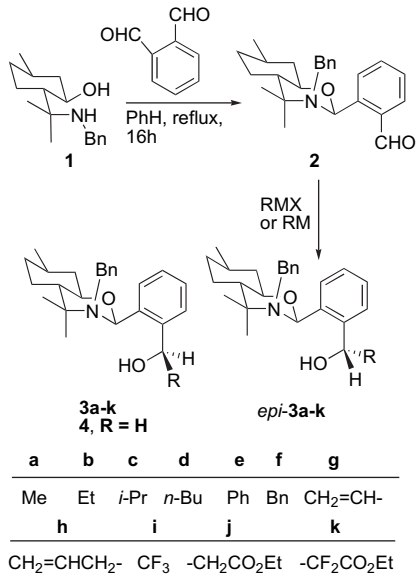


Scheme 1. Synthesis of **2** and reaction with organometallics.

of $TMSCF_3$ catalyzed by anhydrous TBAF.[16] Treatment of **2** with 4 equiv of Reformatsky reagents[17] gave alcohols **3j** and **3k** in good to excellent yields, but they highly decreased when less than 4 equiv of organometallic was used.

The nucleophilic addition of organometallics to aldehyde **2** is also stereochemically noteworthy. In most cases only one diastereoisomer was detected in the ^1H NMR of the reaction mixtures, and the stereochemistry of the novel created stereocenter is independent on the organometallic used in the reaction. An exception to this general behavior was the reactions with allylmagnesium bromide in Et_2O (entries 14 and 15 in Table 1), but a significant improvement in the stereoselection was observed when allylmagnesium chloride, in THF, was used instead (entry 16 in Table 1). The reaction with allylzinc bromide occurred also with moderate diastereoselection.

Table 1. Nucleophilic addition of organometallic reagents to **2**

Entry	Reagent	Temp (°C)	Solvent	Time (h)	Products (%)[a]
1	MeMgI	0	Et_2O	0.5	**3a**:*epi*-**3a** (93:3)
2	MeLi	0	THF	0.5	**3a**:*epi*-**3a** (96:3)
3	EtMgBr	0	THF	1	**3b**:*epi*-**3b** (85:2) **4** (11)
4	*i*-PrMgBr	−30	Et_2O	1.5	**3c** (47) **4**(39)
5	*i*-PrMgBr	−30	Et_2O	1.5	**3c** (52) **4**(43)
6	*n*-BuMgBr	−30	Et_2O	2.3	**3d** (70) **4**(13)
7	*n*-BuLi	−30	THF	0.5	**3d** (98)[b]
8	*n*-BuLi–$CeCl_3$	−78	THF	3	**3d** (96)[b]
9	PhMgBr	25	Et_2O	1.5	**3e** (82)[b]
10	PhLi	−78	THF	0.75	**3e**:*epi*-**3** (95:3)
11	PhMgBr–$CeCl_3$	0	THF	0.5	**3e** (94)[b]
12	$PhCH_2MgBr$	−30	Et_2O	0.75	**3f** (87)[b]
13	CH_2=CHMgBr	25	Et_2O	3	**3g** (96)[b]
14	CH_2=$CHCH_2MgBr$	−30	Et_2O	0.5	**3h**:*epi*-**3h** (43:39)
15	CH_2=$CHCH_2MgBr$	−78	Et_2O	0.5	**3h**:*epi*-**3h** (48:46)
16	CH_2=$CHCH_2MgCl$	−78	THF	0.5	**3h**:*epi*-**3h** (78:14)
17	CH_2=$CHCH_2ZnBr$	25	THF	1.5	**3h**:*epi*-**3h** (77:15)
18	CF_3TMS	0	THF	0.75	**3i** (96)[b]
19	$BrZnCH_2CO_2Et$	0	THF	24	**3j** (72)[b,c]
20	$BrZnCF_2CO_2Et$	0	THF	2	**3k** (95)[b,c]

[a] Yields in parenthesis refer to pure and isolated compounds.
[b] Only one diastereoisomer was observed in the ^1H NMR spectra of the reaction mixtures.
[c] It was necessary to use 4 equiv of the organometallic to achieve complete conversion.

The configuration of the formed quaternary stereocenter was determined as *R* for compound **3a** and *S* for the difluoroacetate derivative **3k** by X-ray diffraction analysis,[18] extended for all the alcohols, and later confirmed after elimination of the chiral auxiliary.

The sense of the excellent 1,4-stereoinduction observed is coincident with that previously observed for related compounds[13] and the described 1,2-stereoinduction for the addition to carbonyl groups placed at the same chiral inductor.[19] It can be explained by accepting that the addition occurs in the conformation shown in Figure 1, determined by the strong (9%) NOE observed between the hydrogen atoms placed at the formyl group and the C-2 of the template. The major adducts are formed by the preferred nucleophilic attack from the less hindered *Re* face of the carbonyl group according to a Felkin–Anh model. Probably a coordination of the organometallic to the oxygen atom of the template occurred prior to the addition of the carbonyl group.[19]

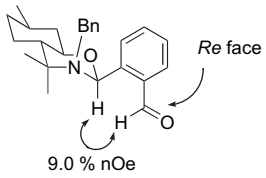
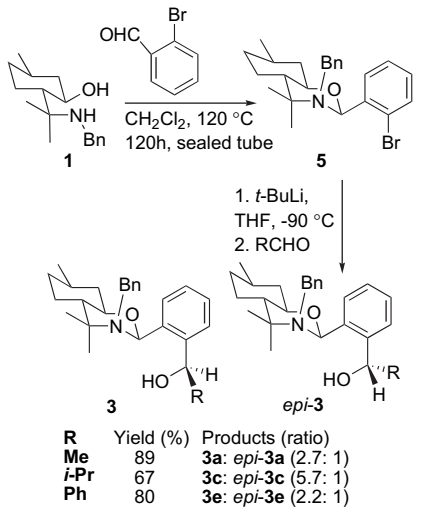


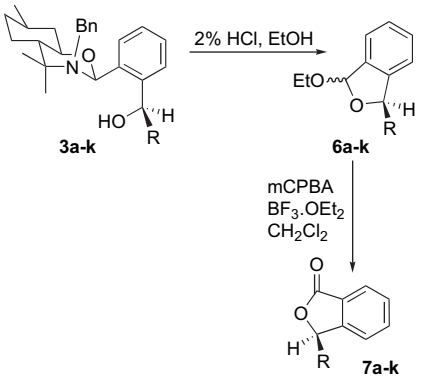
Figure 1. Preferred conformation in $CDCl_3$ solution for compound **2**.

In order to gain further information concerning 1,4-stereoinduction with perhydro-1,3-benzoxazines as chiral templates we explore the chiral aryllithium derived from **5** in an alternative preparation of carbinols **3**. Some chirons bearing a chiral acetal appendage have been previously used in diastereoselective additions to carbonyl compounds.[7d,20] Compound **5** was prepared in 65% yield, as a single diastereoisomer, by condensation of **1** with *o*-bromobenzaldehyde and converting into the organolithium derivative by treatment with 2.2 equiv of *t*-BuLi at −90 °C in THF. Addition of the appropriate aldehyde (1.5 equiv) at that temperature afforded alcohols **3a**, **3c**, and **3e** as major diastereoisomers but in lower yield and diastereoselectivity than those above described (Scheme 2).

The transformation of the benzylic alcohols into the final phthalides **7a–k** was performed in two steps as depicted in Scheme 3. Hydrolytic cleavage of the *N*,*O*-ketal by reaction with dilute (2%) aqueous alcoholic HCl solution allows for the regeneration of the carbonyl group and the formation of the acetals **6a–k** as an equimolar mixture of cis and trans isomers in good to excellent yield. The mixture of these isomers was not isolated but used in the next step after purification. The 3-alkyl derivatives were easily hydrolyzed and transformed into **6a–d** after 12–48 h of reaction (entries 1–4 in Table 2) but in these conditions, the hydrolysis of perhydrobenzoxazines **6e–k** occurred very slowly, and long periods of reaction or refluxing conditions were necessary to obtain the ketals (entries 5–7). The benzyl derivative **3f** was hydrolyzed at rt very slowly (entry 6), but the transformation



Scheme 2. Reaction of the aryllithium derived from **5** with aldehydes.



Scheme 3. Synthesis of phthalides **7a–k**.

Table 2. Hydrolysis of **3a–k** and oxidation to 3*H*-isobenzofuran-1-ones **7a–k**

Entry	Hydrolysis time (h)	Product (%)[a]	Oxidation time (h)	Product (%)[b]
1	12	**6a** (96)	3	**7a** (72)
2	12	**6b** (91)	4	**7b** (71)
3	12	**6c** (97)	4	**7c** (52)[d]
4	48	**6d** (84)	3	**7d** (76)
5	720	**6e** (97)	0.5	**7e** (90)
6	192	**6f** (95)	3.5	**7f** (83)
7	720	**6g** (70)	3	**7g** (—)[e]
8	14[c]	**6h** (95)	3	**7h** (58)
9	12[c]	**6i** (85)	72	**7i** (80)
10	7[c]	**6j** (65)[f]	24	**7j** (62)
11	7[c]	**6k** (62)[f]	48	**7k** (60)

[a] Yields in parenthesis refer to isolated compounds.
[b] Yields in parenthesis refer to total yields, and were calculated from alcohols **3**.
[c] Reflux.
[d] Conversion was total in the oxidation step.
[e] Compound **7g** could not be purified.
[f] As a mixture of 1-ethoxy- and 1-hydroxy-1,3-dihydroisobenzofurans.

of **3h–k** was only possible at reflux of ethanol. The hydrolysis of alcohols **3e** (R=Ph) and **3g** (R=vinyl) was performed at rt for 30 days because under refluxing conditions they lead to a complex mixture of reaction and the desired isobenzofuranes could not be isolated.

Treatment of compounds **6a–k** with MCPBA and $BF_3\cdot OEt_2$[21] in CH_2Cl_2 at rt gave the enantiopure isobenzofuran-1-ones **7a–k** in good to excellent yields (Table 2). All the phthalides were purified by column chromatography or recrystallization, except the vinyl derivative **7g**, which could not be isolated from the reaction crude. The optical rotations of compounds **7a**,[22] **7b**,[23] **7d**,[24] **7e**,[9] and **7h**[25] were compared with those previously described, allowing to confirm the absolute configuration of the stereocenter formed as *R*. This result was consistent with the absolute configuration determined for the alcohols **3a** and **3k**, and therefore, essentially no racemization is taking place during the hydrolysis and oxidation conditions. Enantiomeric excesses for compounds **7a–h** were determined as >99% by HPLC analysis (see Section 3).

In summary, our methodology allows for the preparation of enantiopure 3-substituted phthalides starting from easily accesible compounds. In addition, a great variety of substituents can be introduced merely by changing the nucleophile acting on the formyl group of the aryl substituent at C-2 in the starting chiral perhydro-1,3-benzoxazine.

3. Experimental

3.1. General

All reactions were carried out under an argon atmosphere in an oven-dried glassware. Solvents and bases were dried by standard methods: CH_2Cl_2 was distilled from CaH_2 and benzene, THF and Et_2O from Na. (Trifluoromethyl)trimethylsilane was used as 2 M solution in THF. ^1H NMR (300 MHz), ^{13}C NMR (75 MHz), and ^{19}F (282.38 MHz) spectra were registered in $CDCl_3$ as solvent and TMS or $CFCl_3$ as internal reference, and chemical shifts are given in parts per million. Specific rotations were determined on a digital polarimeter using Na lamp, and concentration is given in grams per 100 mL. Melting points were obtained with open capillary tubes and are uncorrected. TLC was performed on glass-backed plates coated with silica gel 60 with an F_{254} indicator. The chromatograms were visualized under UV light and/or by staining with I_2 or phosphomolybdic acid. Flash chromatography was carried out on silica gel (230–240 mesh).

3.1.1. Synthesis of alcohols 3a–k. General method. To a solution of the aldehyde **2** (5.3 mmol) in the appropriate ethereal solvent (Table 1, 5 mL) under argon and at the temperature showed in Table 1 was slowly added a solution of the appropriate organometallic reagent (5.83 mmol) and the mixture was stirred at that temperature until disappearance of the starting material (TLC). The reaction mixture was quenched with saturated ammonium chloride, and the product was extracted with diethyl ether (3×5 mL). The organic extracts were washed with brine and dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography on silica gel using hexanes–ethyl acetate as eluent.

3.1.1.1. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3]oxazin-2′-yl)phenyl)-ethanol (3a). White solid. Mp: 88–90 °C (from hexane). $[\alpha]_D^{23}$ +9.9 (*c* 1.20, $CHCl_3$); $[\alpha]_D^{23}$ +10.2 (*c* 1.0, CH_2Cl_2).

^{1}H NMR (δ): 0.93 (d, 3H, J=6.5 Hz); 0.97–1.24 (m, 3H); 1.21 (s, 3H); 1.28 (d, 3H, J=6.5 Hz); 1.38 (s, 3H); 1.42–1.60 (m, 1H); 1.62–1.74 (m, 3H); 1.98 (m, 1H); 3.52 (d, 1H, J=16.2 Hz); 3.67 (td, 1H, J_1=10.5 Hz, J_2=4.2 Hz); 3.84 (s, 1H); 3.90 (d, 1H, J=16.2 Hz); 5.33 (q, 1H, J=6.5 Hz); 5.84 (s, 1H); 6.73–6.76 (m, 2H); 6.93–6.99 (m, 3H); 7.10–7.21 (m, 3H); 7.56 (d, 1H, J=7.0 Hz). ^{13}C NMR (δ): 19.0; 20.9; 22.2; 25.0; 28.3; 31.3; 34.9; 41.3; 46.3; 47.1; 58.4; 63.9; 76.2; 86.3; 124.6; 125.7; 126.8; 127.5 (2C); 127.7 (3C); 128.3; 136.8; 142.1; 143.3. IR (Nujol): 3200; 1600; 1590; 820; 780; 750 cm^{-1}. CI/MS (*m*/*z*, %): 394 (M+1, 100). Anal. Calcd for $C_{26}H_{35}NO_2$ (393.56): C, 79.33; H, 8.97; N, 3.56. Found: C, 79.51; H, 8.82; N, 3.44.

3.1.1.2. (1*S*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-ethanol (*epi*-3a). Colorless oil. $[\alpha]_D^{23}$ +8.5 (*c* 0.29, CH_2Cl_2). ^{1}H NMR (δ): 0.83–0.92 (m, 1H); 0.95 (d, 3H, J=6.5 Hz); 0.98–1.16 (m, 3H); 1.25 (s, 3H); 1.31 (s, 3H); 1.32 (d, 3H, J=6.8 Hz); 1.33–1.77 (m, 3H); 1.99 (m, 1H); 3.23 (d, 1H, J=16.0 Hz); 3.61 (td, 1H, J_1=10.5 Hz, J_2=4.2 Hz); 3.87 (d, 1H, J=16.0 Hz); 5.51–5.55 (m, 1H); 5.55 (s, 1H); 6.75–6.79 (m, 2H); 6.97–7.18 (m, 6H); 7.39–7.42 (m, 1H). ^{13}C NMR (δ): 15.1; 22.2; 24.3; 25.2; 28.2; 31.2; 34.9; 41.3; 48.3; 48.7; 58.0; 66.5; 75.6; 90.7; 125.8; 126.2; 126.3; 127.5 (2C); 127.8 (2C); 128.6; 128.9; 135.9; 142.6; 146.5. IR (Film): 3520; 3400; 3060; 3020; 1600; 1590; 750; 720; 700 cm^{-1}. CI/MS (*m*/*z*, %): 394 (M+1, 34); 133(100). Anal. Calcd for $C_{26}H_{35}NO_2$ (393.56): C, 79.33; H, 8.97; N, 3.56. Found: C, 72.25; H, 9.09; N, 3.68.

3.1.1.3. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-propan-1-ol (3b). Colorless oil. $[\alpha]_D^{23}$ +3.3 (*c* 1.08, $CHCl_3$). ^{1}H NMR (δ): 0.95–1.12 (m, 2H); 0.97 (t, 3H, J=7.5 Hz); 0.97 (d, 3H, J=6.5 Hz); 1.18–1.38 (m, 1H); 1.25 (s, 3H); 1.47 (s, 3H); 1.50–1.78 (m, 6H); 2.02 (m, 1H); 2.82 (s, 1H); 3.68 (d, 1H, J=16.2 Hz); 3.73 (td, 1H, J_1=10.5 Hz, J_2=4.0 Hz); 3.90 (d, 1H, J=16.2 Hz); 5.10 (dd, 1H, J_1=8.1 Hz, J_2=5.0 Hz); 5.93 (s, 1H); 6.77–6.80 (m, 2H); 6.97–7.10 (m, 3H); 7.10–7.24 (m, 3H); 7.65 (d, 1H, J=7.5 Hz). ^{13}C NMR (δ): 11.0; 20.3; 22.3; 25.0; 28.3; 29.1; 31.4; 35.0; 41.4; 45.9; 46.5; 58.2; 68.2; 76.6; 85.3; 125.0; 125.7; 126.7; 127.2; 127.6 (2C); 127.7 (2C); 128.2; 136.7; 143.0 (2C). IR (Film): 3560; 3420; 3210; 3060; 1600; 1590; 1570; 820; 750; 730; 710 cm^{-1}. CI/MS (*m*/*z*, %): 408 (M+1, 100). Anal. Calcd for $C_{27}H_{37}NO_2$ (407.59): C, 79.56; H, 9.15; N, 3.44. Found: C, 79.70; H, 9.28; N, 3.32.

3.1.1.4. (1*S*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-propan-1-ol (*epi*-3b). Colorless oil. $[\alpha]_D^{23}$ +0.9 (*c* 1.62, $CHCl_3$). ^{1}H NMR (δ): 0.97 (d, 3H, J=6.9 Hz); 1.00–1.20 (m, 3H); 1.28 (s, 3H); 1.32 (s, 3H); 1.39–1.77 (m, 6H); 2.00 (m, 1H); 3.00 (br s, 1H); 3.38 (d, 1H, J=16.0 Hz); 3.64 (td, 1H, J_1=10.7 Hz, J_2=4.0 Hz); 3.90 (d, 1H, J=16.0 Hz); 5.09 (m, 1H); 5.71 (s, 1H); 6.80–6.83 (m, 2H); 7.00–7.10 (m, 4H); 7.12–7.25 (m, 2H); 7.49 (dd, 1H, J_1=7.3 Hz, J_2= 1.2 Hz). ^{13}C NMR (δ): 11.1; 16.8; 22.3; 25.1; 28.3; 31.2; 31.4; 34.9; 41.3; 47.8; 47.9; 58.3; 73.2; 75.8; 91.0; 125.8; 126.4; 127.1; 127.5 (2C); 127.8 (2C); 128.3; 128.7; 136.2; 142.4 (2C). IR (Film): 3550; 3400; 3050; 1590; 1450; 750; 720; 690 cm^{-1}. CI/MS (*m*/*z*, %): 408 (M+1, 100). Anal. Calcd for $C_{27}H_{37}NO_2$ (407.59): C, 79.56; H, 9.15; N, 3.44. Found: C, 79.62; H, 9.02; N, 3.31.

3.1.1.5. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-2-methylpropan-1-ol (3c). Colorless oil. $[\alpha]_D^{23}$ +1.1 (*c* 0.50, $CHCl_3$). ^{1}H NMR (δ): 0.67–1.00 (m, 2H); 0.70 (d, 3H, J=6.7 Hz); 0.89 (d, 3H, J=6.2 Hz); 0.91 (d, 3H, J=6.4 Hz); 1.10–1.24 (m, 1H); 1.22 (s, 3H); 1.42 (s, 3H); 1.44–1.78 (m, 5H); 1.94 (m, 1H); 2.82 (s, 1H); 3.63 (td, 1H, J_1=10.5 Hz, J_2=4.1 Hz); 3.65 (d, 1H, J=16.4 Hz); 3.77 (d, 1H, J=16.4 Hz); 4.79 (d, 1H, J=7.5 Hz); 5.91 (s, 1H); 6.73–6.77 (m, 2H); 6.90–7.16 (m, 6H); 7.62 (dd, 1H, J_1=7.7 Hz, J_2=1.1 Hz). ^{13}C NMR (δ): 18.4; 19.6; 21.4; 22.3; 25.0; 28.2; 31.4; 34.1; 35.1; 41.4; 45.5; 45.8; 57.8; 73.3; 76.6; 83.8; 125.5; 125.7; 126.4; 126.6; 127.5 (4C); 127.7; 136.7; 142.0; 142.4. IR (Nujol): 3360; 3060; 3020; 1600; 1590; 1450; 780; 750 cm^{-1}. CI/MS (*m*/*z*, %): 422 (M+1, 100). EI/MS (*m*/*z*, %): 421 (M, 0.1); 148 (72); 91 (100). Anal. Calcd for $C_{28}H_{39}NO_2$ (421.61): C, 79.76; H, 9.32; N, 3.32. Found: C, 79.85; H, 9.43; N, 3.21.

3.1.1.6. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-pentan-1-ol (3d). Colorless oil. $[\alpha]_D^{23}$ +17.6 (*c* 1.44, $CHCl_3$). ^{1}H NMR (δ): 0.95 (t, 3H, J=7.5 Hz); 0.97–1.10 (m, 1H); 1.02 (d, 3H, J=6.5 Hz); 1.13–1.49 (m, 5H); 1.30 (s, 3H); 1.50 (s, 3H); 1.50–1.78 (m, 7H); 2.08 (m, 1H); 2.92 (s, 1H); 3.68 (d, 1H, J=16.2 Hz); 3.76 (td, 1H, J_1=10.5 Hz, J_2=4.0 Hz); 3.95 (d, 1H, J=16.2 Hz); 5.25 (m, 1H); 5.97 (s, 1H); 6.81–6.84 (m, 2H); 6.97–7.07 (m, 3H); 7.15–7.28 (m, 3H); 7.68 (d, 1H, J=8.0 Hz). ^{13}C NMR (δ): 14.2; 20.1; 22.3; 22.9; 25.0; 28.3; 28.7; 31.4; 35.0; 36.0; 41.4; 46.0; 46.6; 58.2; 68.2; 76.6; 85.3; 125.0; 125.7; 126.7; 127.2; 127.6 (2C); 127.7 (2C); 128.2; 136.7; 142.7; 143.0. IR (Film): 3560; 3420; 3210; 3060; 3020; 1600; 1590; 1570; 1450; 820; 750; 730; 710 cm^{-1}. CI/MS (*m*/*z*, %): 436 (M+1, 100). Anal. Calcd for $C_{29}H_{41}NO_2$ (435.64): C, 79.95; H, 9.49; N, 3.22. Found: C, 80.07; H, 9.60; N, 3.34.

3.1.1.7. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl) phenyl-methanol (3e). Colorless oil. $[\alpha]_D^{23}$ +14.0 (*c* 0.80, $CHCl_3$). ^{1}H NMR (δ): 1.07–1.12 (m, 1H); 1.07 (d, 3H, J=6.5 Hz); 1.24–1.47 (m, 2H); 1.26 (s, 3H); 1.53 (s, 3H); 1.64–1.81 (m, 4H); 2.06 (m, 1H); 3.76 (d, 1H, J= 16.2 Hz); 3.78 (td, 1H, J_1=10.5 Hz, J_2=4.1 Hz); 4.00 (d, 1H, J=16.2 Hz); 5.07 (s, 1H); 6.18 (s, 1H); 6.30 (s, 1H); 6.70 (d, 1H, J=7.7 Hz); 6.89–6.96 (m, 2H); 7.06–7.18 (m, 3H); 7.20–7.36 (m, 7H); 7.76 (d, 1H, J=7.4 Hz). ^{13}C NMR (δ): 20.8; 22.3; 24.9; 28.5; 31.4; 35.0; 41.3; 42.5; 46.6; 58.9; 71.2; 76.8; 85.0; 125.9; 127.1 (2C); 127.3 (2C); 127.5; 127.6; 127.7 (2C); 128.1 (3C); 128.2 (2C); 137.5; 142.0; 142.2 (2C). IR (Film): 3580; 3220; 3060; 3020; 1720; 1690; 1600; 1570; 1450; 750; 730; 690 cm^{-1}. CI/MS (*m*/*z*, %): 456 (M+1, 92); 195 (100). Anal. Calcd for $C_{31}H_{37}NO_2$ (455.63): C, 81.72; H, 8.19; N, 3.07. Found: C, 81.52; H, 8.30; N, 2.87.

3.1.1.8. (1*S*,2′*S*,4a′*S*,7′*R*,8*a*′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl) phenyl-methanol (*epi*-3e). Colorless oil. $[\alpha]_D^{23}$ −1.6 (*c* 0.61, $CHCl_3$). ^{1}H NMR (δ): 0.87–1.08 (m, 1H); 0.90 (d,

3H, J=6.5 Hz); 1.10–1.37 (m, 2H); 1.13 (s, 3H); 1.18 (s, 3H); 1.39–1.73 (m, 4H); 1.94 (m, 1H); 3.38 (td, 1H, J_1=10.5 Hz, J_2=4.0 Hz); 3.65 (d, 1H, J=16.2 Hz); 4.00 (d, 1H, J=16.2 Hz); 5.62 (s, 1H); 5.90 (s, 1H); 6.89–6.96 (m, 2H); 7.02–7.13 (m, 4H); 7.15–7.33 (m, 8H); 7.76 (d, 1H, J=7.4 Hz). ^{13}C NMR (δ): 20.1; 22.3; 24.7; 28.5; 31.2; 34.9; 41.0; 44.9; 47.2; 59.5; 75.7; 77.5; 85.7; 126.1; 126.4 (2C); 126.6; 127.5; 127.8 (2C); 127.9 (2C); 128.2 (3C); 128.9; 130.2; 137.4; 140.9; 141.2; 144.6. IR (Film): 3253; 3060; 3030; 1603; 1451; 756; 732; 692 cm^{-1}. Anal. Calcd for $C_{31}H_{37}NO_2$ (455.63): C, 81.72; H, 8.19; N, 3.07. Found: C, 81.84; H, 8.30; N, 3.19.

3.1.1.9. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-2-phenylethanol (3f). Colorless oil. $[\alpha]_D^{23}$ +9.7 (*c* 3.10, $CHCl_3$). ^{1}H NMR (δ): 0.96 (d, 3H, J=6.4 Hz); 0.98–1.12 (m, 2H); 1.18–1.26 (m, 1H); 1.21 (s, 3H); 1.37 (s, 3H); 1.40–1.53 (m, 2H); 1.68–1.75 (m, 2H); 1.95 (m, 1H); 2.19 (br s, 1H); 2.78 (dd, 1H, J_1=13.6 Hz, J_2=5.0 Hz); 2.97 (dd, 1H, J_1=13.6 Hz, J_2=8.1 Hz); 3.52 (td, 1H, J_1= 10.4 Hz, J_2=3.8 Hz); 3.62 (d, 1H, J=16.3 Hz); 3.84 (d, 1H, J=16.3 Hz); 5.46 (dd, 1H, J_1=8.1 Hz, J_2=5.0 Hz); 5.57 (s, 1H); 6.77–6.80 (m, 2H); 6.96–7.02 (m, 3H); 7.12–7.24 (m, 8H); 7.62 (d, 1H, J=7.0 Hz). ^{13}C NMR (δ): 20.6; 22.4; 24.9; 25.0; 28.3; 31.4; 35.1; 41.4; 43.9; 46.1; 46.5; 57.9; 69.2; 76.3; 84.9; 125.2; 125.7; 126.2; 126.9; 127.0; 127.5 (4C); 128.2 (3C); 129.4 (2C); 136.6; 138.9; 142.4; 143.2. IR (Film): 3530; 3200; 3060; 3020; 1720; 1600; 1580; 1490; 1450; 760; 730; 700 cm^{-1}. CI/MS (*m*/*z*, %): 470 (M+1, 100). Anal. Calcd for $C_{32}H_{39}NO_2$ (469.66): C, 81.83; H, 8.37; N, 2.98. Found: C, 81.70; H, 8.48; N, 3.09.

3.1.1.10. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-prop-2-en-1-ol (3g). White solid. Mp: 102–103 °C (from ethanol). $[\alpha]_D^{23}$ +29.0 (*c* 0.80, $CHCl_3$). ^{1}H NMR (δ): 0.88 (d, 3H, J=6.5 Hz); 0.92–1.00 (m, 1H); 1.10–1.37 (m, 1H); 1.21 (s, 3H); 1.30 (s, 3H); 1.44–1.45 (m, 1H); 1.56–1.68 (m, 4H); 1.94 (m, 1H); 3.38 (d, 1H, J=16.0 Hz); 3.62 (td, 1H, J_1=10.5 Hz, J_2=4.1 Hz); 3.84 (d, 1H, J=16.0 Hz); 3.87 (s, 1H); 5.11 (dt, 1H, J_1=10.5 Hz, J_2=1.7 Hz), 5.29 (dt, 1H, J_1=17.2 Hz, J_2=1.7 Hz); 5.40–5.65 (m, 1H); 5.67 (s, 1H); 5.80 (br s, 1H); 6.71–6.88 (m, 2H); 6.90–6.97 (m, 4H); 7.06 (dt, 1H, J_1=7.5 Hz, J_2=1.5 Hz); 7.15 (dt, 1H, J_1=6.5 Hz, J_2=1.5 Hz); 7.48 (d, 1H, J=7.3 Hz). ^{13}C NMR (δ): 18.0; 22.2; 25.1; 28.3; 31.3; 34.8; 41.2; 46.7; 46.8; 58.3; 68.9; 76.4; 88.1; 114.4; 125.7; 126.9; 127.1; 127.6 (3C); 127.7 (2C); 128.5; 137.1; 138.6; 141.6; 141.9. IR (Film): 3560; 3400; 3060; 3020; 1635; 1600; 1570; 1450; 750; 730; 710; 690 cm^{-1}. CI/MS (*m*/*z*, %): 406 (M+1, 96); 145 (100). Anal. Calcd for $C_{27}H_{35}NO_2$ (405.57): C, 79.96; H, 8.70; N, 3.45. Found: C, 79.32; H, 8.70; N, 3.01.

3.1.1.11. (1*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-but-3-en-1-ol (3h). Colorless oil. $[\alpha]_D^{23}$ +17.0 (*c* 1.97, $CHCl_3$). ^{1}H NMR (δ): 1.03–1.26 (m, 2H); 1.04 (d, 3H, J=6.5 Hz); 1.28–1.40 (m, 1H); 1.31 (s, 3H); 1.50 (s, 3H); 1.57–1.69 (m, 2H); 1.74–1.83 (m, 2H); 2.02 (m, 1H); 2.30–2.35 (m, 1H); 2.41–2.59 (m, 1H); 2.93 (br s, 1H); 3.68 (d, 1H, J=16.0 Hz); 3.75 (td, 1H, J_1=10.6 Hz, J_2=4.1 Hz); 3.96 (d, 1H, J=16.0 Hz); 5.13 (dt, 1H, J_1=10.0 Hz, J_2=1.0 Hz); 5.18 (dd, 1H, J_1=17.7 Hz, J_2=1.7 Hz); 5.26–5.40 (m, 1H); 5.60–5.88 (m, 1H); 5.95 (s, 1H); 6.84–6.87 (m, 2H); 7.00–7.09 (m, 3H); 7.10–7.28 (m, 3H); 7.71 (d, 1H, J=7.3 Hz). ^{13}C NMR (δ): 20.3; 22.4; 25.1; 28.3; 31.4; 35.1; 40.9; 41.4; 46.1; 46.7; 58.2; 67.8; 76.6; 85.5; 116.8; 124.5; 125.7; 126.9; 127.4; 127.6 (2C); 127.7 (2C); 128.2; 135.8; 136.6; 142.3; 142.7. IR (Film): 3560; 3420; 3060; 3020; 1640; 1600; 1580; 1450; 760; 740; 710 cm^{-1}. CI/MS (*m*/*z*, %): 420 (M+1, 100); 159 (59). Anal. Calcd for $C_{28}H_{37}NO_2$ (419.60): C, 80.15; H, 8.89; N, 3.34. Found: C, 79.42; H, 8.32; N, 3.19.

3.1.1.12. (1*S*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-but-3-en-1-ol (*epi*-3h). Colorless oil. $[\alpha]_D^{23}$ −12.0 (*c* 1.60, $CHCl_3$). ^{1}H NMR (δ): 0.98 (d, 3H, J=6.5 Hz); 1.01–1.21 (m, 2H); 1.29 (s, 3H); 1.33 (s, 3H); 1.40–1.68 (m, 2H); 1.76–1.80 (m, 2H); 1.95 (m, 1H); 2.22–2.32 (m, 1H); 2.43–2.49 (m, 1H); 3.27 (d, 1H, J=16 Hz); 3.64 (td, 1H, J_1=10.5 Hz, J_2=4.0 Hz); 3.80 (d, 1H, J=16 Hz); 5.08–5.18 (m, 2H); 5.39–5.42 (m, 1H); 5.61 (s, 1H); 5.86–5.99 (m, 1H); 6.79–6.81 (m, 2H); 6.99–7.12 (m, 4H); 7.13–7.27 (m, 2H); 7.46 (d, 1H, J=7.2 Hz). ^{13}C NMR (δ): 15.5; 22.3; 25.2; 28.23; 31.2; 34.9; 41.3; 42.9; 43.2; 46.4; 58.1; 70.2; 75.7; 90.1; 116.3; 125.8; 126.7; 126.9; 127.5 (2C); 127.8 (2C); 128.5; 128.9; 136.1; 136.5; 142.4 (2C). IR (Film): 3580; 3410; 3060; 3020; 1640; 1600; 1580; 1450; 760; 720; 700 cm^{-1}. CI/MS (*m*/*z*, %): 420 (M+1, 100). Anal. Calcd for $C_{28}H_{37}NO_2$ (419.60): C, 80.15; H, 8.89; N, 3.34. Found: C, 79.64; H, 8.27; N, 3.63.

3.1.1.13. (1*S*,2′*S*,4a′*S*,7′*R*,8a′*R*)-1-(2-(*N*-Benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-2,2,2-trifluoroethanol (3i). White solid. Mp: 103–104 °C (from methanol). $[\alpha]_D^{23}$ −25.5 (*c* 1.00, $CHCl_3$). ^{1}H NMR (δ): 0.96–1.14 (m, 2H); 1.03 (d, 3H, J=6.5 Hz); 1.26 (q, 1H, J=11.8 Hz); 1.39 (s, 3H); 1.52 (s, 3H); 1.54–1.62 (m, 2H); 1.73–1.83 (m, 2H); 2.08 (m, 1H); 3.77 (td, 1H, J_1=10.5 Hz, J_2=6.0 Hz); 3.82 (s, 2H); 5.92 (q, 1H, J_1=17.1 Hz); 5.96 (s, 1H); 6.80–6.83 (m, 2H); 7.04–7.09 (m, 3H); 7.19–7.26 (m, 2H); 7.31–7.42 (m, 1H); 7.82 (d, 1H, J=7.0 Hz). ^{19}F NMR (δ): −77.1 (br s, 3F). ^{13}C NMR (δ): 20.8; 22.3; 25.1; 28.3; 31.4; 35.2; 41.3; 46.1; 46.48; 57.9; 67.2 (q, $^2J_{CF}$=31.6 Hz); 76.5; 84.1; 126.0; 127.6 (2C); 127.8 (2C); 128.1; 128.2; 128.7; 129.1; 133.4; 137.9; 143.1. IR (KBr): 3554; 3080; 3060; 3030; 1726; 1600; 1493; 1455; 1243; 1154; 1103; 764; 714 cm^{-1}. CI/MS (*m*/*z*, %): 448 (M+1, 100); 447 (M, 11); 428 (23). Anal. Calcd for $C_{26}H_{32}F_3NO_2$ (447.53): C, 69.78; H, 7.21; N, 3.13. Found: C, 69.70; H, 7.34; N, 3.05.

3.1.1.14. (3*R*,2′*S*,4a′*S*,7′*R*,8a′*R*)-Ethyl 3-(2-(*N*-benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-3-hydroxypropanoate (3j). Yellow solid. Mp: 131–132 °C (from hexane). $[\alpha]_D^{23}$ +9.7 (*c* 0.34, $CHCl_3$). ^{1}H NMR (δ): 0.97 (d, 3H, J=6.5 Hz); 1.00–1.09 (m, 2H); 1.21–1.30 (m, 1H); 1.28 (t, 3H, J=7.1 Hz); 1.28 (s, 3H); 1.44 (s, 3H); 1.48–1.55 (m, 1H); 1.62–1.78 (m, 3H); 2.01 (m, 1H); 2.36 (d, 1H, J=15.4 Hz); 2.71 (dd, 1H, J_1=15.4 Hz, J_2=9.9 Hz); 3.45 (br s, 1H); 3.54 (d, 1H, J=16.2 Hz); 3.72 (td, 1H, J_1=10.5 Hz, J_2=4.0 Hz); 3.91 (d, 1H, J=16.2 Hz); 4.14–4.23 (m, 2H); 5.82 (d, 1H, J=9.9 Hz); 5.86 (s, 1H); 6.78–6.80 (m, 2H); 6.79–7.00 (m,

3H); 7.05 (d, 1H, J=7.5 Hz); 7.16 (dt, 1H, J_1=7.5 Hz, J_2=1.3 Hz); 7.23 (dt, 1H, J_1=7.5 Hz, J_2=1.3 Hz); 7.60 (d, 1H, J=7.5 Hz). ^{13}C NMR (δ): 14.2; 22.2; 25.0; 28.2; 31.3; 34.9; 41.4; 41.3; 47.1; 58.2; 60.4; 65.1; 76.5; 86.3; 124.9; 125.7; 127.2; 127.5 (3C); 127.7 (2C); 128.4; 136.7; 141.2; 142.3; 171.7. IR (Nujol): 3460; 3040; 1680; 1600; 1580; 1370; 1320; 770; 730; 700 cm^{-1}. CI/MS (*m*/*z*, %): 466 (M+1, 100). Anal. Calcd for $C_{29}H_{39}NO_4$ (465.62): C, 74.81; H, 8.44; N, 3.00. Found: C, 74.12; H, 8.21; N, 3.02.

3.1.1.15. (3*S*,2′*S*,4a′*S*,7′*R*,8a′*R*)-Ethyl 3-(2-(*N*-benzyl-4′,4′,7′-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazin-2′-yl)phenyl)-2,2-difluoro-3-hydroxypropanoate (3k). White solid. Mp: 140–141 °C (from hexane). $[\alpha]_D^{23}$ −30.0 (*c* 0.83, $CHCl_3$). ^{1}H NMR (δ): 0.96–1.12 (m, 2H); 1.01 (d, 3H, J=6.5 Hz); 1.23–1.30 (m, 1H); 1.36 (s, 3H); 1.37 (t, 3H, J=7.1 Hz); 1.50–1.70 (m, 2H); 1.59 (s, 3H); 1.72–1.81 (m, 2H); 2.08 (m, 1H); 3.76 (td, 1H, J_1=10.5 Hz, J_2=4.0 Hz); 3.81 (s, 2H); 4.32–4.42 (m, 2H); 6.05 (s, 1H); 6.09 (dd, 1H, $^2J_{HF}$=19.6 Hz, $^2J_{HF}$=3.1 Hz); 6.81 (m, 2H); 6.97–7.07 (m, 3H); 7.17–7.27 (m, 2H); 7.32–7.37 (m, 1H); 7.83 (d, 1H, J=7.7 Hz). ^{19}F NMR (δ): −110.3 (d, 1F, J=260.6 Hz); −124.6 (dd, 1F, J_1=260.6 Hz, $^2J_{HF}$=19.6 Hz). ^{13}C NMR (δ): 13.9; 21.4; 22.4; 25.1; 28.3; 31.4; 35.19; 41.4; 45.9; 46.1; 57.7; 62.9; 67.7 (dd, $^2J_{CF}$=32.5 Hz, $^2J_{CF}$= 21.7 Hz); 76.4; 83.7; 114.0 (dd); 125.9; 126.7; 127.4; 127.5 (2C); 127.6 (2C); 127.7; 128.5; 133.4; 138.3; 143.7; 163.5 (dd). IR (Nujol): 3520; 3060; 1770; 1730; 1600; 1590; 1320; 770; 740; 710; 700 cm^{-1}. CI/MS (*m*/*z*, %): 502 (M+1, 100). Anal. Calcd for $C_{29}H_{37}F_2NO_4$ (501.61): C, 69.44; H, 7.43; N, 2.79. Found: C, 68.92; H, 6.97; N, 2.36.

3.1.2. Synthesis of (2*S*,4a*S*,7*R*,8a*R*)-3-benzyl-2-(2-bromophenyl)-4,4,7-trimethyl-octahydro-2*H*-benzo[*e*][1,3] oxazine (5). A solution of (−)-8-*N*-benzylaminomenthol (11.5 mmol) and 2-bromobenzaldehyde (23 mmol) was stirred in a sealed tube at 120 °C until disappearance of the starting aminoalcohol (TLC, 5 days). Then 50 mL of ethanol was added and the mixture ethanol–water was evaporated in vacuo. The residue was chromatographed on silica gel with hexanes–ethyl acetate 1:60 as eluent. Yield 65%. White solid. Mp: 71 °C (from hexane). $[\alpha]_D^{23}$ +54.9 (*c* 1.20, $CHCl_3$). ^{1}H NMR (δ): 0.97–1.16 (m, 2H); 0.98 (d, 3H, J=6.5 Hz); 1.06 (s, 3H); 1.23 (q, 1H, J=11.7 Hz); 1.48 (s, 3H); 1.54–1.70 (m, 3H); 1.75–1.78 (m, 1H); 1.99 (m, 1H); 3.67 (d, 1H, J=17.1 Hz); 3.73 (td, 1H, J_1=10.5 Hz, J_2=4.1 Hz); 3.95 (d, 1H, J=17.1 Hz); 5.99 (s, 1H); 6.90–7.14 (m, 7H); 7.34 (dd, 1H, J_1=7.9 Hz, J_2=1.2 Hz); 7.60 (dd, 1H, J_1=7.8 Hz, J_2=1.7 Hz). ^{13}C NMR (δ): 19.8; 22.4; 25.1; 27.8; 31.5; 35.2; 41.6; 46.0; 46.6; 58.2; 76.7; 87.3; 123.3; 125.4; 126.8; 127.0 (2C); 129.1; 130.2; 132.3; 138.8; 143.7. IR (Film): 3060; 3020; 1600; 1590; 1450; 750; 720; 690 cm^{-1}. CI/MS (*m*/*z*, %): 428 (M+1, 100), 430 (M+2, 90). Anal. Calcd for $C_{24}H_{30}BrNO$ (428.41): C, 67.29; H, 7.06; N, 3.27. Found: C, 67.20; H, 7.21; N, 3.18.

3.1.3. Synthesis of 3*H*-isobenzofuran-1-ones (7a–k). General method. A solution of the corresponding perhydrobenzoxazines **3a–k** (4 mmol) in ethanol (60 mL) and 2% aqueous hydrochloric acid (30 mL) was stirred at rt or refluxing (see experimental conditions in Table 2) until the hydrolysis was complete (TLC). The aqueous layer was extracted with hexane (3×60 mL). The organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting 1,3-dihydroisobenzofurans **6a–k** were redissolved in anhydrous CH_2Cl_2 (10 mL), MCPBA (4.07 mmol) and $BF_3{\cdot}Et_2O$ (0.74 mmol) were added under argon and the mixture was stirred at rt until the oxidation was finished (TLC, Table 2). The reaction mixture was quenched and made alkaline by addition of a saturated solution of sodium bicarbonate. The aqueous layer was extracted with diethyl ether (3×5 mL). The organic extracts were washed with brine and dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography on silica gel using hexanes–ethyl acetate as eluent. The enantiomeric excesses for compounds **7a–h** were determined as >99% by HPLC (Chiralcel OD column 0.46×25 cm; solvent ratio (hexane/IPA) 99:1 except for **7e**,**f** (90/10) and **7d** (98/2); flow rate: 1.1 mL/min for **7a**,**b**,**e**,**f**; 1.0 mL/min for **7c**,**d** and 1.2 mL/min for **7h**; and UV detection at 225 nm for **7a–d**,**h** and at 208 nm for **7e** and **7f**).

The (−)-8-benzylaminomenthol can be recovered in 90–95% from the aqueous phase of the hydrolysis simply by adding a solution of sodium carbonate to pH=8 and extraction with diethyl ether (4×50 mL). The ethereal solution was dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo giving a white solid, which was recrystallized from hexanes.

3.1.3.1. (*R*)-3-Methylisobenzofuran-1(3*H*)-one (7a). White solid. Mp: 47–49 °C (from hexane). $[\alpha]_D^{23}$ +41.4 (*c* 1.39, CH_2Cl_2). ^{1}H NMR (δ): 1.65 (d, 3H, J=6.7 Hz); 5.58 (q, 1H, J=6.7 Hz), 7.45 (dd, 1H, J_1=7.5 Hz, J_2=0.7 Hz); 7.53 (t, 1H, J=7.5 Hz); 7.69 (dt, 1H, J_1=7.6 Hz, J_2= 1.1 Hz); 7.91 (d, 1H, J=7.6 Hz). ^{13}C NMR (δ): 20.3; 77.7; 121.7; 125.3; 125.5; 129.0; 134.1; 151.1; 170.4. IR (Film): 1780; 1600; 1590; 1450; 770; 740; 700 cm^{-1}. MS (*m*/*z*, %): 149 (M+1, 100). EI/MS (*m*/*z*, %): 105 (100); 133 (56). Anal. Calcd for $C_9H_8O_2$ (148.16): C, 72.96; H, 5.44. Found: C, 73.08; H, 5.52. HPLC analysis: 3*S* isomer 24.59 min, 3*R* isomer 25.84 min.

3.1.3.2. (*R*)-3-Ethylisobenzofuran-1(3*H*)-one (7b). Colorless oil. $[\alpha]_D^{23}$ +78.7 (*c* 1.37, $CHCl_3$). ^{1}H NMR (δ): 1.00 (t, 3H, J=7.3 Hz); 1.83 (dq, 1H, J_1=14.5 Hz, J_2= 7.2 Hz); 2.14 (ddq, 1H, J_1=14.5 Hz, J_2=7.2 Hz, J_3= 4.3 Hz); 5.47 (dd, 1H, J_1=7.1 Hz, J_2=4.3 Hz); 7.45 (dd, 1H, J_1=7.5 Hz, J_2=1.0 Hz); 7.53 (t, 1H, J=7.5 Hz); 7.69 (dt, 1H, J_1=7.5 Hz, J_2=1.0 Hz); 7.88 (d, 1H, J=7.5 Hz). ^{13}C NMR (δ): 8.7; 27.5; 82.3; 121.7; 125.4; 126.1; 129.0; 133.9; 149.6; 170.7. IR (Film): 1770; 1600; 1590; 1450; 760; 740; 700; 690 cm^{-1}. EI/MS (*m*/*z*, %): 162 (M, 10); 133 (100); 105 (35). Anal. Calcd for $C_{10}H_{10}O_2$ (162.19): C, 74.06; H, 6.21. Found: C, 74.00; H, 6.02. HPLC analysis: 3*S* isomer 18.74 min, 3*R* isomer 20.16 min.

3.1.3.3. (*R*)-3-Isopropylisobenzofuran-1(3*H*)-one (7c). White solid. Mp: 59–60 °C (from hexane). $[\alpha]_D^{23}$ +73.0 (*c* 1.03, $CHCl_3$). ^{1}H NMR (δ): 0.80 (d, 3H, J=6.8 Hz); 1.13 (d, 3H, J=6.8 Hz); 2.24–2.35 (m, 1H); 5.38 (d, 1H, J=3.7 Hz); 7.47 (dd, 1H, J_1=7.4 Hz, J_2=0.9 Hz); 7.54 (t, 1H, J=7.4 Hz); 7.68 (dt, 1H, J_1=7.4 Hz, J_2=0.9 Hz); 7.88 (d, 1H, J=7.4 Hz). ^{13}C NMR (δ): 15.5; 18.6; 32.2; 85.6; 122.1; 125.5; 126.5; 128.9; 133.8; 149.8; 170.8. IR (Nujol): 1740; 1600; 730; 690 cm^{-1}. CI/MS (*m*/*z*, %): 177 (M+1,

100). Anal. Calcd for $C_{11}H_{12}O_2$ (176.21): C, 74.98; H, 6.86. Found: C, 74.79; H, 6.57. HPLC analysis: 3*S* isomer 11.37 min, 3*R* isomer 12.30 min.

3.1.3.4. (*R*)-3-Butylisobenzofuran-1(3*H*)-one (7d). Colorless oil. $[\alpha]_D^{23}$ +49.1 (*c* 0.65, $CHCl_3$). ^1H NMR (δ): 0.88 (t, 3H, *J*=7.0 Hz); 1.23–1.48 (m, 4H); 1.71–1.76 (m, 1H); 2.00–2.05 (m, 1H); 5.46 (dd, 1H, J_1=7.8 Hz, J_2=4.0 Hz); 7.42 (dd, 1H, J_1=7.5 Hz, J_2=1.0 Hz); 7.50 (t, 1H, *J*=7.5 Hz); 7.66 (dt, 1H, J_1=7.5 Hz, J_2=1.0 Hz); 7.88 (d, 1H, *J*=7.5 Hz). ^{13}C NMR (δ): 13.8; 22.4; 26.8; 34.4; 81.5; 122.7; 125.6; 125.7; 129.0; 134.0; 150.1; 180.7. IR (Film): 1740; 1610; 1590; 1450; 730; 710; 690 cm^{-1}. EI/MS (*m*/*z*, %): 190 (M, 1); 41 (100); 133 (39); 77 (27); 105 (13). Anal. Calcd for $C_{12}H_{14}O_2$ (190.24): C, 75.76; H, 7.42. Found: C, 75.90; H, 7.52. HPLC analysis: 3*S* isomer 11.79 min, 3*R* isomer 12.43 min.

3.1.3.5. (*R*)-3-Phenylisobenzofuran-1(3*H*)-one (7e). White solid. Mp: 152–153 °C (from hexane). $[\alpha]_D^{23}$ −45.5 (*c* 0.4, $CHCl_3$). ^1H NMR (δ): 6.41 (s, 1H); 7.27–7.41 (m, 6H); 7.57 (t, 1H, *J*=7.5 Hz); 7.65 (dt, 1H, J_1=7.5 Hz, J_2=1.1 Hz); 7.98 (d, 1H, *J*=7.5 Hz). ^{13}C NMR (δ): 82.6; 122.8; 125.6; 126.9 (2C); 128.9 (2C); 129.3; 134.3; 136.3; 149.6; 170.5. IR (Nujol): 1760; 1600; 1590; 770; 750; 700 cm^{-1}. CI/MS (*m*/*z*, %): 211 (M+1, 100); 133 (16). Anal. Calcd for $C_{14}H_{10}O_2$ (210.23): C, 79.98; H, 4.79. Found: C, 79.66; H, 5.07. HPLC analysis: 3*S* isomer 9.87 min, 3*R* isomer 12.76 min.

3.1.3.6. (*R*)-3-Benzylisobenzofuran-1(3*H*)-one (7f). White solid. Mp: 93–94 °C (from hexane). $[\alpha]_D^{23}$ +56.0 (*c* 1.12, $CHCl_3$). ^1H NMR (δ): 3.15 (dd, 1H, J_1=14.0 Hz, J_2=7.5 Hz); 3.23 (dd, 1H, J_1=14.0 Hz, J_2=7.5 Hz); 5.67 (t, 1H, *J*=6.4 Hz); 7.18–7.29 (m, 6H); 7.45 (t, 1H, *J*=7.5 Hz); 7.58 (t, 1H, *J*=7.5 Hz); 7.80 (d, 1H, *J*=7.5 Hz). ^{13}C NMR (δ): 40.8; 81.3; 122.4; 125.6; 126.1; 127.1; 128.5 (2C); 129.2; 129.7 (2C); 133.8; 135.0; 149.1; 170.4. IR (Nujol): 1730; 1600; 730; 700 cm^{-1}. CI/MS (*m*/*z*, %): 225 (M+1, 100); 133 (5). Anal. Calcd for $C_{15}H_{12}O_2$ (224.25): C, 80.34; H, 5.39. Found: C, 80.02; H, 5.28. HPLC analysis: 3*S* isomer 16.81 min, 3*R* isomer 17.38 min.

3.1.3.7. (*R*)-3-Allylisobenzofuran-1(3*H*)-one (7h). Colorless oil. $[\alpha]_D^{23}$ +74.5 (*c* 0.55, $CHCl_3$). ^1H NMR (δ): 2.62–2.75 (m, 2H); 5.12–5.16 (m, 1H); 5.20–5.21 (m, 1H); 5.52 (t, 1H, *J*=5.9 Hz); 5.75 (ddt, 1H, J_1=11.0 Hz, J_2=9.7 Hz, J_3=6.7 Hz); 7.27–7.52 (m, 2H); 7.67 (dt, 1H, J_1=7.4 Hz, J_2=1.0 Hz); 7.90 (d, 1H, *J*=7.5 Hz). ^{13}C NMR (δ): 38.6; 80.1; 119.6; 121.9; 125.6; 126.1; 129.1; 131.1; 133.9; 149.3; 170.3. IR (Film): 1750; 1640; 1620; 1600; 1470; 750; 720; 700 cm^{-1}. CI/MS (*m*/*z*, %): 175 (M+1, 100); 133 (31). Anal. Calcd for $C_{11}H_{10}O_2$ (174.20): C, 75.85; H, 5.79. Found: C, 75.67; H, 5.81. HPLC analysis: 3*S* isomer 22.21 min, 3*R* isomer 24.93 min.

3.1.3.8. (*S*)-3-Trifluoromethylisobenzofuran-1(3*H*)-one (7i). White solid. Mp: 44–45 °C (from hexane). $[\alpha]_D^{23}$ +32.7 (*c* 1.13, $CHCl_3$). ^1H NMR (δ): 5.53 (q, 1H, $^2J_{HF}$=5.8 Hz); 7.57 (dt, 2H, J_1=7.5 Hz, J_2=0.7 Hz); 7.68 (dt, 1H, J_1=7.5 Hz, J_2=1.1 Hz); 7.89 (d, 1H, *J*=7.5 Hz). ^{19}F NMR (δ): −77.1 (d, 3F, $^2J_{HF}$=5.8 Hz). ^{13}C NMR (δ): 76.4 (q, $^2J_{CF}$=35.7 Hz); 123.4; 126.3; 131.1; 134.9; 140.6; 168.3; 170.5. IR (Nujol): 1760; 1600; 1590; 1450; 770; 720; 700 cm^{-1}. CI/MS (*m*/*z*, %): 203 (M+1, 100). Anal. Calcd for $C_9H_5F_3O_2$ (202.13): C, 53.48; H, 2.49. Found: C, 53.08; H, 2.79.

3.1.3.9. (*R*)-Ethyl 2-(3-oxo-1,3-dihydroisobenzofuran-1-yl)acetate (7j). Yellow oil. $[\alpha]_D^{23}$ +4.8 (*c* 0.92, $CHCl_3$). ^1H NMR (δ): 1.27 (t, 3H, *J*=7.2 Hz); 2.91 (d, 2H, *J*=6.7 Hz); 4.21 (q, 2H, *J*=7.2 Hz); 5.89 (t, 1H, *J*=6.7 Hz); 7.50 (d, 1H, *J*=7.5 Hz); 7.55 (t, 1H, *J*=7.5 Hz); 7.69 (t, 1H, *J*=7.5 Hz); 7.90 (d, 1H, *J*=7.5 Hz). ^{13}C NMR (δ): 14.1; 39.5; 61.2; 77.0; 122.1; 125.7; 125.8; 129.5; 134.3; 148.7; 169.2; 169.9. IR (Film): 1760; 1730; 1600; 1590; 1450; 770; 740; 700 cm^{-1}. CI/MS (*m*/*z*, %): 221 (M+1, 100). Anal. Calcd for $C_{12}H_{12}O_4$ (220.22): C, 65.45; H, 5.49. Found: C, 65.56; H, 5.59.

3.1.3.10. (*S*)-Ethyl 2,2-difluoro-2-(3-oxo-1,3-dihydroisobenzofuran-1-yl)acetate (7k). Yellow oil. $[\alpha]_D^{23}$ +47.6 (*c* 0.84, $CHCl_3$). ^1H NMR (δ): 1.30 (t, 3H, *J*=7.1 Hz); 4.34 (q, 2H, *J*=7.1 Hz); 5.87 (dd, 1H, $^2J_{1HF}$=14.0 Hz, $^2J_{2HF}$=4.5 Hz); 7.64 (m, 2H); 7.75 (t, 1H, *J*=7.5 Hz); 7.95 (d, 1H, *J*=7.5 Hz). ^{19}F NMR (δ): −121.4 (dd, 1F, J_1=274.8 Hz, $^2J_{HF}$=14.0 Hz); −112.4 (dd, 1F, J_1=274.8 Hz, $^2J_{HF}$=4.5 Hz). ^{13}C NMR (δ): 13.8; 63.8; 76.6 (t, $^2J_{CF}$=32.2 Hz); 112.0 (dd); 123.8; 126.1; 126.3; 130.8; 134.7; 141.6; 161.5 (t); 168.6. IR (Film): 1776; 1760; 1602; 1468; 740; 726; 690 cm^{-1}. CI/MS (*m*/*z*, %): 257 (M+1, 100). Anal. Calcd for $C_{12}H_{10}F_2O_4$ (256.2): C, 56.26; H, 3.93. Found: C, 56.37; H, 4.05.

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A DFT study for the formation of imidazo[1,2-*c*]pyrimidines through an intramolecular Michael addition

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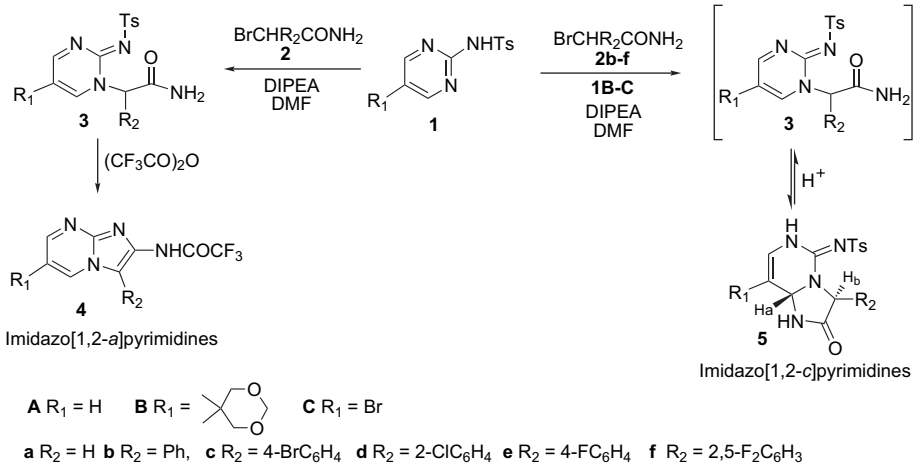
Abstract—The formation of imidazo[1,2-*c*]pyrimidines through a ring closure of 2-(2-sulfonylimino-1,2-dihydro-1-pyrimidinyl) acetamides has been studied using DFT methods. Analysis of the energy results for the cyclization step shows the demand of almost an acid catalyst, which increases the electrophilicity of the dihydropyrimidine moiety, in order to make feasible the intramolecular Michael addition. The substitution on both dihydropyrimidine and amide moieties has also an influence on the cyclization step.

1. Introduction

In a previous paper, we reported the synthesis of a series of imidazo[1,2-*a*]pyrimidines **4** using 2-tosylaminopyrimidines **1** as starting materials.[1] The alkylation of **1A** with 2-bromoacetamides **2a–f** and the alkylation of **1B**,**C** with 2-bromoacetamide **2a** afforded the corresponding dihydropyrimidines **3** that were converted into the target products **4** with trifluoroacetic anhydride. Nevertheless, the reaction of pyrimidines **1B**,**C** with 2-bromoacetamides **2b–f** afforded the hexahydroimidazo[1,2-*c*]pyrimidines **5** (Scheme 1).[1]

Several methods have been applied for the synthesis of imidazo[1,2-*c*]pyrimidines and the most frequently developed procedure involves the condensation of 4(6)-aminopyrimidine derivatives.[2] To the best of our knowledge, our finding was the first example of a synthesis of imidazo[1,2-*c*]pyrimidines starting from 2-aminopyrimidines and constitutes



Scheme 1.

Keywords: Pyrimidines; Cyclization; Michael addition; Electrophilicity; DFT calculations.
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a novel synthetic method for these compounds. The formation of the imidazo[1,2-*c*]pyrimidines **5** is the result of an intramolecular Michael addition of the carboxamide group to the α,β-unsaturated imine system. The detection of the open ring alkylated product **3Be**, by ^{13}C NMR, when imidazopyrimidine **5Be** was stirred with catalytic amounts of *p*-toluenesulfonic acid, showed that the attack of the carboxamide group to the α,β-unsaturated imine is a reversible reaction. The structure of the compound **5Ac** was determined by X-ray crystallography.[1] The trans disposition of the H_a and H_b hydrogens of the imidazopyrimidine **5Ac** confirmed that the addition of the amide to the unsaturated imine is diastereoselective with the nucleophilic attack occurring preferentially on the opposite face to the aryl group present on the amide substituent (Scheme 1). The formation of the hexahydroimidazo[1,2-*c*]pyrimidines **5** as major products only in the case of pyrimidines with R_1 and R_2 different from H was initially attributed to steric factors that induce a favorable conformation for the ring closure. However, they can be also obtained from unsubstituted sulfonamidopyrimidines **2A**. In fact, when the alkylated product **3Ac**, obtained from **1A** and **2c**, was stored in THF solution at room temperature for a week in the presence of catalytic amounts of diisopropylethylamine (DIPEA), the corresponding product **5Ac** was obtained (20%).

The nucleophilic attack of a carboxamide group to an α,β-unsaturated system is not very frequent and there are few references reported in the literature. The most common examples are intramolecular nucleophilic additions of a carboxamide group to strongly electrophilic iminium species, derived from the reaction of amino groups with aldehydes or ketones under acidic conditions[3] or dichloromethane[4] (Scheme 2). An example of an intermolecular addition was reported for the synthesis of the rolitetracycline.[5]

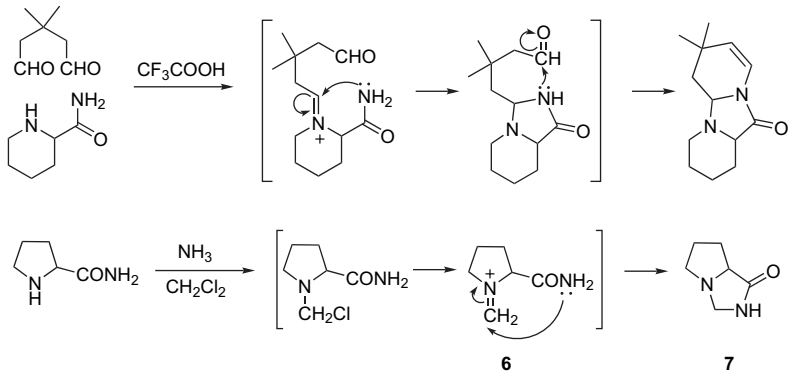
In the present paper, we have carried out a theoretical study for the cyclization reaction of the 2-(2-sulfonylimino-1,2-dihydro-1-pyrimidinyl) acetamides **3** with the formation of the hexahydroimidazo[1,2-*c*]pyrimidines **5**, using DFT methods at the B3LYP/6-31G* level. Two reaction models have been chosen. The first one corresponds to the cyclization of the iminium cation **6** (see Scheme 3). The second one corresponds to the formation of hexahydroimidazo[1,2-*c*]pyrimidines **5** through an intramolecular Michael addition. Finally, a DFT analysis based on the global reactivity indexes of the reactants involved in these intramolecular processes will be performed. Our main interest is to explain the participation of these dihydropyrimidyl acetamides in intramolecular Michael additions.

2. Computational methods

DFT calculations were carried out using the B3LYP[6] exchange-correlation functionals, together with the standard 6-31G* basis set.[7] The optimizations were carried out using the Berny analytical gradient optimization method.[8] The stationary points were characterized by frequency calculations in order to verify that the transition structures (TSs) have one and only one imaginary frequency. Inclusion of thermal corrections to enthalpies and entropies (computed at 25 °C and 1 atm) to the energies, increases the activation free energies for the intermolecular Michael additions in a narrow range from 0.4 to 1.0 kcal/mol. Thus, energy discussions will be made on the basis of the relative energies. The intrinsic reaction coordinate (IRC)[9] 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.[10] The electronic structures of stationary points were analyzed by the NBO method.[11] All calculations were carried out with the Gaussian 03 suite of programs.[12]

These reactions are carried out in polar solvents, and as solvent can modify the gas-phase activation energy, their effects have been studied. The solvent effects have been considered by B3LYP/6-31G* single point calculations on the gas-phase stationary points using a relatively simple self-consistent reaction field (SCRF) method[13] based on the polarizable continuum model (PCM) of the Tomasi's group.[14] The solvent used in the experimental work is dimethylformamide (DMF), ε=38.25. We have used dimethylsulfoxide (DMSO), which has also a large dielectric constant, ε=46.7.

The global electrophilicity index,[15] ω, which measures the stabilization energy when the system acquires an additional electronic charge ΔN from the environment, has been given by the following simple expression,[15] $\omega=(\mu^2/2\eta)$, 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.[16]

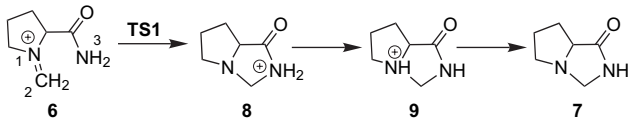


Scheme 2.

3. Results and discussions

3.1. Study of the cyclization reaction of the iminium cation 6

Firstly, the cyclization reaction of the iminium cation **6** was studied. This cyclization reaction is a one-step process associated with the nucleophilic attack of the amide N3 nitrogen atom to the iminium C2 carbon atom with formation of the C2–N3 bond (see Scheme 3). Therefore, the iminium cation **6**, the TS associated with the nucleophilic attack, **TS1**, and the intermediates **8** and **9** were located and characterized. The total energies in gas-phase and in DMSO are summarized in Table 1, while a schematic representation for the reaction profile of this cyclization is given in Figure 1.



Scheme 3.

Since some paths involve TSs and intermediates with charges, and solvent effects can stabilize these species, the energetic discussion will be performed using the energies obtained in DMSO. The activation energy associated with the nucleophilic attack of the amide N3 nitrogen atom to the iminium C2 carbon atom with the formation of the five-membered heterocycle is 21.4 (25.0) kcal/mol (energies in parenthesis correspond to the gas-phase calculations). Formation of the intermediate **8** is endothermic in 13.6 (20.5) kcal/mol. However, a proton transfer process from the amide N3 nitrogen atom to the amine N1 nitrogen atom converts the intermediate **8** into **9**, which is 24.8 (20.5) kcal/mol more stable (see Fig. 1). This large stabilization is related to the more basic character of the amine N1 nitrogen atom than the amide N3 one. Finally, an acid/base reaction will transform **9** into the final product **7**.

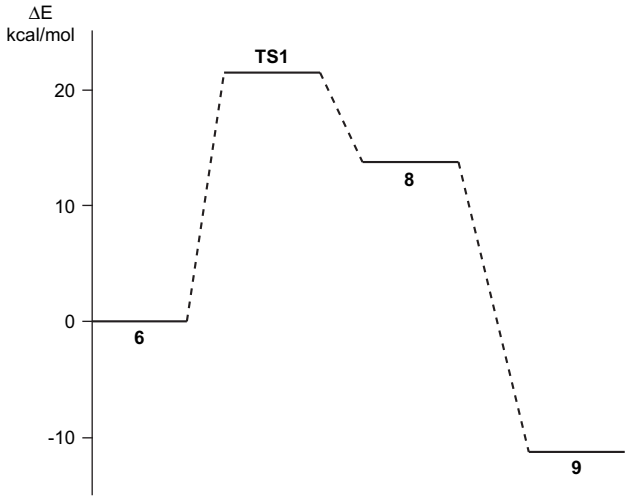


Figure 1. Reaction profile, in DMSO, for the cyclization reaction of the iminium cation **6**.

The geometry of **TS1** is given in Figure 2. The length of the C2–N3 forming bond at **TS1** is 1.967 Å, while at the intermediates **8** and **9** this length is 1.612 and 1.424 Å, respectively. The extent of bond-formation along a reaction pathway is provided by the concept of bond order (BO).[17] The BO value of the C2–N3 forming bond at **TS1** is 0.44, while the N1–C2 BO value is 1.37.

3.2. Study of the transformation of the 2-mesylaminopyrimidines 10 into imidazo[1,2-*c*]pyrimidines 15

The transformation of the 2-mesylaminopyrimidines **10** into imidazo[1,2-*c*]pyrimidines **15** requires two consecutive reactions: (i) the first one is a nucleophilic substitution of the 2-bromoacetamide derivatives **12** by the 2-mesylaminopyrimidines **11**, which are obtained by deprotonation of **10**, to give the *N*-substituted dihydropyrimidines **13**; (ii) the second reaction is a cyclization reaction of the dihydropyrimidines **13** to give the zwitterionic intermediate **14**, which by an acid/base process gives **15** (see Scheme 4). For these

Table 1. B3LYP/6-31G* total energies (in au) in gas-phase and in DMSO for the stationary points involved in the cyclization reactions of the iminium cation **6** and the dihydropyrimidines **13a–c**

	In gas-phase	In DMSO		In gas-phase	In DMSO
6	−419.754306	−419.841906	**18a**	−1115.950536	−1116.045935
TS1	−419.714505	−419.807827	**18b**	−1346.994052	−1347.089565
8	−419.721655	−419.820269	**18c**	−1653.477152	−1653.565623
9	−419.754241	−419.859759	**TS5a**	−1115.899471	−1116.006436
11a	−907.019956	−907.117406	**TS5b**	−1346.954958	−1347.055554
11b	−907.019956	−907.117406	**TS5c**	−1653.441006	−1653.530485
11c	−1213.493973	−1213.595640	**TS6a**	−1115.896746	−1116.005921
12a	−2780.315365	−2780.328801	**TS6b**	−1346.944796	−1347.045044
12b	−3011.366853	−3011.384590	**19a**	−1115.895976	−1116.008533
12c	−3011.366853	−3011.384590	**19b**	−1346.956359	−1347.059178
TS2a	−3687.327589	−3687.428311	**19c**	−1653.443485	−1653.536675
TS2b	−3918.379001	−3918.480054	**20a**	−1115.899883	−1116.009713
TS2c	−4224.861350	−4224.960196	**20b**	−1346.956628	−1347.061592
13a[a]	−3687.377833	−3687.484147	**20c**	−1653.448134	−1653.539694
13b[a]	−3918.426477	−3918.529842	**21a**	−1115.897050	−1116.008856
13c[a]	−4224.890958	−4225.007929	**21b**	−1346.948691	−1347.055303
16	−1695.979992	−1696.060038			
TS4	−1695.944237	−1696.019777			
17	−1696.007098	−1696.070158			

[a] Compound **13** plus Br^- total energies.

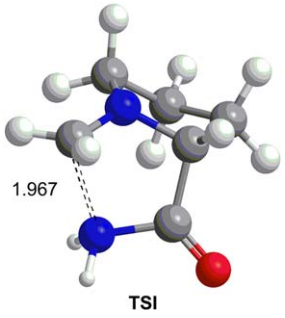


Figure 2. Transition structure, **TS1**, associated with the cyclization of the iminium cation **6**. The distance is given in Å.

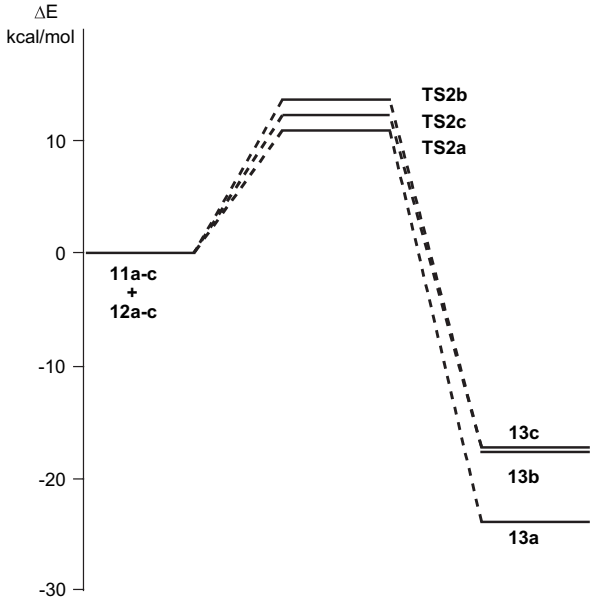


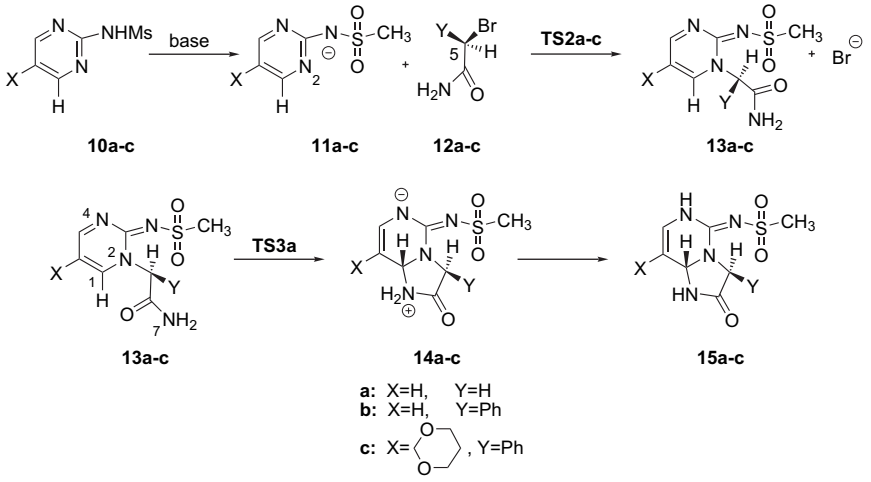
Figure 3. Reaction profiles, in DMSO, for the nucleophilic substitutions on the 2-bromoacetamides derivatives **12a–c**.

reactions, three computational models have been considered, which are related to the X (H or dioxane) and Y (H or Ph) substitutions on the pyrimidine and the acetamide moieties, respectively (*Model I* (X=H, Y=H), *Model II* (X=H, Y=Ph), and *Model III* (X=dioxane, Y=Ph)). In these computational models, the experimental *p*-toluenesulfonyl group has been substituted by the methanesulfonyl one.

3.2.1. Nucleophilic substitutions on the 2-bromoacetamides derivatives 12. The attachment of the acetamide on the 2-mesylaminopyrimidines **10** is carried out by a nucleophilic attack of the latter to the corresponding 2-bromoacetamide derivative **12**, to afford the dihydropyrimidines **13** (see Scheme 4). The total energies in gas-phase and in DMSO are summarized in Table 1, while a schematic representation for the reaction profiles of these nucleophilic substitutions is given in Figure 3.

The activation energies for the nucleophilic attacks of **11** to **12** are 11.2 (4.9) kcal/mol for *Model I*, 13.8 (4.9) kcal/mol for *Model II*, and 12.6 (−0.3) kcal/mol for *Model III* (see Fig. 3). These low values make the substitution reaction on the α position of the carboxamide group very favorable. Note that, in gas-phase, **TS2c** is located below reagents; however, the inclusion of diffuse functions by single point calculation at the B3LYP/6-31+G* level makes its barrier slightly positive (0.49 kcal/mol). These reactions are exothermic in −17 to −24 (−19 to −27) kcal/mol. Therefore, formation of the dihydropyrimidines **13** are kinetically and thermodynamically favored process. Inclusion of thermal corrections to enthalpies and entropies to the gas-phase energies, increases the activation free energies for the nucleophilic substitutions in a range from 13.7 to 15.3 kcal/mol, as a consequence of the bimolecular nature of these processes.

The geometries of the TSs are given in Figure 4. The lengths of the N2–C5 forming and C5–Br breaking bonds at the TSs are 1.954 and 2.626 Å at **TS2a**, 1.973 and 2.806 Å at **TS2b**, and 2.102 and 2.658 Å at **TS2c**, respectively. The N2–C5–Br angle at these TSs are 172.0° at **TS2a**, 169.0° at **TS2b**, and 166.5° at **TS2c**. For these S_N2 reactions there is a deviation of the linear rearrangement with the increase of the substitution on both reagents. The BO values of the N2–C5 forming and C5–Br breaking bonds at the TSs are 0.45 and 0.38 at **TS2a**, 0.46 and 0.25 at **TS2b**, and 0.35 and 0.35 at **TS2c**, respectively. While **TS2a** and **TS2b**, correspond to asynchronous processes where the C5–Br breaking bond is



Scheme 4.

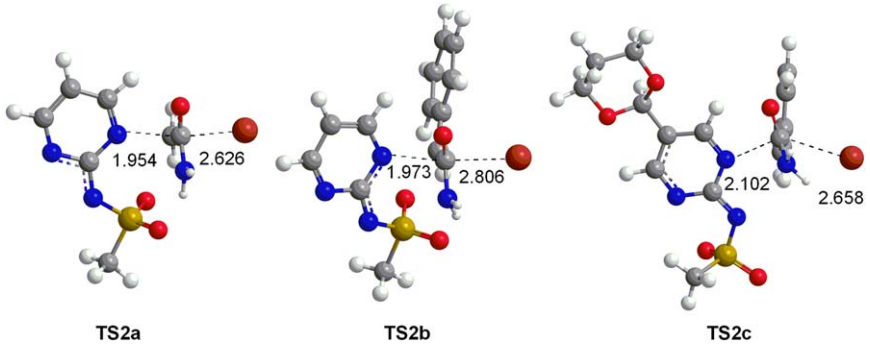


Figure 4. Transition structures, **TS2a–c**, associated with the nucleophilic substitutions on the 2-bromoacetamides derivatives **12a–c**. The distances are given in Å.

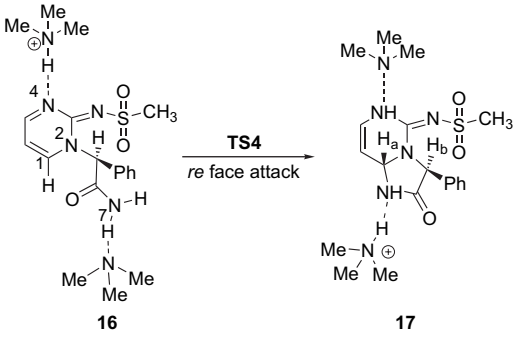
more advanced than the N2–C5 forming bond, **TS2c** corresponds to a synchronous process.

3.3. Study of the cyclization reaction of the dihydropyrimidines 13

The cyclization reaction of the dihydropyrimidines **13** to give the imidazo[1,2-*c*]pyrimidines **15** involves an intramolecular Michael addition of the amide N7 nitrogen atom to the C1 conjugate position of **13**. All attempts to locate the TSs and adducts associated with this intramolecular Michael addition at the three models were unsuccessful. Only HF/6-31G* calculations in DMSO allowed to find a TS for the reaction *Model I*, **TS3a**, but it presented a very high activation energy, 41.9 kcal/mol. Further optimizations of this structure at the B3LYP/6-31G* level yielded the dihydropyrimidine **13a**. These unfavorable energy results, which prevent the cyclization process on **13**, can be related to the low electrophilic character of the dihydropyrimidine ring and the low nucleophilic character of the amide residue (see later), as well as to the large energy associated with the formation of the zwitterionic structure **14**.

Then, the cyclization step with formation of the imidazo[1,2-*c*]pyrimidines **15** must take place through an acid/base catalysis. The acid/base catalysis has two favorable roles. While the acid catalysis increases the electrophilicity of the dihydropyrimidine moiety of **13**, favoring the Michael addition, the basic catalysis increases the nucleophilicity of the amide group. To study the effects of the acid/base catalysts in these cyclizations, two molecular models were considered. The first one corresponds to a basic catalysis achieved by the inclusion of a trimethylamine molecule, as a reduced model of DIPEA, hydrogen-bonded to an amide hydrogen atom. However, at DFT level it was not possible to find the TS associated with this base catalyzed cyclization; the energy for this process was estimated in ca. 50 kcal/mol. In addition, all attempts to find the corresponding product were unsuccessful because the ring cleavage to give the dihydropyrimidine **13** takes place without an appreciable barrier. These facts caused to discard the base catalyzed process. The second one is an acid/base catalysis where a trimethylammonium cation was also hydrogen-bonded to the pyrimidine N4 nitrogen atom.

For the acid/base catalyzed cyclization reaction of the *Model II*, both **TS4**, associated with the intramolecular addition by the *re*-face of the planar C1 carbon and the corresponding product **17** were located and characterized (see Scheme 5). The total energies in gas-phase and in DMSO are summarized in Table 1, while a schematic representation for the reaction profile of the acid/base catalyzed reaction is given in Figure 5.



Scheme 5.

The activation energy associated with the acid/base catalyzed intramolecular Michael addition of **16** via **TS4** is 25.3 (22.4) kcal/mol. This energy result suggests that the

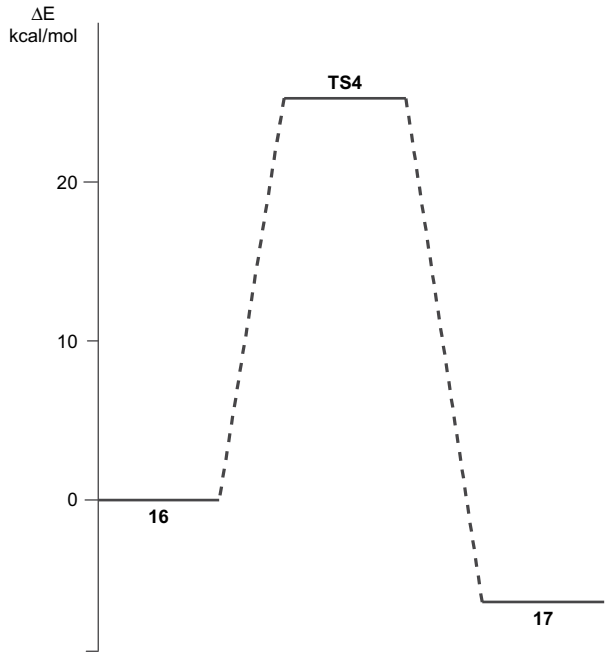


Figure 5. Reaction profile, in DMSO, for the acid/base catalyzed cyclization reaction of the dihydropyrimidine **13b**.

acid catalysis has a relevant role on the cyclization reaction of the dihydropyrimidines **13**, being essential the electrophilic activation of the pyrimidine ring. This value is ca. 4 kcal/mol larger than that obtained for the cyclization of iminium cation **6**, via **TS1**.

The geometry of **TS4** is given in Figure 6. The lengths of the C1–N7 forming bond at the TS of the acid/base catalyzed process is 1.864 Å. At this TS, the length of the amide N7–H breaking and the N4–H forming bonds are 1.098 and 1.632 Å, respectively. The BO value of the C1–N7 forming bond at this TS is 0.52, while the BO values for the amide N7–H breaking and the N4–H forming bonds are 0.60 and 0.18, respectively. In this catalyzed process N7–H breaking bond process is more advanced than the N4–H forming bond one.

Finally, in view of the necessity of an electrophilic activation of the dihydropyrimidine moiety to make possible the cyclization process, a simple acid catalyst model in which the N4 nitrogen of the dihydropyrimidines **13** was protonated, was considered (see Scheme 6). This reduced model allowed to perform a comparative study of the effects of the substitution on the cyclization step, as well as to study the diastereoselectivity found at the cyclizations of **5Ac**. Thus, one TSs, **TS5**, and two intermediates, **19** and **20**, for each reaction model were studied and characterized. The total energies in gas-phase and in DMSO are given in Table 1, while a schematic representation for the reaction profiles of the acid catalyzed reactions is given in Figure 7.

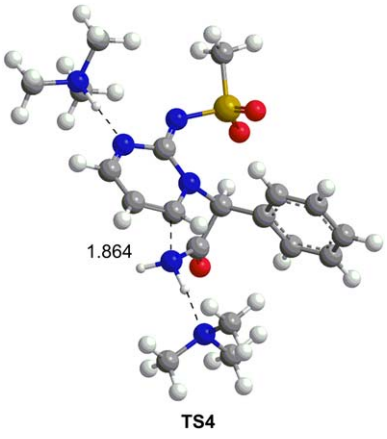


Figure 6. Transition structure, **TS4**, associated with the acid/base catalyzed cyclization of the dihydropyrimidine **13b**. The distance is given in Å.

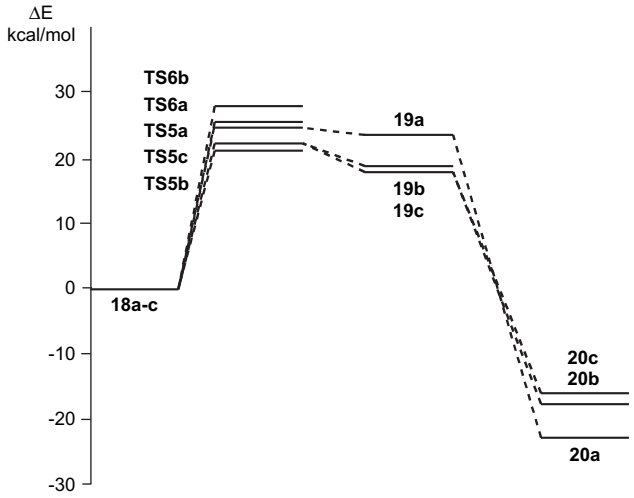
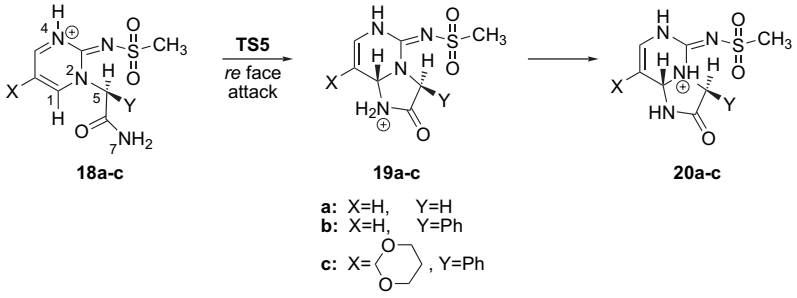


Figure 7. Reaction profiles, in DMSO, for the acid catalyzed cyclization reactions of the dihydropyrimidines **13a** and **b**.

The activation energies associated with the three cyclization models are 24.8 (32.0) kcal/mol for **TS5a**, 21.3 (24.5) kcal/mol for **TS5b**, and 22.0 (22.7) kcal/mol for **TS5c**. These values are closer to that obtained for the cyclization of the iminium cation **6** via **TS1**. These energy results indicate the requirement of a strong electrophilic activation of these dihydropyrimidines to make feasible the corresponding Michael addition. The presence of the phenyl substituent on the α position of the amide group decreases the activation energy of **TS5b** by 3.5 (7.5) kcal/mol relative to **TS5a**. Note that the effect of the phenyl substitution is larger in gas-phase. These acid catalyzed cyclization reactions are strongly endothermic, between 18 and 24 kcal/mol. However, the proton transfer process from the amide N7 nitrogen in **19** to the amine N2 one in **20** does these catalyzed processes exothermic in −16 to −23 kcal/mol (see Fig. 7).

The geometries of TSs are given in Figure 8. The length of the C1–N7 forming bond at the TSs is 1.757 Å at **TS5a**, 1.826 Å at **TS5b**, and 1.939 Å at **TS5b**. There is an increase of the length with the decrease of the gas-phase relative energy of the TS. At **TS5b** the C1–N7 length is closer to that for **TS1**. At the intermediates **19** and **20** the C1–N7 length is 1.618 and 1.444 Å at the *Model I*, 1.593 and 1.445 Å at the *Model II*, and 1.539 and 1.443 Å at the *Model III*. The C1–N7 lengths at the protonated amides **19** are larger than at protonated amines **20**, in clear agreement with the strong endothermic character of the intermediates **19**. The BO values of the C1–N7 forming bond at the TSs



Scheme 6.

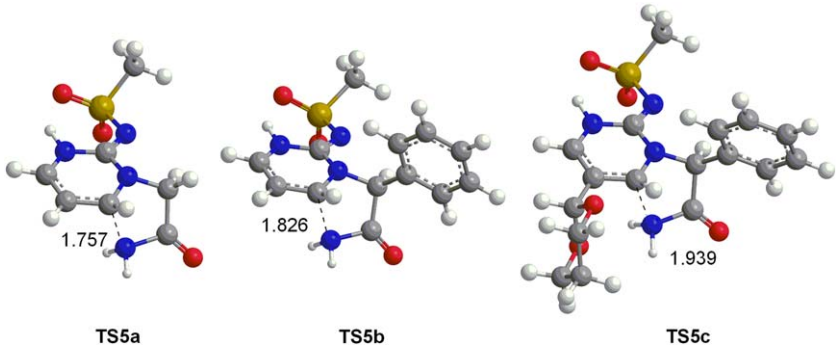
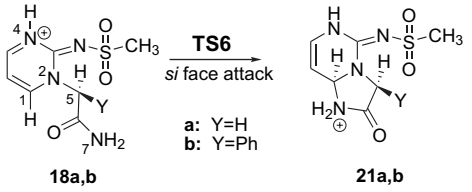


Figure 8. Transition structures, **TS5a–c**, associated with the acid catalyzed cyclizations of the dihydropyrimidines **13a** and **b**. The distances are given in Å.

are 0.61 at **TS5a**, 0.55 at **TS5b**, and 0.45 at **TS5c**. With the phenyl and dioxane substitutions, the TS for the cyclization becomes more earlier. This is in agreement with the lesser endothermic character of the corresponding protonated amide intermediate **19**.[18]

3.3.1. Study of the diastereoselectivity of the cyclization of the dihydropyrimidine 13c. The X-ray crystallographic analysis of a single crystal of **5Ac** confirmed unequivocally the structure and also the stereochemistry of the cyclization. The trans disposition of the hydrogen atoms attached to C1 and C5 indicates that the Michael addition is diastereoselective with the nucleophilic attack of the amide nitrogen occurring preferentially over the *re*-face of the planar C1 carbon (see Scheme 6). In order to explain the diastereoselectivity of this reaction, the TSs associated with the cyclization of **18a** and **18b** over the *si*-face of the planar C1 carbon were studied (see Scheme 7). The total energies in gas-phase and in DMSO are summarized in Table 1.



Scheme 7.

The activation energies associated with **TS6a** and **TS6b** are 25.1 (33.8) and 27.9 (30.9) kcal/mol. In DMSO, these TSs are 0.3 and 6.6 kcal/mol higher in energy than **TS5a** and **TS5b**, respectively (see Fig. 7). Thus, while in the absence of the phenyl group both diastereoisomeric TSs have similar energies, in the presence of the phenyl group on C5, the ring closure caused by the attack over the *si*-face is 6.6 kcal/mol higher in energy than the attack over the *re*-face, in clear agreement with the complete diastereoselectivity observed in the cyclization of the dihydropyrimidine **1Ac**. The low difference found at **TS6a** can be associated with the unlike conformations adopted by the cyclic five-membered **TS5a** and **TS6a**.

The geometries of the two diastereoisomeric TSs are given in Figure 9. The length of the C1–N7 forming bond at these TSs are 1.748 (**TS6a**) and 1.810 (**TS6b**) Å. These values are slightly shorter than that at the two diastereoisomeric TSs

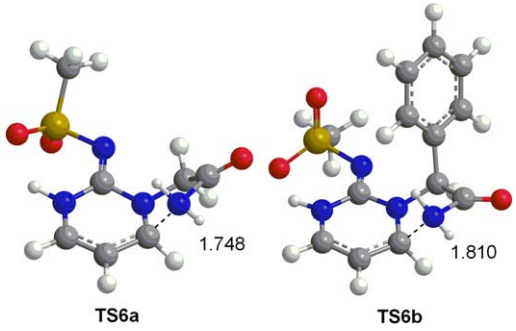


Figure 9. Transition structures, **TS6a** and **b**, associated with the diastereoisomeric paths of the acid catalyzed cyclizations of the dihydropyrimidines **13a** and **b**. The distances are given in Å.

TS5a and **TS5b**. Analysis of the geometry of **TS6b** indicates that the methanesulfonyl group is located over the plain of the phenyl group. The distance between both groups is of 3.8 Å. Therefore, the unfavorable interactions that appear between both groups at **TS6b**, which are absent at **TS5b**, are responsible for the diastereoselectivity found at the cyclization of **1Ac**. Finally, the BO values of the C1–N7 forming bond at these TSs are 0.63 (**TS6a**) and 0.56 (**TS6b**).

3.4. Analysis of the electrophilicity of the Michael acceptors

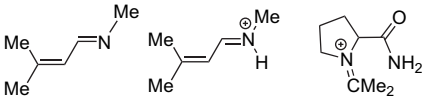
A recent study carried out on electrophilically activated C–C double bonds has shown that the analysis of the electrophilicity index, ω, is a powerful tool to analyze the participation of the C–C double bond in nucleophilic addition reactions such as the Michael addition.[19] Thus, the electrophilicity of some compounds related to these intramolecular cyclizations has been analyzed. In Table 2, the global reactivity indexes are given.

The α,β-unsaturated imine **24** has an electrophilicity value ω of 1.10 eV (see Table 2). It is classified as a moderate electrophile within the electrophilicity scale.[20] The dihydropyrimidine **13a** has electrophilicity value of 2.26 eV. Although it is classified as a strong electrophile, its relatively low value indicates that it will participate as Michael acceptor only toward strong nucleophiles. Note that the amide is a very poor nucleophile.

Protonation of the nitrogen atom of the imine **24** strongly increases notably the electrophilicity of iminium cation **22**,

Table 2. Global indexes of some Michael-type acceptors

	μ (au)	η (au)	ω (eV)
18a	−0.3364	0.1414	10.89
22	−0.3383	0.1810	8.60
6	−0.3318	0.1804	8.30
23	−0.3070	0.1919	6.68
13a	−0.1622	0.1586	2.26
24	−0.1281	0.2024	1.10



24 **22** **23**

ω=8.60 eV. This value is closer to that for the cyclic iminium cation **6**, 8.30 eV, for which the activation energy for the cyclization has been estimated in 21.4 kcal/mol. Note that methyl substitution on the terminal methylene of **6** decreases the electrophilicity of **23** to 6.68 eV, as a consequence of the electron-releasing character of the methyl groups.

Finally, protonation of the N4 nitrogen atom of the dihydropyrimidine **13a** increases the electrophilicity of **18a** to 10.89 eV, being the stronger electrophile of the series given in Table 2. This strong electrophilic activation of the pyrimidine ring accounts for the acid catalysis demanded for the cyclization of these dihydropyrimidines.

4. Conclusions

The transformation of 2-(2-sulfonylimino-1,2-dihydro-1-pyrimidinyl) acetamides into imidazo[1,2-*c*]pyrimidines through a ring closure reaction has been studied using DFT calculations at the B3LYP/6-31G* level. Calculations carried out on the neutral species indicate that the cyclization step has very high activation energy as a consequence of the low electrophilic character of the dihydropyrimidine moiety and the very low nucleophilic character of the appended amide. In addition, the solitary base catalysis is not sufficient to allow the cyclization process, being necessary an acid. This acid catalysis allows the intramolecular Michael addition by a strong electrophilic activation of the α,β-unsaturated imine system present on the dihydropyrimidine ring. In addition, the substitution on both dihydropyrimidine and amide moieties has also an influence on the activation energy associated with the cyclization process. Thus, the presence of the phenyl group on the amide, reduces the activation energies as well makes the cyclization to be diastereoselective.

Analysis of the electrophilicity index at some reagents involved in these cyclizations allows an understanding of the role of the acid catalyst. We can conclude that, although the carboxamide group can act as nucleophile and the α,β-unsaturated imine residue of the dihydropyrimidine ring can act as electrophile, a strong electrophilic activation of the dihydropyrimidine ring is necessary in order to make possible the corresponding intramolecular Michael addition.

Acknowledgements

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Arene-promoted lithiation of 1,*n*-dihaloalkanes (*n*=2–6): a comparative study

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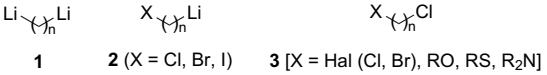
Abstract—The reaction of 1,*n*-dichloroalkanes **3a** (*n*=2–6) with an excess of lithium powder and a catalytic amount of 4,4′-di-*tert*-butylbiphenyl (DTBB; 2.5 mol %) in the presence of different carbonyl compounds [Bu^{t}CHO, PhCHO, Et_2CO, $(CH_2)_4CO$, $(CH_2)_5CO$, $(CH_2)_7CO$, (−)-menthone], in THF at −78 °C leads, after hydrolysis with water, to the expected 1,(*n*+2)-diols **4**, yields being <25% for *n*=2, 3 and in the range of 45–79% for *n*=4–6. When the same protocol is applied to 1,*n*-bromochloroalkanes **3b** and 1,*n*-dibromoalkanes **3c** (*n*=2–6), diols **4** are obtained in general with lower yields.

1. Introduction

From a synthetic point of view, the generation of dilithio compounds[1] of the type **1** (Chart 1) would be of great interest because their reaction with two molecules of an electrophile would allow the simultaneous introduction of two electrophilic fragments in the starting molecule through a single synthetic operation. The halogen–lithium exchange[2] is the most commonly used method to generate these intermediates, but this methodology cannot be applied in this case because the initially formed halogen-lithium compound **2** (Chart 1) is extremely unstable and suffers spontaneous elimination of lithium halide, thus preventing the second lithiation step.[3] Thus, **2** with *n*=1, the so-called lithium carbenoids, undergo α-elimination giving a carbene, which either decompose or can be trapped by an appropriate reagent.[4] Probably, the most dramatic situation appears for **2** with *n*=2, where the β-elimination affording an olefin occurs rapidly, even at very low temperatures (<−100 °C),[5] making impossible to prepare this type of intermediates and, consequently, the corresponding 1,2-dilithio compounds. In the case of *n*=3, it has been shown that the γ-elimination that gives a cyclopropane derivative works not so easily, so in some cases, the corresponding γ-functionalised organolithium compound can be trapped under mild reaction conditions.[6] For intermediates **2** with *n*=4–6 the elimination can be avoided partially under controlled reaction conditions,[7] which in general consist in performing the lithiation using an arene as electron carrier[8] at low temperature and in the presence of the corresponding electrophile (Barbier-type reaction conditions[9]). In the frame of our continuous interest on the lithiation of compounds of type **3**[10] (Chart 1) we report here the 4,4′-di-*tert*-butylbiphenyl (DTBB)-catalysed lithiation of different 1,*n*-dihaloalkanes **3a–c**[11] and their use as 1,*n*-dianionic synthetic equivalents in the reaction with different carbonyl compounds as electrophiles.



1 **2** (X = Cl, Br, I) **3** [X = Hal (Cl, Br), RO, RS, R_2N]

Chart 1.

2. Results and discussion

2.1. Lithiation of 1,*n*-dihaloalkanes 3 under Barbier-type reaction conditions (Method A)

The reaction of commercially available 1,*n*-dichloroalkanes **3a** with an excess of lithium (1:10 molar ratio; theoretic 1:4 molar ratio) and a catalytic amount of DTBB (1:0.1 molar ratio, 2.5 mol %) in the presence of different carbonyl compounds (1:3 molar ratio) in THF at −78 °C for ca. 3 h, followed by hydrolysis with water at temperatures ranging between −78 °C and room temperature (Method A), led to the diols **4** (Scheme 1 and Table 1).

As expected, yields are low for compounds **4** with *n*=2, 3 due to the above commented elimination side reaction problems, which gave ethylene and cyclopropane, respectively (Table 1, entries 1–6). However, in the other cases (**4** with *n*=4–6) this problem was extensively overcome, so the

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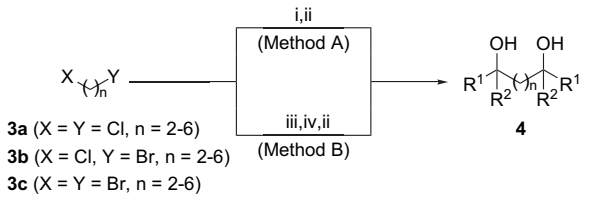


Scheme 1. Reagents and conditions: (i) Li, DTBB (2.5 mol %), R^1R^2CO, THF, −78 °C; (ii) H_2O, −78 to 20 °C; (iii) Li, DTBB (2.5 mol %), THF, −78 °C; (iv) R^1R^2CO, −78 °C.

expected diols were the main products isolated (Table 1, entries 7–17). When aldehydes were used as prochiral electrophiles, the corresponding ca. 1:1 mixtures of diastereomers (NMR) were obtained (Table 1, entries 1, 5, 7, 14 and 15, and footnote c). In the case of (−)-menthone, the attack of the organolithium intermediate to the upper less hindered face of the chiral electrophile[12] was exclusively observed, so the corresponding enantiomerically pure diol was the only reaction product obtained (Table 1, entries 4, 6, 10, 13 and 17, and footnote d).

Concerning a possible mechanistic pathway for the reaction shown in the Scheme 1, we think that after the first lithiation, the chloro-lithio intermediate **2** with X=Cl initially formed, which has a great tendency to undergo elimination of lithium chloride (see above, especially for n=2, 3), can also react with the electrophile present in the reaction medium to give the chloro-alkoxide **5**. This new intermediate then suffers a new chlorine–lithium exchange to afford the functionalised organolithium species **6**, which in the presence of the electrophile gives the corresponding dialkoxide **7**, precursor of the diols **4** by final hydrolysis (Chart 2). On the other hand, the participation of dilithium intermediates of the

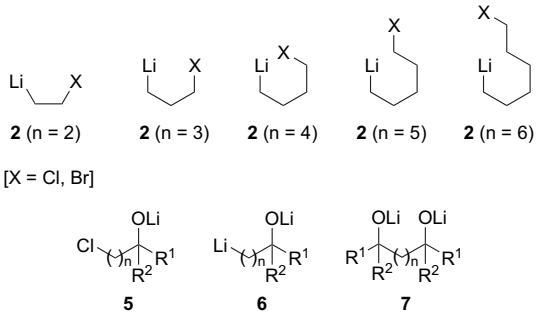
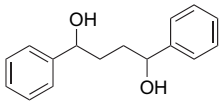
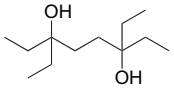
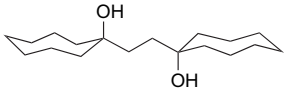
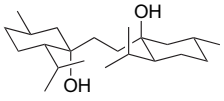
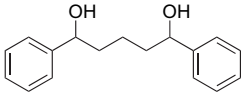
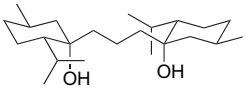
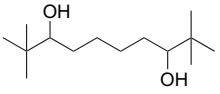
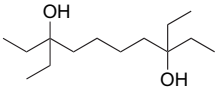


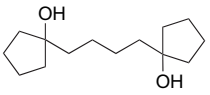
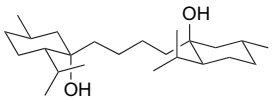
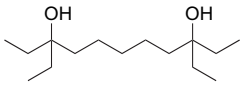
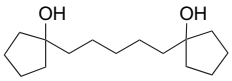
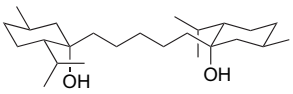
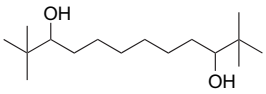
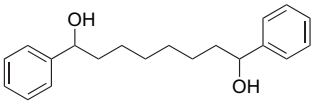
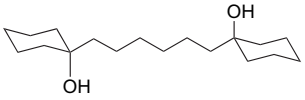
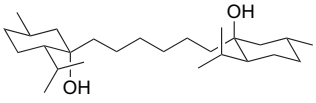
Chart 2.

Table 1. Double lithiation of 1,*n*-dichloroalkanes **3a** (preparation of compounds **4** (Method A))

Entry	*n*	Electrophile	Product[a]		
			No.	Structure	Yield (%)[b]
1	2	PhCHO	**4a**		23[c]
2	2	Et_2CO	**4b**		18
3	2	$(CH_2)_7CO$	**4c**		9
4	2	(−)-Menthone	**4d**		25[d]
5	3	PhCHO	**4e**		20[c]
6	3	(−)-Menthone	**4f**		15[d]
7	4	Bu^{t}CHO	**4g**		79[c]
8	4	Et_2CO	**4h**		45

(*continued*)

Table 1. (*continued*)

Entry	*n*	Electrophile	Product[a]		
			No.	Structure	Yield (%)[b]
9	4	$(CH_2)_4CO$	**4i**		62
10	4	(−)-Menthone	**4j**		67[d]
11	5	Et_2CO	**4k**		46
12	5	$(CH_2)_4CO$	**4l**		57
13	5	(−)-Menthone	**4m**		63[d]
14	6	Bu^tCHO	**4n**		64[c]
15	6	PhCHO	**4o**		78[c]
16	6	$(CH_2)_5CO$	**4p**		65
17	6	(−)-Menthone	**4q**		72[d]

[a] All products **4** were >95% pure (GLC and/or 300 MHz ^{1}H NMR) and were fully characterised by spectroscopic means (IR, ^{1}H and ^{13}C NMR, and LR and HR mass spectrometry).
[b] Isolated yields of compounds **4** after column chromatography (silica gel, hexane/ethyl acetate).
[c] Obtained as a ca. 1:1 mixture of diastereomers (NMR).
[d] The diastereomer shown in this table was exclusively obtained (see text).

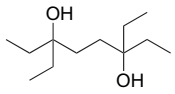
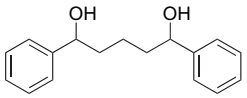
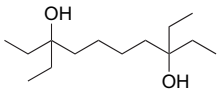
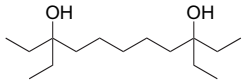
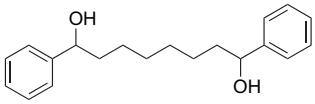
type **1** could be ruled out because the second lithiation of the already chlorinated organolithium intermediate is much more difficult than either the decomposition by elimination or the reaction with the electrophile present in the reaction medium.[13] The reaction conditions [(a) low temperature, (b) slow addition of the reagents (see Section 4.2) and (c) Barbier-type conditions] are essential for the preparation of diols **4** from the corresponding 1,*n*-dihaloalkane **3**.

The double lithiation under the same reaction conditions (Method A) of other starting materials, such as 1-bromo-*n*-chloroalkanes **3b** and 1,*n*-dibromoalkanes **3c** (both commercially available), was also studied. The results of this comparative study are summarised in Table 2. In general, yields are lower for compounds **3b** and **3c**, dichloro derivatives **3a** being the best substrates in this kind of processes. In the case of 1-bromo-*n*-chloroalkanes **3b**, we think that after the first lithiation, the choro-lithio intermediate **2** with X=Cl is formed due to the higher reactivity of the carbon–bromine bond towards the lithiation reagent. We have reported recently on the selective monolithiation of bromochloroalkanes (**2**, *n*=4, 5 and 6) (carbon–bromine bond undergoes reductive cleavage faster than carbon–chlorine bond) and also on the one-pot tandem introduction of two different electrophiles under careful reaction conditions.[7]

2.2. Lithiation of 1,*n*-dihaloalkanes 3 under Grignard-type reaction conditions (Method B)

In order to determine the stability of the intermediates **2** (X=Cl, Br; Chart 2), we studied the lithiation of compounds **3a–c** in the absence of the electrophile (Grignard-type

Table 2. Double lithiation of 1,*n*-dihaloalkanes **3a–c** (preparation of compounds **4**)

Entry	Starting material [X–$(CH_2)_n$–Y]				Electrophile	Product[a]			
						Yield (%)[b]		No.	Structure
	No.	X	Y	*n*		Method A	Method B		
1	**3a**	Cl	Cl	2		18	0		
2	**3b**	Cl	Br	2	Et_2CO	12	0	**4b**	
3	**3c**	Br	Br	2		2[c]	0		
4	**3a**	Cl	Cl	3		20	0		
5	**3b**	Cl	Br	3	PhCHO	7	0	**4e**	
6	**3c**	Br	Br	3		23	0		
7	**3a**	Cl	Cl	4		45	3[c]		
8	**3b**	Cl	Br	4	Et_2CO	31	2[c]	**4h**	
9	**3c**	Br	Br	4		22	0		
10	**3a**	Cl	Cl	5		46	4[c]		
11	**3b**	Cl	Br	5	Et_2CO	30	0	**4k**	
12	**3c**	Br	Br	5		16	0		
13	**3a**	Cl	Cl	6		78	23		
14	**3b**	Cl	Br	6	PhCHO	67	17	**4o**	
15	**3c**	Br	Br	6		53	0		

[a] All products **4** were >95% pure (GLC and/or 300 MHz ^1H NMR) and were fully characterised by spectroscopic means (IR, ^1H and ^{13}C NMR, and LR and HR mass spectrometry).
[b] Isolated yields of compounds **4** after column chromatography (silica gel, hexane/ethyl acetate).
[c] Yield determined by GLC analysis.

conditions). So, there would be a correlation between the yield of compounds **4** and the stability of the corresponding intermediates **2**. The reaction of compounds **3a–c** with an excess of lithium (1:10 molar ratio; theoretic 1:4 molar ratio) and a catalytic amount of DTBB (1:0.1 molar ratio, 2.5 mol %) in THF at −78 °C for 1 h, followed by addition of 2.2 equiv of a carbonyl compound at the same temperature and final hydrolysis with water at temperatures ranging between −78 °C and room temperature (Method B), would lead to the expected diols **4** (Scheme 1 and Table 2).

In the case of 1,2-dihalo derivatives **3a–c** (*n*=2, entries 1–3, Table 2), intermediates **2** (*n*=2) decomposed rapidly before a second lithiation took place, so, after the addition of 3-pentanone as electrophile, nothing of the expected diol **4b** was isolated or detected by tandem GC–MS analysis. This indicates, as previously commented, that intermediates of type **2** (*n*=2, 3) show a high tendency to undergo elimination. Such elimination takes place almost exclusively (Scheme 1, Table 2, entries 1–3) even in the presence of the electrophile (Barbier-type conditions), this probably being the main reason for the low yields. The possible participation of β-haloradicals in the synthesis of 1,4-diols **4** cannot be completely ruled out.

Intermediates **2** with *n*=3 seem to be also highly unstable because 1,5-diol **4e** was not observed after addition of benzaldehyde as electrophile (Table 2, entries 4–6).

Very low yield was also obtained from 1,4- and 1,5-dihaloalkanes **3a–c** (*n*=4, 5) when 3-pentanone was used as electrophile, the expected reaction products **4h** and **4k** not being isolated but detected by tandem GC–MS (Table 2, entries 7–9 and 10–12). This indicates that 4-halo- and 5-haloalkyllithium intermediates (**2**, *n*=4, 5, X=Cl, Br) were not stable species under these reaction conditions. Finally, when benzaldehyde was used as electrophile, low yields of diol **4o** were obtained from 1,6-dichloro- and 1-bromo-6-chlorohexanes **3a** and **3b** (*n*=6) (Table 2, entries 13 and 14), meanwhile, diol **4o** was not detected when starting from 1,6-dibromohexane (**3c**, *n*=6) (Table 2, entry 15). Paying attention to these experimental results, we can assume that the 6-chloro derivative (**2**, *n*=6, X=Cl) is more stable than the 6-bromoalkyllithium intermediate **2** (*n*=6, X=Br) under the commented reaction conditions. The best yields of 1,4-, 1,6-, 1,7- and 1,8-diols **4** were obtained from dichloroalkanes **3a** (*n*=2, 4, 5 and 6). The preparation of 1-chloro-4-lithiobutane[14] (**2**, *n*=4, X=Cl) and 1-chloro-6-lithiohexane[15] (**2**, *n*=6, X=Cl) by monolithiation of the corresponding *n*-chloro-1-iodoalkanes with butyllithium has been previously reported.

3. Conclusions

In conclusion, we report here for the first time the controlled lithiation of 1,*n*-dihaloalkanes under DTBB-promoted conditions, which allows the preparation of symmetrically substituted diols **4** by using carbonyl compounds as electrophiles. The reaction is especially of interest for *n*=2, 3 (even working with low yields in all cases)[16] due to the outstanding problems concerning the decomposition of the halogen-lithio intermediates by elimination, which are partially overcome in this study. The best yields are always obtained starting from 1,*n*-dichloroalkanes **3a**.

4. Experimental

4.1. General

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

4.2. Double lithiation of compounds 3a–c in the presence of a carbonyl compound as electrophile (Barbier-type reaction conditions, Method A). Preparation of diols 4

4.2.1. Isolation of compounds 4. Method A: general procedure. To a blue suspension of lithium powder (0.070 g, 10 mmol) and a catalytic amount of DTBB (0.027 g, 0.1 mmol) in THF (3 mL), a solution of the corresponding 1,*n*-dihaloalkane **3a–c** (1.0 mmol) and the corresponding carbonyl compound (R^1R^2CO, 3.0 mmol) in THF (1.2 mL) was slowly added (ca. 3 h) at −78 °C. After the addition, the reaction mixture was stirred for 15 min at the same temperature. Then, it was hydrolysed with water (4 mL) and extracted with ethyl acetate (3×10 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated (15 Torr). The residue was purified by column chromatography (silica gel; hexane/ethyl acetate) to yield pure products **4**. Yields and structures are included in Tables 1 and 2. Physical and spectroscopic data as well as literature references follow.

4.2.1.1. 1,4-Diphenylbutane-1,4-diol (4a).[17] Diastereomeric mixture. Colourless oil; R_f 0.19 (hexane/ethyl acetate: 2/1); ν (film) 3580–3170 (OH), 3060, 3029, 2935, 2875, 1460 cm^{-1}; δ_H 1.43 (2H, quintet, J=7.9 Hz, $CH_2CH_2CH_2$), 1.75–1.81 (4H, m, CH_2CH_2), 2.94 (2H, br s, 2×OH), 4.63–4.67 (2H, m, 2×C*H*OH), 7.23–7.31 (10H, m, ArH); δ_C 35.0, 35.9 (CH_2), 74.05, 74.45 (CHOH), 125.8, 127.35, 127.4, 128.35, 144.45 (ArC); *m*/*z* 224 (M^+-H_2O, 13%), 120 (73), 118 (100), 107 (38), 105 (22), 104 (16), 79 (48), 77 (44).

4.2.1.2. 3,6-Diethyloctane-3,6-diol (4b).[18] Colourless oil; R_f 0.21 (hexane/ethyl acetate: 2/1); ν (film) 3520–3190 (OH), 2972, 2935, 2880, 1460 cm^{-1}; δ_H 0.86 (12H, t, J=7.5 Hz, 4×CH_3), 1.45 [4H, s, $(CH_2)_2$], 1.48 (8H, q, J=7.5 Hz, 2×CH_3CH_2), 1.75 (2H, br s, 2×OH); δ_C 7.8 (CH_3), 30.9, 31.3 (CH_2), 74.4 (COH); *m*/*z* 166 (M^+-2H_2O, 1%), 156 (11), 155 (100), 137 (52), 98 (33), 95 (18), 87 (56), 83 (26), 69 (31), 57 (91), 55 (30); HRMS: M^+-H_2O, found 184.1833. $C_{12}H_{24}O$ requires 184.1827.

4.2.1.3. 1-[2-(1-Hydroxycyclooctyl)ethyl]cyclooctanol (4c).[19] White solid; mp 118–119 °C (dichloromethane/hexane); R_f 0.16 (hexane/ethyl acetate: 2/1); ν (KBr) 3470–3230 (OH), 2932, 2919, 2852, 1460 cm^{-1}; δ_H 1.25–1.78 (34H, m, 16×CH_2, 2×OH); δ_C 22.4, 25.0, 28.25, 34.2, 36.3 (CH_2), 74.65 (COH); *m*/*z* 264 (M^+-H_2O, 5%), 193 (42), 180 (29), 127 (100), 122 (46), 110 (54), 109 (56), 95 (29), 81 (63), 67 (51), 55 (59).

4.2.1.4. (1*S*,2*S*,5*R*,1′*S*,2′*S*,5′*R*)-1-[2-(1′-Hydroxy-2′-isopropyl-5′-methylcyclohexyl)ethyl]-2-isopropyl-5-methylcyclohexanol (4d). White solid; mp 122–123 °C (dichloromethane/hexane) (found: C, 77.96; H, 12.37. $C_{22}H_{42}O_2$ requires: C, 78.05; H, 12.50); R_f 0.64 (hexane/ethyl acetate: 2/1); ν (KBr) 3510–3280 (OH), 2965, 2954, 2868, 1455, 1371 cm^{-1}; δ_H 0.85–1.00 (20H, m, 6×CH_3, 2×CH), 1.05–1.67 (13H, m), 1.70–1.81 (6H, m), 1.90–2.15 (3H, m); δ_C 18.1 (CH_3), 19.9 (CH_3), 20.4 (CH_2), 22.5, 23.5 (CH_3), 24.3 (CH_2), 24.7, 25.25, 25.3 (CH), 25.8 (CH_2), 28.0, 29.85 (CH), 33.9, 35.05, 46.8, 47.1 (CH_2), 47.4, 54.4 (CH), 75.2, 75.4 (COH); *m*/*z* 320 (M^+-H_2O, 8%), 236 (17), 235 (100), 217 (18), 166 (21), 155 (64), 150 (51), 138 (51), 136 (31), 123 (30), 109 (21), 108 (17), 95 (51), 81 (42), 69 (38), 55 (29). $[\alpha]_D^{20}$ −15.9 (*c* 0.80, dichloromethane).

4.2.1.5. 1,5-Diphenylpentane-1,5-diol (4e).[20] Diastereomeric mixture. Colourless oil; R_f 0.19 (hexane/ethyl acetate: 2/1); ν (film) 3530–3180 (OH), 3062, 3029, 2980, 2938, 2865, 1454 cm^{-1}; δ_H 1.43 (2H, quintet, J=7.9 Hz, $CH_2CH_2CH_2$), 1.52–1.81 (4H, m, 2×CH_2CH), 2.56 (2H, br s, 2×OH), 4.58 (2H, t, J=7.3 Hz, 2×C*H*OH), 7.23–7.33 (10H, m, ArH); δ_C 22.05, 22.15, 38.6, 38.7 (CH_2), 74.1, 74.3 (CHOH), 125.8, 127.4, 128.35, 144.7 (ArC); *m*/*z* 238 (M^+-H_2O, 4%), 129 (22), 105 (20), 104 (100), 91 (18), 77 (21).

4.2.1.6. (1*S*,2*S*,5*R*,1′*S*,2′*S*,5′*R*)-1-[3-(1′-Hydroxy-2′-isopropyl-5′-methylcyclohexyl)propyl]-2-isopropyl-5-methylcyclohexanol (4f). Colourless oil; R_f 0.71 (hexane/ethyl acetate: 2/1); ν (film) 3630–3340 (OH), 2952, 2868, 1464, 1373 cm^{-1}; δ_H 0.82–1.78 (42H, m), 2.06–2.11 (2H, m); δ_C 18.0 (CH_2), 18.15 (CH_3), 20.5 (CH_2), 22.4, 23.6 (CH_3), 25.5, 28.0 (CH), 35.1, 41.9, 46.7 (CH_2), 48.1 (CH), 75.0 (COH); *m*/*z* 334 (M^+-H_2O, 1%), 165 (15), 164 (100), 163 (18), 149 (26), 137 (25), 135 (35), 121 (27), 109 (38), 107 (17), 95 (53), 93 (26), 83 (17), 81 (58), 69 (48), 67 (34), 55 (56), 43 (56), 41 (58); HRMS: M^+-H_2O, found 334.3233. $C_{23}H_{42}O$ requires 334.3236. $[\alpha]_D^{20}$ +4.2 (*c* 1.07, dichloromethane).

4.2.1.7. 2,2,9,9-Tetramethyldecane-3,8-diol (4g).[10c] Diastereomeric mixture. White solid; mp 113–114 °C (dichloromethane/hexane) (found: C, 72.70; H, 13.39. $C_{14}H_{30}O_2$ requires: C, 72.99; H, 13.39); R_f 0.44 (hexane/ethyl acetate: 2/1); ν (KBr) 3580–3220 (OH), 2972, 2865, 1470, 1390, 1371 cm^{-1}; δ_H 0.89 (18H, s, 6×CH_3), 1.26–1.54 (10H, m, 4×CH_2, 2×OH), 3.20 (2H, dd, J=8.3, 2.1 Hz, 2×C*H*OH); δ_C 25.6 (CH_3), 26.9, 27.1, 31.4 (CH_2), 34.9 (C), 79.7, 79.9 (CHOH); *m*/*z* 212 (M^+-H_2O, 1%), 155 (24), 137 (100), 99 (17), 97 (25), 95 (49), 83 (24), 81 (69), 71 (31), 69 (46), 67 (17), 57 (77), 55 (19).

4.2.1.8. 3,8-Diethyldecane-3,8-diol (4h).[21] White solid; mp 72–73 °C (dichloromethane/hexane); R_f 0.25 (hexane/ethyl

acetate: 2/1); ν (KBr) 3520–3230 (OH), 2963, 2943, 2878, 1461 cm^{-1}; δ_H 0.85 (12H, t, J=7.5 Hz, 4×CH_3), 1.27–1.40 [10H, m, $(CH_2)_4$, 2×OH], 1.46 (8H, q, J=7.5 Hz, 4×CH_2); δ_C 7.75 (CH_3), 24.0, 31.0, 38.15 (CH_2), 74.6 (COH); *m/z* 194 (M^+-2H_2O, 1%), 183 (13), 165 (65), 109 (23), 97 (43), 95 (20), 87 (100), 85 (24), 69 (38), 57 (77), 55 (21).

4.2.1.9. 1-[4-(1-Hydroxycyclopentyl)butyl]cyclopentanol (4i).[22] White solid; mp 98–99 °C (dichloromethane/hexane) (found: C, 74.20; H, 11.75. $C_{14}H_{26}O_2$ requires: C, 74.29; H, 11.58); R_f 0.13 (hexane/ethyl acetate: 2/1); ν (KBr) 3440–3180 (OH), 2957, 2869, 1434 cm^{-1}; δ_H 1.43–1.81 (26H, m, 12×CH_2, 2×OH); δ_C 23.7, 25.2, 39.55, 41.4 (CH_2), 82.4 (COH); *m/z* 208 (M^+-H_2O, 1%), 121 (14), 113 (13), 108 (100), 95 (15), 93 (31), 85 (28), 79 (12), 67 (31), 55 (18).

4.2.1.10. (1*S*,2*S*,5*R*,1′*S*,2′*S*,5′*R*)-1-[4-(1′-Hydroxy-2′-isopropyl-5′-methylacyclohexyl)butyl]-2-isopropyl-5-methylcyclohexanol (4j). White solid; mp 56–57 °C (dichloromethane/hexane) (found: C, 78.21; H, 12.54. $C_{24}H_{46}O_2$ requires: C, 78.63; H, 12.65); R_f 0.69 (hexane/ethyl acetate: 2/1); ν (KBr) 3610–3330 (OH), 2941, 2866, 1455, 1366 cm^{-1}; δ_H 0.83–0.98 (20H, m, 6×CH_3, 2×CH), 1.06–1.52 (17H, m), 1.60–1.77 (6H, m), 1.86–2.17 (3H, m); δ_C 18.1 (CH_3), 20.5 (CH_2), 22.4, 23.6 (CH_3), 24.7 (CH_2), 25.45, 28.0 (CH), 35.1, 41.3, 46.8 (CH_2), 47.7 (CH), 75.05 (COH); *m/z* 348 (M^+-H_2O, 25%), 330 (12), 196 (18), 194 (19), 178 (78), 163 (27), 155 (78), 137 (100), 135 (49), 95 (46), 83 (15), 81 (54), 69 (49), 55 (30). $[\alpha]_D^{20}$ +6.1 (*c* 0.84, dichloromethane).

4.2.1.11. 3,9-Diethylundecane-3,9-diol (4k). White solid; mp 82–83 °C (dichloromethane/hexane); R_f 0.27 (hexane/ethyl acetate: 2/1); ν (KBr) 3450–3210 (OH), 2968, 2933, 2878, 1466 cm^{-1}; δ_H 0.85 (12H, t, J=7.5 Hz, 4×CH_3), 1.25–1.42 [12H, m, $(CH_2)_5$, 2×OH], 1.45 (8H, q, J=7.5 Hz, 4×CH_2); δ_C 7.7 (CH_3), 23.3, 30.9, 30.95, 38.1 (CH_2), 74.55 (COH); *m/z* 208 (M^+-2H_2O, 2%), 197 (17), 179 (64), 123 (21), 111 (42), 109 (28), 97 (20), 95 (28), 87 (100), 85 (31), 69 (45), 57 (90), 55 (26).

4.2.1.12. 1-[5-(1-Hydroxycyclopentyl)pentyl]cyclopentanol (4l).[23] Colourless oil; R_f 0.18 (hexane/ethyl acetate: 2/1); ν (film) 3530–3210 (OH), 2968, 2933, 2859, 1462 cm^{-1}; δ_H 1.26–1.82 (28H, m, 13×CH_2, 2×OH); δ_C 23.8, 24.6, 30.7, 39.65, 41.4 (CH_2), 82.6 (COH); *m/z* 222 (M^+-H_2O, 1%), 204 (6), 136 (20), 135 (62), 123 (20), 122 (100), 121 (26), 108 (34), 95 (45), 93 (47), 85 (77), 81 (49), 80 (58), 67 (71), 57 (29), 55 (51).

4.2.1.13. (1*S*,2*S*,5*R*,1′*S*,2′*S*,5′*R*)-1-[5-(1′-Hydroxy-2′-isopropyl-5′-methylcyclohexyl)pentyl]-2-isopropyl-5-methylcyclohexanol (4m). White solid; mp 68–69 °C (dichloromethane/hexane) (found: C, 78.23; H, 12.60. $C_{25}H_{48}O_2$ requires: C, 78.88; H, 12.71); R_f 0.74 (hexane/ethyl acetate: 2/1); ν (KBr) 3560–3390 (OH), 2949, 2866, 2840, 1469, 1368 cm^{-1}; δ_H 0.86–0.91 (18H, m, 6×CH_3), 1.03–1.11 (4H, m), 1.22–1.49 (18H, m), 1.62–1.77 (6H, m), 2.03–2.12 (2H, m); δ_C 18.1 (CH_3), 20.45 (CH_2), 22.45, 23.6 (CH_3), 23.9 (CH_2), 25.45, 28.0 (CH), 31.0, 35.1, 41.3, 46.8 (CH_2), 47.6 (CH), 75.1 (COH); *m/z* 362 (M^+-H_2O, 14%), 344 (13), 319 (26), 208 (19), 165 (45), 155 (64), 137 (100), 123 (14), 109 (30), 97 (18), 95 (47), 81 (51), 69 (42), 55 (25). $[\alpha]_D^{20}$ +5.8 (*c* 0.91, dichloromethane).

4.2.1.14. 2,2,11,11-Tetramethyldodecane-3,10-diol (4n).[7] Diastereomeric mixture. White solid; mp 104–105 °C (dichloromethane/hexane) (found: C, 74.99; H, 13.81. $C_{16}H_{34}O_2$ requires: C, 74.36; H, 13.26); R_f 0.58 (hexane/ethyl acetate: 2/1); ν (KBr) 3580–3210 (OH), 2970, 2864, 1469, 1389, 1367 cm^{-1}; δ_H 0.89 (18H, s, 6×CH_3), 1.25–1.55 (14H, m, 6×CH_2, 2×OH), 3.18 (2H, dd, J=9.9, 2.0 Hz, 2×C*H*OH); δ_C 25.7 (CH_3), 27.1, 29.7, 31.4, 31.45 (CH_2), 34.9 (C), 79.9, 79.95 (CHOH); *m/z* 240 (M^+-H_2O, 1%), 183 (22), 165 (29), 109 (100), 97 (23), 95 (62), 83 (58), 81 (22), 71 (29), 69 (39), 67 (17), 57 (74), 55 (24).

4.2.1.15. 1,8-Diphenyloctane-1,8-diol (4o).[7] Diastereomeric mixture. White solid; mp 83–84 °C (dichloromethane/hexane); R_f 0.30 (hexane/ethyl acetate: 2/1); ν (KBr) 3520–3180 (OH), 3083, 3058, 3022 cm^{-1} (ArH); δ_H 1.19–1.69 (14H, m, 6×CH_2, 2×OH), 4.54 (2H, dd, J=7.2, 6.1 Hz, 2×C*H*OH), 7.21–7.29 (10H, m, 2×ArH); δ_C 25.1, 28.8, 38.4 (CH_2), 73.9 (CHOH), 125.3, 126.8, 127.8, 144.3 (ArC); *m/z* 280 (M^+-H_2O, 2%), 207 (37), 174 (64), 158 (15), 117 (52), 107 (100), 105 (26), 104 (83), 91 (30), 79 (61), 77 (36).

4.2.1.16. 1-[6-(1-Hydroxycyclohexyl)hexyl]cyclohexanol (4p).[23] White solid; mp 89–90 °C (dichloromethane/hexane) (found: C, 76.11; H, 12.36. $C_{18}H_{34}O_2$ requires: C, 76.54; H, 12.13); R_f 0.35 (hexane/ethyl acetate: 2/1); ν (KBr) 3570–3240 (OH), 2945, 1442 cm^{-1}; δ_H 1.25–1.60 (34H, m, 16×CH_2, 2×OH); δ_C 22.2, 22.7, 25.8, 30.2, 37.3, 42.3 (CH_2), 71.3 (COH); *m/z* 246 (M^+-2H_2O, 13%), 166 (26), 109 (21), 99 (100), 96 (39), 95 (20), 94 (21), 83 (17), 82 (17), 81 (59), 67 (24), 55 (35).

4.2.1.17. (1*S*,2*S*,5*R*,1′*S*,2′*S*,5′*R*)-1-[6-(1′-Hydroxy-2′-isopropyl-5′-methylcyclohexyl)hexyl]-2-isopropyl-5-methylcyclohexanol (4q). White solid; mp 54–55 °C (dichloromethane/hexane); R_f 0.77 (hexane/ethyl acetate: 2/1); ν (KBr) 3590–3370 (OH), 2952, 2866, 1455, 1366 cm^{-1}; δ_H 0.86–0.91 (18H, m, 6×CH_3), 1.02–1.12 (4H, m), 1.22–1.49 (20H, m), 1.64–1.77 (6H, m), 2.05–2.10 (2H, m); δ_C 18.1 (CH_3), 20.4 (CH_2), 22.45, 23.6 (CH_3), 23.8 (CH_2), 25.4, 27.95 (CH), 30.2, 35.1, 41.3, 46.8 (CH_2), 47.5 (CH), 75.1 (COH); *m/z* 376 (M^+-H_2O, 38%), 358 (18), 333 (65), 315 (18), 222 (13), 179 (36), 155 (81), 137 (100), 123 (20), 109 (30), 97 (21), 95 (49), 81 (55), 69 (43), 55 (25); HRMS: M^+-H_2O, found 376.3707. $C_{26}H_{48}O$ requires 376.3705. $[\alpha]_D^{20}$ +6.3 (*c* 1.09, dichloromethane).

4.3. Double lithiation of compounds 3a–c followed by reaction with carbonyl compounds as electrophiles (Grignard-type reaction conditions, Method B). Preparation of diols 4

4.3.1. Isolation of compounds 4. Method B: general procedure. To a blue suspension of lithium powder (0.070 g, 10 mmol) and a catalytic amount of DTBB (0.027 g, 0.1 mmol) in THF (3 mL) was added the corresponding 1,*n*-difunctionalised alkane **3a–c** (1.0 mmol) at −78 °C. The reaction mixture was stirred for 1 h at the same temperature and after that, the corresponding carbonyl compound

(2.2 mmol) was added dropwise and after 15 min it was hydrolysed with water (4 mL) and extracted with ethyl acetate (3×10 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated (15 Torr). The residue was purified by column chromatography (silica gel; hexane/ethyl acetate) to yield pure products **4**. Yields and structures are included in Table 2. Physical and spectroscopic data as well as literature references are shown above.

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Enantioselective total synthesis of both diastereomers of preclavulone-A methyl ester

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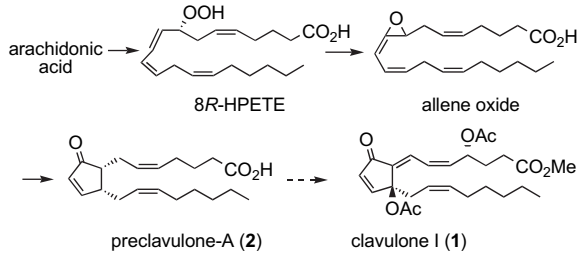
Abstract—The enantioselective total synthesis of preclavulone-A methyl ester and its diastereomer was achieved from enantiomerically pure **5** in a stereocontrolled manner. The absolute stereochemistry of naturally occurring preclavulone-A methyl esters was determined by comparison of the $[\alpha]_D$ value.

1. Introduction

Clavulones[1] (claviridenones[2]) and related marine prostanoids,[3] isolated from the Okinawan soft coral, *Clavularia viridis*, have received much attention owing to their structural features, significant biological activities, and unique biosynthesis. Corey et al. proposed a biosynthetic pathway of clavulones starting from oxidation of arachidonic acid by lipoxygenase (LOX) through (8*R*)-HPETE, allene oxide, and preclavulone-A (**2**) as shown in Scheme 1.[4]



Scheme 1. Biosynthetic pathway of calvulones proposed by Corey et al.

This biosynthesis was based on experimental results showing that **2** was obtained by treating labeled arachidonic acid as well as labeled (8*R*)-HPETE with the cell-free extract or acetone powder prepared from *C. viridis*, although the absolute configuration of **2** could not be determined due to its small amount. The trans diastereomer of preclavulone-A was also obtained in this biosynthetic experiment, but its absolute configuration was not determined.

Very recently, a trace amount of preclavulone-A methyl ester (**4**) and its diastereomer **3** was isolated from the methanol extract of *C. viridis* by our group (Fig. 1).[5] Interestingly, both compounds **4** and **3** were found to be an enantiomeric mixture (**4**: 8% ee, **3**: 46% ee) from the HPLC analysis using a chiral column. Preclavulone-A derivatives **3** and **4** were previously synthesized by Corey and Xiang (**4**: enantiomerically pure form)[6] and Traverso et al. (**3**: racemic form).[7] Although the determination of absolute stereochemistry of **4** was achieved by the comparison of $[\alpha]_D$ value reported by Corey et al., the absolute stereochemistry of trans isomer **3** could not be determined. Therefore, stereocontrolled synthesis of **3** as an enantiomerically pure form was required to establish the stereochemistry of the natural compounds and to clarify the detailed stereochemical course of the biosynthesis of clavulones. We have recently developed a novel stereocontrolled synthetic method for (±)-**3** and (±)-**4** in a highly stereoselective manner via the common intermediate **5**.[8] Now, both compounds **3** and **4** were prepared from an enantiomerically pure **5** and the predominant enantiomer of natural **3** and **4** was determined.

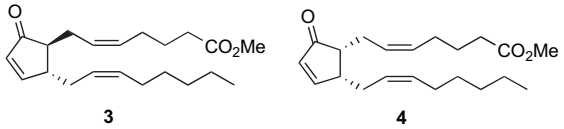


Figure 1.

2. Results and discussions

2.1. Retrosynthetic analysis

Our synthetic plan of compounds **3** and **4** each in enantiomerically pure form was the same as that for our previously

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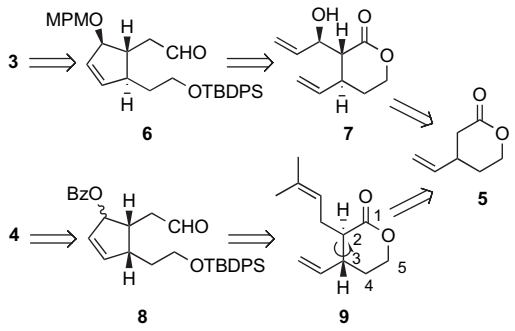
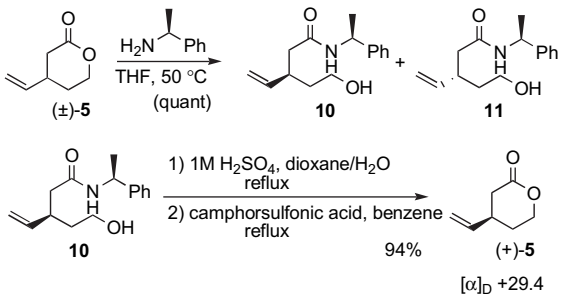


Figure 2. Retrosynthetic pathway of compounds **3** and **4**.

reported synthesis of racemic **3** and **4** as shown in Figure 2.[8] The α- and ω-chains of compounds **3** and **4** could be constructed from the corresponding aldehyde by Wittig reaction with high *Z*-selectivity. So, it was important to prepare compounds **6** and **8** in a highly stereocontrolled manner. For the construction of the cyclopentene ring of **6** and **8**, ring-closing olefin metathesis was employed. Compound **7** having a trans-relationship between the α- and β-substituents on the lactone was prepared from **5** through diastereoselective Mukaiyama aldol reaction using boron enolate. On the other hand, compound **9** could be prepared by the trans-selective α-alkylation of compound **5**. The 3-methyl-2-butenyl group was chosen as an alkylating agent because tri-substituted carbon–carbon double bond should not be reacted during the ring-closing olefin metathesis and is possible for the site-selective epoxidation. Introduction of vinyl group to carbonyl moiety and following ring-closing olefin metathesis could be obtained cis-disubstituted cyclopentene derivative **8**.

2.2. Synthesis of the common key intermediate 5

The preparation of optically pure compound **5** was essential for the enantioselective synthesis of **3** and **4**. Our main purpose in the synthesis of compounds **3** and **4** as enantiomerically pure form is for the determination of the absolute stereochemistry of natural **3** and **4**. So, both enantiomers of **5** could be applied for the synthesis of **3** and **4**. Additionally, racemic **5** was easily obtained by the 1,4-addition of vinylmagnesium chloride to 5,6-dihydro-2*H*-pyran-2-one.[9] Therefore, optical resolution of racemic **5** was selected for the preparation of enantiomerically pure **5** as shown in Scheme 2. Racemic **5** was reacted with (*S*)-1-phenylethylamine and

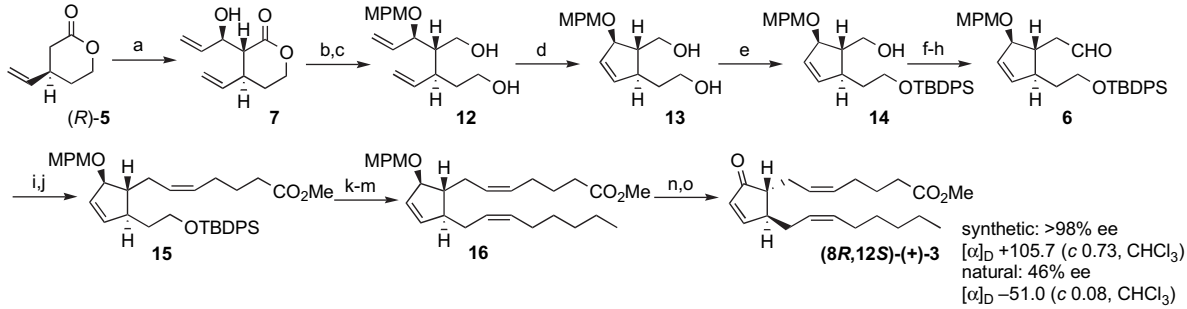


Scheme 2. Synthesis of enantiomerically pure **5**.

compounds **10** and **11** were obtained as a diastereomeric mixture (Scheme 2). After separation of each diastereomer by HPLC, compound **10** was converted to (+)-**5** ($[\alpha]_D$ +29.4) by acid hydrolysis and following lactonization using an acid catalyst in 91% yield. Compound (−)-**5** ($[\alpha]_D$ −29.2) was also prepared from compound **11** in the same manner. Absolute stereochemistry of compound **5** was determined by comparing optical rotation value of the literature after conversion of **5** to 4-ethyltetrahydropyran-2-one.[10]

2.3. Enantioselective and stereocontrolled total synthesis of 3

The total synthesis of (+)-**3** from (*R*)-(+)-**5** is shown in Scheme 3. The construction of **7** having two trans-related substituents on the lactone was achieved by Mukaiyama aldol reaction through boron enolate using dibutylboron triflate and diisopropylethylamine in a highly diastereoselective manner (80% yield, minor isomer could not be detected by crude ^{1}H NMR).[11] Although other metals (lithium, zinc, and magnesium) for the enolate were examined, the control of the stereochemistry of the newly formed chiral centers could not be achieved. After the conversion of compound **7** to **12** by protection of hydroxyl group and reduction of lactone moiety, the ring-closing olefin metathesis of **12** by the use of first generation Grubbs catalyst[12] proceeded to give compound **13** in 76% yield. One carbon elongation of the α-chain was achieved as follows. Mono-protection of the hydroxyl groups to obtain compound **14**, oxidation of another hydroxyl group, Wittig reaction by the use of methoxymethyltriphenylphosphonium chloride with butyllithium, and following acid hydrolysis of the resulting enol ether

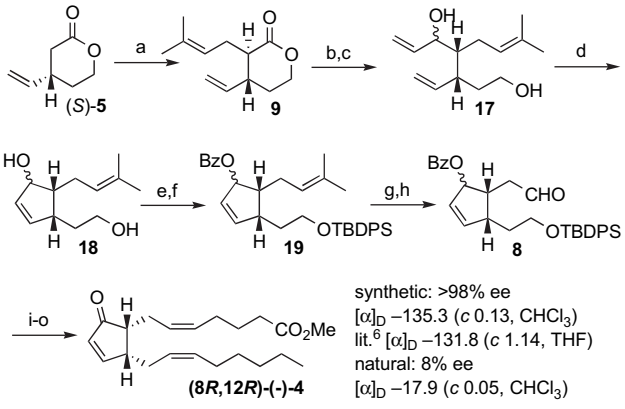


Scheme 3. Reagents and conditions: (a) Bu_2BOTf, $EtNiPr_2$, CH_2Cl_2, −78 °C, then acrolein, −78 °C to 0 °C, 80%; (b) *p*-methoxybenzyl trichloroacetimidate, camphorsulfonic acid, CH_2Cl_2, 0 °C to rt; (c) $LiAlH_4$, ether, rt, two steps 50%; (d) first Grubbs catalyst, toluene, 50 °C, 78%; (e) TBDPSCl, imidazole, DMF, rt, 53%; (f) Dess–Martin periodinane, CH_2Cl_2, rt, 95%; (g) $Ph_3P^+Cl^-CH_2OMe$, *n*-BuLi, THF, rt; (h) TsOH, acetone, rt, two steps, 50%; (i) $Ph_3P^+Br^-(CH_2)_4CO_2H$, NaHMDS, THF, rt, 78%; (j) CH_2N_2, ether, 0 °C, 90%; (k) TBAF, THF, rt, 99%; (l) Dess–Martin periodinane, CH_2Cl_2, rt, 89%; (m) $Ph_3P^+Br^-(CH_2)_5CH_3$, NaHMDS, THF, rt, 87%; (n) DDQ, CH_2Cl_2/H_2O, rt, 95%; and (o) Dess–Martin periodinane, CH_2Cl_2, rt, 98%.

gave compound **6**. Construction of the α- and ω-chains by Wittig reaction proceeded with high *Z*-selectivity (minor isomer could not be detected by ^{1}H NMR) to give compound **16**. Total synthesis of enantiomerically pure (8*R*,12*S*)-**3** (>98% ee, determined by HPLC using chiralcel OD-H) was achieved by deprotection of MPM group by treating with DDQ and following oxidation of resulting allylic alcohol moiety using Dess–Martin periodinane. Comparison of the optical rotation values of the synthetic and natural compounds indicated that the synthetic (8*R*,12*S*)-**3** was a minor enantiomer of natural **3** and a major enantiomer was (8*S*,12*R*)-**3**.

2.4. Enantioselective and stereocontrolled total synthesis of 4

Although the enantioselective total synthesis of **4** was achieved by Corey and Xiang,[6] an alternative total synthesis of **4** as an enantiomerically pure form was established by our developed synthetic route from (*S*)-(−)-**5** as shown in Scheme 4. Two chiral centers of **4** were constructed diastereoselectively through the α-alkylation of **5** by employing 1-bromo-3-methyl-2-butene. The reaction proceeded to give compound **9** as a single diastereomer in 94% yield. Compound **9** was converted to the precursor of the ring-closing olefin metathesis by half-reduction of the lactone moiety and following addition of vinylmagnesium chloride. The ring-closing metathesis of **17** was examined by the use of second generation Grubbs catalyst[13] and compound **18** was obtained as a diastereomeric mixture in 80% yield. If first generation of Grubbs catalyst was employed in this step, the reaction did not proceed. After the protection of two hydroxyl groups, the site-selective oxidative cleavage of the tri-substituted carbon–carbon double bond was achieved to give compound **8**. Stereoselective construction of both side chains was achieved in the same manner as mentioned for the synthesis of the trans isomer **3** through the Wittig reaction, and (8*R*,12*R*)-**4** was obtained as an enantiomerically pure form (>98% ee, determined by HPLC using chiralcel OD-H). Comparison of the optical rotation values of the synthetic and natural compounds indicated that (8*R*,12*R*)-**4** is a major enantiomer of the natural **4** and was in good accordance with the reported value of synthetic **4**.[6]



Scheme 4. Reagents and conditions: (a) LiHMDS, THF, −78 °C, then 1-bromo-3-methyl-2-butene, −78 °C to 0 °C, 94%; (b) DIBALH, ether, −78 °C to −20 °C, 98%; (c) vinylmagnesium chloride, THF, 0 °C to rt, 94%; (d) second Grubbs catalyst, CH_2Cl_2, rt, 80%; (e) TBDPSCl, triethylamine, DMAP, CH_2Cl_2, 0 °C, 98%; (f) benzoyl chloride, pyridine, DMAP, CH_2Cl_2, 0 °C, 78%; (g) *m*-CPBA, CH_2Cl_2, 0 °C, 80%; (h) HIO_4, H_2O, *t*-BuOH, rt, 99%; (i) $Ph_3P^+Br^-(CH_2)_4CO_2H$, NaHMDS, THF, −78 °C to rt, 76%; (j) CH_2N_2, ether, 0 °C, 90%; (k) TBAF, THF, rt, 98%; (l) Dess–Martin periodinane, CH_2Cl_2, rt, 99%; (m) $Ph_3P^+Br^-(CH_2)_5CH_3$, NaHMDS, THF, −78 °C to 0 °C, 98%; (n) NaOMe, MeOH, 50 °C, 88%; and (o) MnO_2, CH_2Cl_2, rt, 95%.

3. Conclusion

The enantioselective total syntheses of preclavulone-A methyl ester and its diastereomer were achieved from enantiomerically pure common intermediate **5** and the absolute stereochemistries of the predominant enantiomers of naturally occurring **3** and **4** were determined as shown in Figure 1. These results are an important information for the elucidation of the biosynthetic pathway of clavulones.

4. Experimental

4.1. General

All reactions were carried out under an argon atmosphere. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV-300 and the chemical shifts are given in parts per million using $CHCl_3$ (7.26 ppm) in $CDCl_3$ for ^{1}H NMR and $CDCl_3$ (77.0 ppm) for ^{13}C NMR as an internal standard. IR spectra were taken with a Perkin–Elmer PARAGON 1000 FT-IR and only noteworthy absorptions were listed. Mass spectra were measured on a Micromass LCT.

4.1.1. 3-(2-Hydroxyethyl)-4-pentenoic acid (1-phenylethyl) amide (10, 11). To a solution of racemic **5** (6.9 g, 54.7 mmol) in THF (20 mL) was added (*S*)-phenylethylamine (9 mL, 71.1 mmol) at ambient temperature and the mixture was stirred at 50 °C for 2 days. The mixture was concentrated under vacuum and the resulting residue was purified by silica gel column chromatography (AcOEt) and the compounds **10** and **11** were obtained as a diastereomeric mixture (13.5 g, 54.6 mmol, 99%). The resulting diastereomeric mixture was separated by HPLC (AcOEt/IPA, 10:1). Compound **10**: colorless oil. $[\alpha]_D^{24}$ −66.0 (*c* 0.65, $CHCl_3$). IR (neat) ν cm^{-1}: 699, 915, 995, 1050, 1548, 1643, 2930, 3284. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (3H, d, *J*=7.1 Hz), 1.63 (2H, q, *J*=6.4 Hz), 2.00 (1H, br s), 2.22 (1H, dd, *J*=7.3, 14.4 Hz), 2.28 (1H, dd, *J*=6.8, 14.4 Hz), 2.74 (1H, sext, *J*=7.2 Hz), 3.63 (2H, t, *J*=5.7 Hz), 5.04 (1H, dd, *J*=1.2, 10.2 Hz), 5.08 (1H, dd, *J*=1.2, 17.2 Hz), 5.13 (1H, quint, *J*=7.1 Hz), 5.68 (1H, ddd, *J*=8.3, 10.2, 17.2 Hz), 5.80 (1H, br d, *J*=7.2 Hz), 7.23–7.38 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.6, 37.1, 37.5, 42.0, 48.8, 60.5, 115.5, 126.2, 127.4, 128.7, 141.0, 143.0, 170.8. HREIMS calcd for $C_{15}H_{22}NO_2$: 248.1651 (M+H)$^+$, found: 248.1663. Compound **11**: colorless oil. $[\alpha]_D^{24}$ −96.0 (*c* 0.65, $CHCl_3$). IR (neat) ν cm^{-1}: 699, 916, 994, 1050, 1449, 1548, 1643, 2930, 3284. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (3H, d, *J*=6.9 Hz), 1.62 (2H, q, *J*=6.4 Hz), 2.20 (1H, dd, *J*=7.5, 14.3 Hz), 2.29 (1H, dd, *J*=6.7, 14.3 Hz), 2.73 (1H, sext, *J*=7.3 Hz), 3.57–3.72 (2H, m), 5.01 (1H, d, *J*=10.2 Hz), 5.05 (1H, d, *J*=17.2 Hz), 5.11

(1H, quint, J=7.1 Hz), 5.65 (1H, ddd, J=8.4, 10.2, 17.2 Hz), 6.01 (1H, br s), 7.21–7.37 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.6, 36.9, 37.4, 41.9, 48.8, 60.2, 115.5, 126.2, 127.4, 128.6, 140.9, 142.9, 171.2.

4.1.2. (4*R*)-4-Vinyltetrahydropyran-2-one ((+)-5). The mixture of **10** (1.07 g, 8.46 mmol) and H_2SO_4 (1 M in water, 10.2 mL, 10.2 mmol) in dioxane (10 mL) and water (10 mL) was heated under reflux for 1 h. After cooling at ambient temperature, the mixture was extracted with AcOEt three times and the resulting organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The mixture was diluted with benzene (10 mL) and camphorsulfonic acid (213 mg, 0.85 mmol) was added to the mixture. The mixture was refluxed with removal of water for 15 h. Then, saturated aqueous $NaHCO_3$ was added to the mixture and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography (hexane/AcOEt, 2:1) to afford the compound (+)-**5** as an enantiomerically pure form (1.54 g, 7.91 mmol, 94%). Colorless oil. $[\alpha]_D^{24}$ +29.4 (c 0.52, $CHCl_3$). IR (neat) ν cm^{-1}: 921, 996, 1737, 2977. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.56–1.73 (1H, m), 1.88–2.02 (1H, m), 2.28 (1H, dd, J=11.2, 18.8 Hz), 2.54–2.69 (2H, m), 4.22 (1H, ddd, J=3.9, 9.8, 11.4 Hz), 4.35 (1H, td, J=4.8, 11.4 Hz), 5.01 (1H, dd, J=1.0, 17.4 Hz), 5.02 (1H, dd, J=1.0, 10.1 Hz), 5.71 (1H, ddd, J=6.1, 10.1, 17.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.9, 35.5, 35.9, 68.7, 115.4, 140.0, 171.0.

4.1.3. (+)-4-Ethyltetrahydropyran-2-one. The mixture of (+)-**5** (20 mg, 0.16 mmol) and catalytic amount of 5% Pd/C in MeOH (2 mL) was stirred at ambient temperature under hydrogen atmosphere for 2 h. The reaction mixture was filtered and the filtrate was concentrated under vacuum and the resulting residue was purified by silica gel column chromatography (hexane/AcOEt, 3:1) to afford 4-ethyltetrahydropyran-2-one (9.1 mg, 0.071 mmol) in 44% yield. $[\alpha]_D^{24}$ +17.9 (c 0.52, $CHCl_3$). Colorless oil. $[\alpha]_D^{24}$ +27.8 (c 8.1, $CHCl_3$).[10] The spectral data was same as reported value.[10]

4.1.4. 3-(1-Hydroxy-2-propenyl)-4-vinyltetrahydropyran-2-one (7). To a solution of (*R*)-**5** (550 mg, 4.35 mmol) in CH_2Cl_2 (10 mL) was added dibutylboron trifluoromethanesulfonate (1 M in dichloromethane, 8.7 mL, 8.7 mmol) and diisopropylethylamine (1.86 mL, 10.9 mmol) was added at −78 °C. After being stirred for 2 h at the same temperature, acrolein (0.58 mL, 8.7 mmol) was added to the mixture and the resulting mixture was stirred at the same temperature for 1 h. After being stirred for further 2 h at 0 °C, phosphate buffer (pH 6.86, 10 mL), methanol (20 mL), and H_2O_2 (10 mL) were added to the mixture and the resulting mixture was stirred at ambient temperature for 16 h. The mixture was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 2:1) to afford **7** (630 mg, 3.46 mmol) in 80% yield. Colorless oil. $[\alpha]_D^{24}$ +43.1 (c 1.37, $CHCl_3$). IR (neat) ν cm^{-1}: 1122, 1727, 2918, 3432. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.68–1.82 (1H, m), 1.95–2.08 (1H, m), 2.51 (1H, dd, J=3.1, 9.4 Hz), 2.77 (1H, quint, J=7.6 Hz), 3.06 (1H, br s), 4.23–4.35 (2H, m), 4.35 (1H, br d, J=4.2 Hz), 5.13 (1H, d, J=10.3 Hz), 5.14 (1H, d, J=17.2 Hz), 5.15 (1H, d, J=10.3 Hz), 5.24 (1H, d, J=17.2 Hz), 5.74 (1H, ddd, J=8.1, 10.3, 17.2 Hz), 6.08 (1H, ddd, J=6.2, 10.3, 17.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 29.0, 37.7, 50.3, 67.0, 72.2, 116.0, 116.5, 138.8, 139.5, 172.3. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 65.60; H, 8.03. HREIMS calcd for $C_{10}H_{15}O_3$: 183.1021 $(M+H)^+$, found: 183.1012.

4.1.5. 4-Hydroxymethyl-5-(4-methoxybenzyloxy)-3-vinyl-6-hepten-1-ol (12). To a solution of **7** (1.0 g, 5.48 mmol) in CH_2Cl_2 (5 mL) was added a solution of MPM imidate (10 mmol) in cyclohexane (5 mL) and camphorsulfonic acid (125.2 mg, 0.5 mmol) and the mixture was stirred at ambient temperature for 24 h. The resulting mixture was filtered through Celite and concentrated under vacuum. To the residue, which was diluted with ether (20 mL) was added $LiAlH_4$ (249.6 mg, 6.58 mmol) at ambient temperature. After being stirred for 2 h, saturated aqueous Rochelle salt was added to the mixture and stirred for 1 h. The mixture was extracted with AcOEt and the organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 1:1) to afford **12** (839.5 mg, 2.74 mmol) in 50% yield. Colorless oil. $[\alpha]_D^{24}$ −15.8 (c 1.06, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33–1.49 (2H, m), 1.81–1.96 (1H, m), 1.94 (1H, br s), 2.53–2.66 (1H, m), 3.49–3.75 (3H, m), 3.80 (3H, s), 3.94 (1H, dd, J=1.8, 11.8 Hz), 4.07–4.16 (1H, m), 4.18 (1H, d, J=11.0 Hz), 4.51 (1H, d, J=10.0 Hz), 4.96 (1H, dd, J=1.8, 17.0 Hz), 5.06 (1H, dd, J=1.8, 10.2 Hz), 5.28 (1H, d, J=17.0 Hz), 5.33 (1H, d, J=9.8 Hz), 5.60 (1H, dt, J=9.8, 17.0 Hz), 5.84 (1H, ddd, J=7.0, 10.2, 17.0 Hz), 6.86 (2H, d, J=8.5 Hz), 7.21 (2H, d, J=8.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 33.7, 39.1, 48.3, 55.2, 60.2, 61.3, 70.7, 82.1, 113.8, 116.7, 117.8, 129.5, 129.9, 137.4, 141.0, 159.3. HREIMS calcd for $C_{18}H_{26}O_4Na$: 329.1729 $(M+Na)^+$, found: 329.1749.

4.1.6. 2-[5-Hydroxymethyl-4-(4-methoxybenzyloxy)-2-cyclopentenyl]ethanol (13). To a solution of **12** (227 mg, 0.74 mmol) in toluene (7 mL) was added benzylidenebis(tricyclohexylphosphine)dichlororuthenium (60.9 mg, 0.07 mmol) at 50 °C. After being stirred for 1.5 h at the same temperature, water was added to the mixture and the mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 1:1) to afford **13** (161.5 mg, 0.58 mmol) in 78% yield. Colorless oil. $[\alpha]_D^{24}$ −46.0 (c 1.51, $CHCl_3$). IR (neat) ν cm^{-1}: 1060, 1248, 1585, 1612, 2931, 3361. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.52–1.65 (2H, m), 1.80–1.93 (1H, m), 2.18–2.28 (1H, m), 2.52–2.62 (1H, m), 3.53 (1H, t, J=9.4 Hz), 3.68–3.78 (3H, m), 3.80 (3H, s), 4.26 (1H, br s), 4.49 (2H, s), 5.81 (1H, td, J=1.9, 5.6 Hz), 5.88 (1H, dd, J=1.5, 5.6 Hz), 6.87 (2H, d, J=8.6 Hz), 7.27 (2H, d, J=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 37.7, 45.1, 51.5, 55.3, 60.8, 65.4, 70.5, 86.6, 113.8, 129.1, 129.4, 130.4, 138.9, 159.2. HREIMS calcd for $C_{16}H_{23}O_4$: 279.1596 $(M+H)^+$, found: 279.1624.

4.1.7. [5-[2-(*tert*-Butyldiphenylsilanyloxy)ethyl]-2-(4-methoxybenzyloxy)-3-cyclopentenyl]methanol (14). To a solution of **13** (155 mg, 0.558 mmol) in DMF (2 mL) were added imidazole (83.6 mg, 1.228 mmol) and TBDPSCl (0.16 mL, 0.614 mmol) at 0 °C. After being stirred for 1 h, water was added to the reaction mixture. The mixture was extracted with AcOEt and the organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to afford **14** (150 mg, 0.29 mmol) in 53% yield. Colorless oil. $[\alpha]_D^{24}$ −48.8 (*c* 1.15, $CHCl_3$). IR (neat) ν cm^{-1}: 702, 1036, 1111, 1247, 1513, 1612, 2929, 3450. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.05 (9H, s), 1.53–1.67 (2H, m), 1.67–1.81 (1H, m), 2.03–2.14 (1H, m), 2.49–2.60 (1H, m), 3.65 (2H, d, *J*=7.0 Hz), 3.70–3.80 (2H, m), 3.82 (3H, s), 4.35 (1H, br s), 4.50 (2H, s), 5.78 (1H, dt, *J*=1.9, 5.7 Hz), 5.83 (1H, d, *J*=5.7 Hz), 6.87 (2H, d, *J*=8.6 Hz), 7.27 (2H, d, *J*=8.6 Hz), 7.34–7.47 (6H, m), 7.67 (4H, dd, *J*=1.5, 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.2, 38.2, 44.3, 52.8, 55.3, 62.5, 65.3, 70.5, 86.8, 113.8, 127.7, 129.3, 129.4, 129.7, 130.8, 133.5, 133.6, 135.5, 138.3. HREIMS calcd for $C_{32}H_{40}O_4NaSi$: 539.2594 $(M+Na)^+$, found: 539.2580.

4.1.8. [5-[2-(*tert*-Butyldiphenylsilanyloxy)ethyl]-2-(4-methoxybenzyloxy)-3-cyclopentenyl]acetaldehyde (6). To a solution of **14** (265 mg, 0.512 mmol) in CH_2Cl_2 (5 mL) was added Dess–Martin periodinane (239 mg, 0.564 mmol) at ambient temperature. After being stirred for 30 min, the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to afford aldehyde (251 mg, 0.487 mmol) in 95% yield. A solution of *n*-butyllithium (1.58 M in hexane, 1.74 mL, 2.7 mmol) was added to a suspension of methoxymethyltriphenylphosphonium chloride (858 mg, 2.5 mmol) in THF (15 mL) at −40 °C and the mixture was stirred at the same temperature for 1 h. A solution of aldehyde (247 mg, 0.481 mmol) in THF (5 mL) was added to the reaction mixture at the same temperature and the mixture was stirred at ambient temperature for 2 h. Saturated aqueous ammonium chloride was added to the reaction mixture and the mixture was extracted with ether. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was used for next step without further purification. To a solution of crude material in acetone (5 mL) was added a catalytic amount of *p*-toluenesulfonic acid (20 mg) and the mixture was stirred at ambient temperature for 1 h. Water was added to the mixture and the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to afford **6** (127 mg, 0.24 mmol) in 50% yield. Colorless oil. $[\alpha]_D^{24}$ −20.4 (*c* 1.11, $CHCl_3$). IR (neat) ν cm^{-1}: 702, 1111, 1248, 1722. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.04 (9H, s), 1.58–1.83 (2H, m), 2.21–2.33 (1H, m), 2.38–2.58 (3H, m), 3.73 (2H, t, *J*=6.3 Hz), 3.79 (3H, s), 4.21 (1H, d, *J*=3.0 Hz), 4.46 (2H, s), 5.79 (1H, td, *J*=1.8, 5.8 Hz), 5.87 (1H, br d, *J*=5.8 Hz), 6.86 (2H, d, *J*=8.7 Hz), 7.23 (2H, d, *J*=8.7 Hz), 7.33–7.47 (6H, m), 7.65 (4H, d, *J*=7.7 Hz), 9.73 (1H, t, *J*=2.6 Hz). Anal. Calcd for $C_{33}H_{40}O_4Si$: C, 74.68; H, 7.98. Found: C, 74.58; H, 7.70.

4.1.9. 7-[2-[2-(*tert*-Butyldiphenylsilanyloxy)ethyl]-5-(4-methoxybenzyloxy)-3-cyclopentenyl]-5-heptenoic acid methyl ester (15). To a suspension of 4-hydroxycarbonylbutyltriphenylphosphonium bromide (45.3 mg, 0.102 mmol) in THF (1.2 mL) was added a solution of NaHMDS (1 M in THF, 0.2 mL, 0.2 mmol) at 0 °C and the mixture was stirred at ambient temperature for 1 h. A solution of **6** (18 mg, 0.034 mmol) in THF (0.3 mL) was added to the mixture at 0 °C and the mixture was stirred at ambient temperature for 1 h. Saturated aqueous ammonium chloride was added to the reaction mixture and the resulting mixture was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 2:1) to afford carboxylic acid (16 mg, 0.026 mmol) in 76% yield. Colorless oil. $[\alpha]_D^{24}$ −16.1 (*c* 0.58, $CHCl_3$). IR (neat) ν cm^{-1}: 702, 1247, 1587, 1707, 3069. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.60–1.82 (4H, m), 1.83–1.92 (1H, m), 2.00–2.21 (4H, m), 2.32 (2H, t, *J*=7.5 Hz), 2.35–2.45 (1H, m), 3.73 (2H, t, *J*=6.4 Hz), 3.80 (3H, s), 4.17 (1H, br s), 4.46 (2H, s), 5.30–5.54 (2H, m), 5.76 (1H, td, *J*=1.9, 5.7 Hz), 5.58 (1H, dd, *J*=1.3, 5.7 Hz), 6.86 (2H, d, *J*=8.7 Hz), 7.26 (2H, d, *J*=8.7 Hz), 7.34–7.47 (6H, m), 7.68 (4H, d, *J*=7.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.2, 24.5, 26.6, 26.8, 31.3, 33.4, 38.7, 46.9, 49.9, 55.2, 62.5, 70.4, 88.8, 113.6, 127.6, 128.9, 129.3, 129.5, 130.9, 133.9, 135.5, 138.6, 159.0, 179.5. Anal. Calcd for $C_{38}H_{48}O_5Si$: C, 74.47; H, 7.89. Found: C, 74.44; H, 7.82. To a solution of carboxylic acid (16 mg, 0.026 mmol) in ether (2 mL) was added a solution of diazomethane in ether at 0 °C and the mixture was stirred for 15 min at the same temperature. After concentration under the reduced pressure, crude material was purified by silica gel column chromatography (hexane/AcOEt, 3:1) to afford **15** (14.7 mg, 0.023 mmol) in 90% yield. Colorless oil. $[\alpha]_D^{24}$ −10.0 (*c* 1.62, $CHCl_3$). IR (neat) ν cm^{-1}: 702, 1111, 1247, 1737, 2930. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.59–1.79 (4H, m), 1.79–1.92 (1H, m), 1.96–2.21 (4H, m), 2.29 (2H, t, *J*=7.5 Hz), 2.35–2.44 (1H, m), 3.66 (3H, s), 3.73 (2H, t, *J*=6.5 Hz), 3.80 (3H, s), 4.17 (1H, br s), 4.45 (2H, s), 5.34–5.52 (2H, m), 5.76 (1H, td, *J*=1.9, 5.8 Hz), 5.87 (1H, dd, *J*=1.3, 5.8 Hz), 6.86 (2H, d, *J*=8.6 Hz), 7.25 (2H, d, *J*=8.6 Hz), 7.34–7.48 (6H, m), 7.67 (4H, d, *J*=7.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 19.2, 24.8, 26.5, 26.7, 26.8, 31.3, 33.4, 38.7, 46.9, 49.9, 51.4, 55.2, 62.5, 70.4, 88.8, 113.7, 127.6, 127.7, 128.8, 129.2, 129.3, 129.5, 129.7, 131.0, 133.9, 134.8, 135.5, 138.5, 159.0, 174.0. Anal. Calcd for $C_{39}H_{50}O_5Si$: C, 74.72; H, 8.04. Found: C, 74.53; H, 8.06.

4.1.10. 7-[2-(4-Methoxybenzyloxy)-5-(2-octenyl)cyclopent-3-enyl]-5-heptenoic acid methyl ester (16). To a solution of **15** (74.6 mg, 0.119 mmol) in THF (2 mL) was added a solution of tetrabutylammonium fluoride (1 M in THF, 0.14 mL, 0.14 mmol) at ambient temperature. After being stirred for 2 h at the same temperature, the mixture was poured onto water and the mixture was extracted with

AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 2:1) to afford the alcohol (45.7 mg, 0.118 mmol) in 99% yield. Colorless oil. $[\alpha]_D^{24}$ −29.2 (*c* 1.17, $CHCl_3$). IR (neat) ν cm^{-1}: 1057, 1247, 1513, 1736, 2930, 3441. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.61–1.78 (4H, m), 1.94–2.16 (5H, m), 2.30 (2H, t, *J*=7.5 Hz), 2.29–2.42 (1H, m), 3.65 (3H, s), 3.62–3.72 (2H, m), 3.78 (3H, s), 4.15 (1H, br s), 4.45 (1H, d, *J*=11.4 Hz), 4.47 (1H, d, *J*=11.4 Hz), 5.35–5.51 (2H, m), 5.81 (1H, td, *J*=1.9, 5.7 Hz), 5.90 (1H, dd, *J*=2.0, 5.7 Hz), 6.85 (2H, *J*=8.7 Hz), 7.24 (2H, *J*=8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 24.7, 26.6, 31.8, 33.4, 37.8, 47.3, 49.0, 51.5, 55.2, 60.8, 70.5, 88.8, 113.7, 128.6, 129.3, 129.6, 130.1, 130.6, 138.6, 159.1, 174.1. Anal. Calcd for $C_{23}H_{32}O_5$: C, 71.11; H, 8.30. Found: C, 70.73; H, 8.26. To a solution of alcohol (39 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) was added Dess–Martin periodinane (55.1 mg, 0.13 mmol) at ambient temperature. After being stirred for 30 min, the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 3:1) to afford aldehyde (34.4 mg, 0.089 mmol) in 89% yield. To a suspension of hexyltriphenylphosphonium bromide (119.2 mg, 0.279 mmol) in THF (2 mL) was added a solution of NaHMDS (1 M in THF, 0.28 mL, 0.28 mmol) at 0 °C and the mixture was stirred at ambient temperature for 1 h. A solution of aldehyde (36 mg, 0.093 mmol) in THF (1 mL) was added to the mixture at 0 °C and the mixture was stirred at ambient temperature for 2 h. Saturated aqueous ammonium chloride was added to the reaction mixture and the resulting mixture was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 7:1) to afford **16** (36.8 mg, 0.081 mmol) in 87% yield. Colorless oil. $[\alpha]_D^{24}$ +5.6 (*c* 1.55, $CHCl_3$). IR (neat) ν cm^{-1}: 1247, 1513, 1613, 1739, 2926. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J*=6.8 Hz), 1.19–1.39 (6H, m), 1.68 (2H, quint, *J*=7.4 Hz), 1.84–1.92 (1H, m), 1.97–2.26 (9H, m), 2.31 (2H, t, *J*=7.5 Hz), 3.66 (3H, s), 3.80 (3H, s), 4.18 (1H, br s), 4.47 (2H, s), 5.32–5.52 (4H, m), 5.77 (1H, td, *J*=1.6, 5.7 Hz), 5.86 (1H, d, *J*=5.7 Hz), 6.86 (2H, d, *J*=8.6 Hz), 7.27 (2H, d, *J*=8.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.1, 22.6, 24.8, 26.7, 27.3, 29.4, 31.3, 31.5, 33.4, 33.5, 49.5, 50.3, 51.5, 55.3, 70.5, 89.0, 113.7, 127.5, 128.8, 129.3, 129.6, 129.7, 131.0, 131.2, 138.3, 159.0. HREIMS calcd for $C_{29}H_{42}O_4Na$: 477.2981 $(M+Na)^+$, found: 477.2955. Anal. Calcd for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 76.78; H, 9.19.

4.1.11. *trans*-Preclavulone-A methyl ester (3). To a solution of **16** (32 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (58.5 mg, 0.21 mmol) at ambient temperature. After being stirred for 1 h, water was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to afford an alcohol (22.2 mg, 0.067 mmol) in 95% yield. Colorless oil. $[\alpha]_D^{24}$ −20.3 (*c* 0.51, $CHCl_3$). IR (neat) ν cm^{-1}: 1260, 1731, 2924, 3446. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J*=6.8 Hz), 1.17–1.39 (6H, m), 1.60–1.70 (2H, m), 1.70 (2H, quint, *J*=7.4 Hz), 2.01 (2H, q, *J*=6.8 Hz), 2.10 (2H, q, *J*=7.2 Hz), 2.15–2.34 (5H, m), 2.33 (2H, t, *J*=7.4 Hz), 3.67 (3H, s), 4.43 (1H, d, *J*=1.9 Hz), 5.28–5.57 (4H, m), 5.72 (1H, td, *J*=1.9, 5.7 Hz), 5.80 (1H, td, *J*=1.5, 5.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.1, 22.6, 24.8, 26.7, 27.3, 29.3, 30.6, 31.5, 32.8, 33.4, 50.2, 51.5, 53.7, 82.7, 127.1, 128.7, 130.0, 131.5, 132.4, 137.3, 166.9. HREIMS calcd for $C_{21}H_{33}O_2$: 317.2481 $(M-H_2O+H)^+$, found: 317.2471. To a solution of the alcohol (22.0 mg, 0.066 mmol) in CH_2Cl_2 (1 mL) was added Dess–Martin periodinane (42.4 mg, 0.1 mmol) at ambient temperature. After being stirred for 30 min, the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 7:1) to afford **3**[5] (21.5 mg, 0.0647 mmol) in 98% yield. Colorless oil. $[\alpha]_D^{24}$ +105.4 (*c* 0.74, $CHCl_3$).

4.1.12. (3*R*,4*R*)-3-(3-Methyl-2-butenyl)-4-vinyltetrahydropyran-2-one (9). To a solution of (*S*)-**5** (1.07 g, 8.46 mmol) in THF (20 mL) was added a solution of LiHMDS (1 M in THF, 10.2 mL, 10.2 mmol) at −78 °C and the mixture was stirred at the same temperature for 1 h. 1-Bromo-3-methyl-2-butene (1.51 g, 10.2 mmol) was added to the reaction mixture at the same temperature and the resulting mixture was stirred for 3 h. Then, saturated aqueous ammonium chloride was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 10:1) to afford **9** (1.54 g, 7.91 mmol) in 94% yield. Colorless oil. $[\alpha]_D^{24}$ −24.0 (*c* 2.25, $CHCl_3$). IR (neat) ν cm^{-1}: 1190, 1732, 2922. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.61 (3H, s), 1.70 (3H, s), 1.70–1.82 (1H, m), 1.88–1.97 (1H, m), 2.26–2.58 (4H, m), 4.24 (1H, ddd, *J*=3.7, 9.1, 11.2 Hz), 4.35 (1H, ddd, *J*=4.4, 5.3, 11.2 Hz), 5.05–5.15 (3H, m), 5.70 (1H, ddd, *J*=6.4, 10.5, 16.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.6, 26.4, 28.3, 29.7, 40.3, 46.2, 68.0, 116.6, 121.0, 134.9, 140.4, 173.7.

4.1.13. 4-(3-Methyl-2-butenyl)-3-vinyl-6-heptene-1,5-diol (17). To a solution of **9** (1.26 g, 6.41 mmol) in THF (20 mL) was added a solution of DIBAL (0.93 M in hexane, 8.15 mL, 7.58 mmol) at −78 °C. After being stirred at −20 °C for 4 h, saturated aqueous potassium sodium tartrate was added to the reaction mixture and the mixture was stirred at ambient temperature for 1 h. The mixture was extracted with AcOEt and the organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to afford lactol (1.32 g, 6.73 mmol) in 98% yield. Colorless oil. ^1H NMR (300 MHz, $CDCl_3$)

δ: 1.20–2.41 (6H, m), 1.59 (3H, s), {1.67 (s), 1.68 (s), 3H}, {2.84 (dd, *J*=1.0, 3.2 Hz), 3.35 (d, *J*=6.2 Hz), 1H}, 3.45–3.62 (1H, m), 3.92–4.13 (1H, m), 4.49 (0.4H, dd, *J*=6.0, 8.2 Hz), 4.97–5.23 (3.6H, m), {5.58 (ddd, *J*=8.7, 10.0, 17.1 Hz), 5.64 (ddd, *J*=8.5, 10.2, 17.2 Hz), 1H}. To a solution of lactol (1.26 g, 6.41 mmol) in THF (20 mL) was added a solution of vinylmagnesium chloride (1.38 M in THF, 18.6 mL, 25.7 mmol) at 0 °C. After being stirred for 1 h, saturated aqueous ammonium chloride was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 1:1) to afford **17** (1.36 g, 6.06 mmol) in 94% yield. Compound **17a**: colorless oil. IR (neat) ν cm^{-1}: 916, 994, 1044, 1436, 2915, 3345. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.55–1.83 (3H, m), 1.60 (3H, s), 1.68 (3H, s), 2.01–2.20 (2H, m), 2.46 (1H, sept, *J*=4.7 Hz), 3.60 (1H, ddd, *J*=6.2, 7.2, 10.7 Hz), 3.68 (1H, td, *J*=6.1, 10.7 Hz), 4.23 (1H, t, *J*=5.4 Hz), 5.06 (1H, dd, *J*=2.0, 17.2 Hz), 5.07 (1H, dd, *J*=2.0, 10.3 Hz), 5.14 (1H, d, *J*=10.3 Hz), 5.15–5.22 (1H, m), 5.22 (1H, d, *J*=17.2 Hz), 5.73 (1H, ddd, *J*=9.4, 10.3, 17.2 Hz), 5.90 (1H, ddd, *J*=6.0, 10.3, 17.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 25.5, 25.9, 35.5, 41.6, 48.1, 61.2, 75.2, 115.4, 116.4, 124.0, 132.3, 139.8, 140.6. Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 74.72; H, 10.56. Compound **17b**: colorless oil. IR (neat) ν cm^{-1}: 918, 995, 1440, 2926, 3346. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.50–1.72 (3H, m), 1.61 (3H, s), 1.69 (3H, s), 1.86 (1H, br s), 1.99–2.21 (2H, m), 2.55–2.67 (1H, m), 3.54–3.71 (2H, m), 4.13 (1H, br t, *J*=7.1 Hz), 5.06–5.15 (3H, m), 5.18 (1H, td, *J*=1.5, 10.5 Hz), 5.26 (1H, td, *J*=1.6, 17.2 Hz), 5.76–5.87 (1H, m), 5.91 (1H, ddd, *J*=5.3, 10.5, 17.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.9, 25.2, 25.8, 35.9, 40.4, 48.5, 61.1, 74.6, 115.0, 116.7, 123.6, 132.8, 140.6, 141.4. Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78. Found: C, 74.72; H, 10.56.

4.1.14. 4-(2-Hydroxyethyl)-5-(3-methyl-2-butenyl)-2-cyclopenten-1-ol (18). To a solution of **17** (1.21 g, 5.39 mmol) in CH_2Cl_2 (50 mL) was added second generation of Grubbs catalyst (229 mg, 0.27 mmol) and the mixture was stirred at ambient temperature for 1.5 h. Water was added to the mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 1:1) to afford **18** as a diastereomeric mixture (846 mg, 4.31 mmol) in 80% yield. Compound **18a**: colorless oil. $[\alpha]_D^{24}$ −31.3 (*c* 0.46, $CHCl_3$). IR (neat) ν cm^{-1}: 1117, 1275, 2918, 3418. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.45–1.59 (1H, m), 1.67 (3H, s), 1.72 (3H, s), 1.78–1.91 (3H, m), 2.08–2.23 (2H, m), 2.25–2.40 (1H, m), 2.63–2.73 (1H, m), 3.61–3.79 (2H, m), 4.53 (1H, dd, *J*=2.8, 5.6 Hz), 5.19–5.24 (1H, m), 5.99 (1H, ddd, *J*=1.5, 2.5, 5.8 Hz), 6.16 (1H, dd, *J*=2.8, 5.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 24.1, 25.8, 35.0, 43.1, 45.7, 61.2, 76.5, 123.1, 132.4, 132.6, 140.4. Compound **18b**: colorless oil. $[\alpha]_D^{24}$ −191.4 (*c* 0.47, $CHCl_3$). IR (neat) ν cm^{-1}: 1117, 1275, 2918, 3418. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.25–1.43 (2H, m), 1.64 (3H, s), 1.72 (3H, s), 1.77–1.88 (1H, m), 1.97–2.28 (3H, m), 2.83–2.93 (1H, m), 3.59–3.76 (2H, m), 4.51 (1H, br d, *J*=4.8 Hz), 5.18–5.26 (1H, m), 5.80 (1H, td, *J*=1.6, 5.8 Hz), 6.00 (1H, ddd, *J*=1.3, 2.4, 5.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 25.8, 26.9, 33.8, 42.8, 51.8, 61.9, 81.8, 123.1, 133.0, 133.6, 137.7.

4.1.15. Benzoic acid 4-[2-(*tert*-butyldiphenylsilanyloxy)-ethyl]-5-(3-methyl-2-butenyl)-2-cyclopentenyl ester (19). To a solution of **18** (647 mg, 3.29 mmol) in DMF (10 mL) were added triethylamine (367 mg, 0.5 mL, 3.63 mmol), *tert*-butyldiphenylchlorosilane (998 mg, 0.85 mL, 3.63 mmol), and 4-dimethylaminopyridine (22.2 mg, 0.18 mmol) at 0 °C and the mixture was stirred at same temperature for 1 h. Water was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 15:1) to afford silyl ether as a diastereomeric mixture (1.4 g, 3.22 mmol) in 98% yield. *a Isomer*: colorless oil. $[\alpha]_D^{24}$ −39.1 (*c* 2.88, $CHCl_3$). IR (neat) ν cm^{-1}: 1111, 1427, 2930, 3331. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.23–1.39 (1H, m), 1.68 (3H, s), 1.73 (3H, s), 1.87–2.00 (1H, m), 2.04–2.37 (3H, m), 2.63–2.73 (1H, m), 3.64–3.82 (2H, m), 4.50 (1H, dd, *J*=2.6, 5.4 Hz), 5.21–5.28 (1H, m), 5.93 (1H, ddd, *J*=1.3, 2.5, 5.7 Hz), 6.13 (1H, dd, *J*=2.8, 5.8 Hz), 7.35–7.48 (6H, m), 7.64–7.72 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 19.2, 24.0, 25.8, 26.9, 36.0, 42.6, 45.9, 62.6, 123.2, 127.6, 129.6, 132.1, 132.2, 133.9, 135.6, 141.3. HREIMS calcd for $C_{28}H_{38}O_2NaSi$: 457.2539 $(M+Na)^+$, found: 457.2532. *b Isomer*: colorless oil. $[\alpha]_D^{24}$ −102.2 (*c* 4.34, $CHCl_3$). IR (neat) ν cm^{-1}: 1111, 1472, 2929, 3331. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.22–1.36 (1H, m), 1.65 (3H, s), 1.73 (3H s), 1.80–2.25 (4H, m), 2.86–2.97 (1H, m), 3.60–3.76 (2H, m), 4.47 (1H, br s), 5.17–5.23 (1H, m), 5.73 (1H, td, *J*=1.6, 5.8 Hz), 5.92 (1H, ddd, *J*=1.3, 2.5, 5.8 Hz), 7.35–7.48 (6H, m), 7.67 (4H, d, *J*=7.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 19.2, 25.8, 26.8, 26.9, 33.6, 42.7, 51.8, 62.8, 81.9, 123.3, 127.6, 129.6, 132.7, 133.1, 133.9, 135.5, 138.2. HREIMS calcd for $C_{28}H_{38}O_2NaSi$: 457.2539 $(M+Na)^+$, found: 457.2565. To a solution of silyl ether (1.38 g, 3.17 mmol) in CH_2Cl_2 (15 mL) were added pyridine (377 mg, 0.38 mL, 4.76 mmol), benzoyl chloride (669 mg, 0.55 mL, 4.76 mmol), and 4-dimethylaminopyridine (38.7 mg, 0.32 mmol) at 0 °C. After being stirred at the same temperature for 2 h, methanol and saturated sodium hydrogen carbonate were added to the reaction mixture. The resulting mixture was extracted with AcOEt and the organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 15:1) to afford **19** as a diastereomeric mixture (1.34 g, 2.49 mmol) in 78% yield. Compound **19a**: colorless oil. $[\alpha]_D^{24}$ −177.5 (*c* 2.51, $CHCl_3$). IR (neat) ν cm^{-1}: 702, 1110, 1271, 1601, 1715, 2929. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.07 (9H, s), 1.30–1.46 (1H, m), 1.61 (3H, s), 1.63 (3H, s), 1.85–1.98 (1H, m), 2.15 (2H, t, *J*=7.4 Hz), 2.38–2.49 (1H, m), 2.99–3.10 (1H, m), 3.65–3.81 (2H, m), 5.15 (1H, t, *J*=6.3 Hz), 5.64 (1H, br d, *J*=5.7 Hz), 5.86 (1H, br d, *J*=5.8 Hz), 6.06 (1H, td, *J*=1.0, 5.8 Hz), 7.36–7.48 (8H, m), 7.53 (1H, t, *J*=7.4 Hz),

7.68 (4H, t, J=7.4 Hz), 8.03 (2H,d, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.9, 19.2, 25.7, 26.7, 26.9, 33.4, 42.4, 47.5, 62.8, 84.6, 122.7, 127.6, 128.2, 129.4, 129.5, 129.6, 132.6, 132.7, 133.9, 135.5, 140.6, 166.5. Anal. Calcd for $C_{32}H_{42}O_3Si$: C, 78.02; H, 7.86. Found: C, 77.94; H, 7.90. HREIMS calcd for $C_{32}H_{42}O_3NaSi$: 561.2801 $(M+Na)^+$, found: 561.2800. Compound **19b**: colorless oil. $[\alpha]_D^{24}$ +72.5 (c 0.23, $CHCl_3$). IR (neat) ν cm^{-1}: 707, 1109, 1271, 1604, 1715, 2929. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.41–1.58 (1H, m), 1.47 (3H, s), 1.66 (3H, s), 1.98–2.40 (4H, m), 2.76–2.86 (1H, m), 3.68–3.84 (2H, m), 5.11–5.18 (1H, m), 5.68 (1H, dd, J=2.6, 5.6 Hz), 6.02 (1H, ddd, J=1.0, 2.4, 5.8 Hz), 6.25 (1H, dd, J=2.8, 5.8 Hz), 7.31–7.46 (8H, m), 7.52 (1H, t, J=7.4 Hz), 7.64–7.72 (4H, m), 7.98 (2H, d, J=7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.9, 19.2, 24.1, 25.8, 26.9, 35.4, 42.8, 45.4, 62.5, 77.2, 122.4, 127.6, 128.3, 129.1, 129.5, 129.6, 132.4, 132.7, 134.0, 135.5, 143.6. Anal. Calcd for $C_{32}H_{42}O_3Si$: C, 78.02; H, 7.86. Found: C, 77.94; H, 7.90. HREIMS calcd for $C_{32}H_{42}O_3NaSi$: 561.2801 $(M+Na)^+$, found: 561.2813.

4.1.16. Benzoic acid 4-[2-(*tert*-butyldiphenylsilanyloxy)-ethyl]-5-(2-oxoethyl)-2-cyclopentenyl ester (8). To a solution of **19** (651 mg, 1.21 mmol) in CH_2Cl_2 (10 mL) was added *m*-chloroperbenzoic acid (230 mg, 1.33 mmol) at 0 °C. After being stirred at the same temperature for 30 min, saturated aqueous sodium thiosulfate and saturated aqueous sodium hydrogen carbonate. The resulting mixture was extracted with ether and the organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 15:1) to give epoxide as a diastereomeric mixture (529 mg, 0.954 mmol) in 80% yield. Colorless oil. IR (neat) ν cm^{-1}: 702, 1110, 1271, 1714. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.16–1.32 (6H, m), 1.34–2.13 (4H, m), {2.48–2.67 (m), 2.77–2.86 (m), 2H}, {2.80–2.94 (m), 3.02–3.16 (m), 1H}, 3.64–3.87 (2H, m), 5.66–5.82 (1H, m), {5.83–5.90 (m), 6.02–6.12 (m), 6.27 (ddd, J=2.8, 5.7, 8.2 Hz), 2H}, 7.31–7.48 (8H, m), 7.50–7.59 (1H, m), 7.62–7.71 (4H, m), 7.93–8.07 (2H, m). Anal. Calcd for $C_{32}H_{42}O_4Si$: C, 75.77; H, 7.63. Found: C, 75.53; H, 7.56. To a solution of epoxide (504 mg, 0.909 mmol) in aqueous *t*-BuOH (5 mL) was added sodium periodate (228 mg, 1.0 mmol). After being stirred at ambient temperature for 2 h, saturated aqueous sodium hydrogen carbonate was added to the reaction mixture. The resulting mixture was extracted with AcOEt and the organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 10:1) to give **8** as a diastereomeric mixture (466 mg, 0.906 mmol) in 99% yield. Colorless oil. IR (neat) ν cm^{-1}: 1109, 1270, 1715, 1731, 2932. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.08 (9H, s), 1.25–1.53 (1H, m), 1.65–1.88 (1H, m), {2.52–2.77 (m), 2.87–2.98 (m), 3.11–3.22 (m), 4H}, 3.62–3.83 (2H, m), {5.67 (dd, J=1.3, 6.0 Hz), 5.80 (dd, J=2.5, 5.8 Hz), 1H}, {5.86 (dt, J=1.8, 5.9 Hz), 6.01 (dd, J=1.7, 5.9 Hz), 1H}, {6.08 (dd, J=2.3, 5.9 Hz), 6.23 (dd, J=2.0, 5.9 Hz), 1H}, 7.33–7.48 (8H, m), 7.52–7.59 (1H, m), 7.63–7.71 (4H, m), 7.92–8.06 (2H, m), 9.80 (1H, br s). HREIMS calcd for $C_{32}H_{36}O_4NaSi$: 535.2281 $(M+Na)^+$, found: 535.2300.

4.1.17. *cis*-Preclavulone-A methyl ester (4). To a solution of hydroxycarbonylbutyltriphenylphosphonium bromide (1.17 g, 2.63 mmol) in THF (20 mL) was added a solution of NaHMDS (1 M in THF, 4.82 mL, 4.82 mmol) at 0 °C and the mixture was stirred at ambient temperature for 1 h. A solution of **8** in THF (5 mL) was added to the reaction mixture at 0 °C and the resulting mixture was stirred at ambient temperature for 2 h. Saturated aqueous NH_4Cl was added to the reaction mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to give carboxylic acid as a diastereomeric mixture (397 mg, 0.66 mmol) in 76% yield. Colorless oil. IR (neat) ν cm^{-1}: 707, 1111, 1272, 1713, 2930, 3069. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.33–1.70 (3H, m), 1.79–2.48 (9H, m), {2.76–2.87 (m), 2.98–3.10 (m), 1H}, 3.63–3.85 (2H, m), 5.38–5.51 (2H, m), 5.64–5.71 (1H, m), {5.85 (dt, J=1.7, 5.8 Hz), 5.98–6.06 (m), 6.24 (dd, J=2.8, 5.8 Hz), 2H}, 7.30–7.47 (8H, m), {7.47 (1H, t, J=7.4 Hz), 7.97 (d, J=6.9 Hz), 8.00 (d, J=7.1 Hz), 7H}. Anal. Calcd for $C_{39}H_{44}O_5Si$: C, 74.72; H, 7.59. Found: C, 74.60; H, 7.56. A solution of carboxylic acid (304 mg, 0.51 mmol) in ether (20 mL) was treated with a solution of diazomethane to give methyl ester (280 mg, 0.458 mmol). Colorless oil. IR (neat) ν cm^{-1}: 702, 1109, 1270, 1713, 2931. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06 (9H, s), 1.44–1.75 (3H, m), 1.82–2.50 (8H, m), {2.77–2.88 (m), 2.99–3.11 (m), 1H}, 3.64 (3H, s), 3.65–3.85 (2H, m), 5.32–5.50 (2H, m), {5.64 (br d, J=4.5 Hz), 5.68 (dd, J=2.5, 5.5 Hz), 1H}, {5.85 (td, J=1.7, 5.8 Hz), 6.02 (dd, J=1.5, 5.5 Hz), 1H}, {6.06 (dd, J=1.7, 5.8 Hz), 6.25 (dd, J=2.8, 5.8 Hz), 1H}, 7.32–7.48 (8H, m), 7.54 (1H, t, J=7.4 Hz), 7.63–7.70 (4H, m), {7.96 (d, J=8.6 Hz), 8.02 (d, J=7.5 Hz), 2H}. Anal. Calcd for $C_{40}H_{46}O_5Si$: C, 74.72; H, 7.59. Found: C, 74.79; H, 7.76. To a solution of methyl ester (25 mg, 0.04 mmol) in THF (0.5 mL) was added a solution of TBAF (1 M in THF, 0.05 mL, 0.05 mmol) at ambient temperature. After being stirred at the same temperature for 2 h, water was added to the mixture and the resulting mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 2:1) to give alcohol as a diastereomeric mixture (16 mg, 0.04 mmol) in 98% yield. Colorless oil. IR (neat) ν cm^{-1}: 713, 1069, 1272, 1714, 1737, 2947, 3456. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36–1.74 (4H, m), 1.82–2.13 (3H, m), 2.14–2.55 (5H, m), {2.72–2.82 (m), 2.96–3.07 (m), 1H}, {3.62 (s), 3.65 (s), 3H}, 3.58–3.85 (2H, m), 5.29–5.53 (2H, m), 5.66–5.74 (1H, m), {5.88–5.94 (m), 6.06–6.12 (m), 1H}, {6.15 (ddd, J=0.8, 2.3, 5.8 Hz), 6.36 (dd, J=2.8, 5.8 Hz), 1H}, 7.38–7.47 (2H, m), 7.55 (1H, t, J=7.4 Hz), 7.95–8.06 (2H, m). Anal. Calcd for $C_{22}H_{28}O_5$: C, 70.94; H, 7.58. Found: C, 70.91; H, 7.56. To a solution of alcohol (14.3 mg, 0.038 mmol) was added Dess–Martin periodinane (19.3 mg, 0.046 mmol) at ambient temperature and the mixture was stirred at the same temperature for 30 min. The mixture was diluted with ether and poured onto saturated aqueous sodium hydrogen carbonate. The mixture was extracted with AcOEt and the organic layer

was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to give aldehyde as a diastereomeric mixture (14.0 mg, 0.038 mmol) in 99% yield. Colorless oil. IR (neat) ν cm^{-1}: 712, 1110, 1270, 1452, 1713, 1731, 1738, 2949. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.50–1.74 (3H, m), 1.88–2.63 (7H, m), 2.66–2.87 (1H, m), {3.13–3.24 (m), 3.39–3.52 (m), 1H}, {3.62 (s), 3.65 (s), 3H}, 5.30–5.49 (2H, m), {5.66 (dd, J=1.0, 5.2 Hz), 5.68 (dd, J=1.6, 5.8 Hz), 1H}, {5.93 (dt, J=1.9, 5.8 Hz), 6.05–6.15 (m), 6.32 (dd, J=2.8, 5.8 Hz), 2H}, 7.39–7.49 (2H, m), 7.52–7.60 (1H, m), 7.95–8.06 (2H, m), {9.84 (s), 9.87 (s), 1H}. Anal. Calcd for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.02; H, 7.06. A solution of NaHMDS (1 M in THF, 1.5 mL, 1.5 mmol) was added to the suspension of hexyltriphenylphosphonium bromide (641 mg, 1.5 mmol) in THF (10 mL) at 0 °C and the mixture was stirred at ambient temperature for 1 h. Then, a solution of aldehyde (183.5 mg, 0.495 mmol) in THF (5 mL) was added to the reaction mixture at 0 °C and the mixture was stirred at ambient temperature for 2 h. Saturated aqueous ammonium chloride was added to the mixture and the mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to give benzoic acid 5-(6-methoxycarbonyl-2-hexenyl)-4-(2-octenyl)-2-cyclopentenyl ester as a diastereomeric mixture (212 mg, 0.483 mmol) in 98% yield. Colorless oil. IR (neat) ν cm^{-1}: 711, 1110, 1270, 1451, 1715, 1738, 2953. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.82–0.93 (3H, m), 1.15–1.38 (6H, m), 1.49–1.74 (2H, m), 1.92–2.53 (11H, m), {2.61–2.72 (m), 2.86–2.98 (m), 1H}, {3.62 (s), 3.65 (s), 3H}, 5.28–5.54 (4H, m), {5.70 (dd, J=1.1, 5.6 Hz), 5.72 (dd, J=2.6, 5.4 Hz), 1H}, {5.89 (td, J=1.8, 5.8 Hz), 6.05 (dd, J=1.3, 5.7 Hz), 1H}, {6.08 (ddd, J=1.0, 2.3, 5.8 Hz), 6.29 (dd, J=2.8, 5.7 Hz), 1H}, 7.43 (2H, t, J=7.5 Hz), 7.56 (1H, t, J=7.4 Hz), 8.03 (2H, d, J=7.0 Hz). Anal. Calcd for $C_{28}H_{38}O_4$: C, 76.68; H, 8.73. Found: C, 76.65; H, 8.67. To a solution of benzoic acid 5-(6-methoxycarbonyl-2-hexenyl)-4-(2-octenyl)-2-cyclopentenyl ester (32 mg, 0.07 mmol) in MeOH (2 mL) was added NaOMe (58.5 mg, 0.21 mmol) at ambient temperature and the mixture was stirred at 50 °C for 10 h. Water was added to the mixture and the mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Filtration and concentration of the mixture under reduced pressure gave crude material, which was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to give alcohol as a diastereomeric mixture (21.2 mg, 0.062 mmol) in 88% yield. Colorless oil. IR (neat) ν cm^{-1}: 702, 1109, 1270, 1715, 1736, 2930, 3404. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, J=6.6 Hz), 1.11–1.79 (10H, m), 1.79–2.40 (10H, m), {2.54–2.65 (m), 2.75–2.86 (m), 1H}, 3.67 (3H, s), 4.47–4.52 (1H, m), 5.25–5.60 (4H, m), {5.78 (dt, J=1.5, 5.7 Hz), 5.97 (ddd, J=1.3, 2.4, 5.8 Hz), 1H}, {5.95 (br d, J=5.7 Hz), 6.12 (dd, J=2.7, 5.8 Hz), 1H}. To a solution of alcohol (32 mg, 0.096 mmol) in CH_2Cl_2 (2 mL) was added MnO_2 (83.5 mg, 0.96 mmol) at ambient temperature and the mixture was stirred at the same temperature for 2 days. The mixture was filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography (hexane/AcOEt, 5:1) to give **4**[5] (30 mg, 0.091 mmol) in 95% yield. Colorless oil. $[\alpha]_D^{24}$ −165.8 (*c* 0.13, $CHCl_3$), $[\alpha]_D^{24}$ −135.3 (*c* 0.13, THF).

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Cell penetrating silica nanoparticles doped with two-photon absorbing fluorophores

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Abstract—Organosilica nanoparticles, doped with two-photon absorbing distyrylbenzene derivatives, were prepared and studied as cell staining agents. Two dyes were used, bearing either two peripheral dimethylamino groups or one dimethylamino and one cyano group. Due to the internal charge transfer character of their excited state, the dyes employed show a red-shifted quenched emission in polar solvents. Once included in the particles, the properties of the two dyes undergo a substantial variation. Particles doped with the cyano substituted distyrylbenzene show a remarkable emission quantum yield in water, probably due to solvent exclusion from the nanoparticle core. To the contrary, the emission of the particles containing the dye substituted with two dimethylamino groups is substantially quenched. Fluorescence emission induced by two-photon absorption follows the same behaviour. The doped nanoparticles can be rapidly internalized by tumour cells with accumulation limited to the cytoplasm and show no cytotoxicity at low concentrations.

1. Introduction

The synthetic procedure to prepare nearly monodisperse silica nanoparticles by condensation of tetralkoxysilanes is known since the late '60s from the work of Stöber.[1] Later on, several modifications of this procedure have been proposed to prepare dye-doped silica nanoparticles (DDNs).[2] Such materials have been attracting an increasing interest in the last years for their potential applications in biology, medicine and as sensors. DDNs are easy to prepare, cheap, water soluble and provide several compartments (bulk, surface, mesopores, shells, etc.) that can be conveniently engineered to perform different functions. As a consequence, DDNs are used to stain cells,[2b,3] in immunoassays,[4] as traceable vectors capable of delivering drugs[2c,5] or DNA[6] within cells and as scaffolds for the realization of complex fluorescent nanosensors.[7]

Among the great variety of dyes available for inclusion in functional nanostructures, two-photon absorbing (TPA) fluorescent dyes have gained increasing relevance for applications in the bio-imaging field.[8] TPA bio-imaging is a non-invasive, non-destructive technique that employs near IR light (usually in the 700–1000 nm range), which is not absorbed by biological tissues, as pumping source. Another advantage is that TPA-induced fluorescence allows a better 3D spatial resolution than other imaging methods based on one-photon processes, as the non-linear nature of the TPA phenomenon limits the emitting sample volume to the close vicinity of the focal point of the IR laser beam.[9] Moreover, the use of long wavelength light allows deeper penetration of the tissues and low scattering of the excitation beam. Many research efforts are focusing on the development of chromophores with large TPA cross sections in order to allow the use of low laser intensities.[10] Most of these chromophores are, however, complex molecular structures that are often insoluble in water and require multistep syntheses.

DDNs are a valuable alternative to pristine dyes. In fact, a single particle can be loaded with several dye molecules with an overall improvement of absorptivity and emission characteristics of these systems, when compared to those of isolated molecules.[2b,11] Furthermore, inclusion of the dye in the particle could overcome possible solubility problems of the dye itself, while surface functionalization can give the DDN assembly a cell penetrating ability and even specificity.[2–6,12] The toxicity of the dye to the host organism should be minimized since the fluorophore is not released from the particle. Finally, segregation from the solvent, if

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effective, could lead to other advantages such as decreased photobleaching and solvent quenching of the emission.[2b] Besides such advantages, the use of DDNs could present problems, such as dye aggregation within the particles, giving rise to concentration self-quenching of the emission, or particle toxicity.

Examples of silica nanoparticles doped with fluorescent dyes optimized for two-photon absorption are however quite scarce. Prasad and co-workers anchored a thiol modified hemicyanine dye either to ZnS[2c] or Fe_2O_3[12] nanoparticles and coated them with a silica shell. Recently, the same authors included a naphthalenylvinylpyridine derivative modified with a trialkoxysilane moiety into silica particles, obtaining a ratiometric pH fluorescent probe.[7g] In this paper we describe the preparation and properties of organosilica nanoparticles doped with TPA fluorophores of the distyrylbenzene (DSB) class, and their cell permeation ability.

2. Results and discussion

2.1. Synthesis and properties of the dyes

The DSB molecular structure was selected as its derivatives are known to have good two-photon cross sections and noticeable fluorescence quantum yield.[10a,13] Moreover, the synthesis of DSB derivatives is quite straightforward under Wittig or Wadsworth–Emmons coupling conditions. Compounds **1** and **2** (Chart 1) were selected in order to investigate the effect of peripheral functional groups with different electronic demands. Dye **1** was prepared as reported in the literature.[14] Compound **2** was synthesized in 58% yield from phosphonate **3** and 4-(dimethylamino)benzaldehyde through a Wadsworth–Emmons coupling reaction (Scheme 1).

Absorption spectra of **1** and **2**, typical of substituted DSB derivatives,[15] are reported in Figure 1. They show a strong absorption band at about 400–420 nm and a weaker one at 320 nm, with the maxima of **2** slightly red-shifted relative to that of **1**. The position of the absorption maxima is almost insensitive to solvent polarity with the exception of DMSO (not shown), where a 14–16 nm red shift was observed. On the contrary, the emission spectra of the two fluorophores are strongly sensitive to the solvent (Fig. 2): in both cases, as the solvent polarity increases, the spectra are red-shifted, broader and much less intense. A comparable behaviour has been already observed in similar systems and was attributed to a strong ICT (Internal Charge Transfer) character of the excited state.[15] Changing the solvent from hexane to DMSO, the fluorescence quantum yield decreases from 0.36 to 0.23 and from 0.21 to 0.04, respectively, for **1** and **2**. The effect on **2** is more evident as may be expected if one considers that the ICT character of the excited state is enhanced by the presence of the electron withdrawing cyano group.

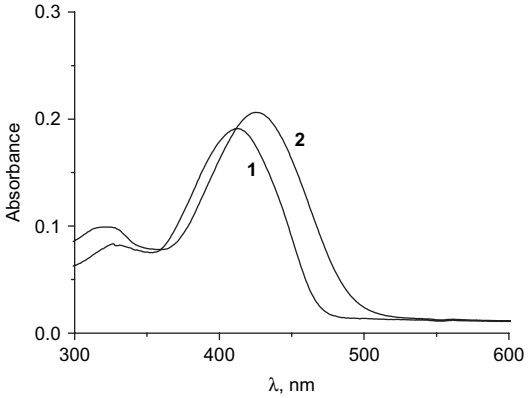


Figure 1. Absorption spectra of dyes **1** and **2** in ethanol. Selected data: [dye]=2.5×10^{-6} M, **1**: λ_{max}=413 nm ($\varepsilon=6.9\times10^4$ M^{-1} cm^{-1}), **2**: λ_{max}=425 nm ($\varepsilon=7.3\times10^4$ M^{-1} cm^{-1}).

2.2. Preparation and characterization of DDNs

The inclusion of dyes **1** and **2** into silica particles was performed following a procedure reported by Prasad and co-workers[2d] modified as follows. Propyltriethoxysilane (PTES) and 3-aminopropyltriethoxysilane (APTES) were co-polymerized in water in the presence of AOT (sodium bis(2-ethylhexyl)sulfosuccinate) micelles loaded with the dye. Polymerization of the organosilanes takes place in the non-polar core of the micellar aggregates, which also incorporate the poorly water soluble dye, and results in the formation of dye-doped organisilica (ORMOSIL) particles. This method appeared not only simple but also suitable for the preparation of DDNs since it does not require any chemical modification of the fluorophore before its incorporation in the particles.

The formation of the colloids was followed by Dynamic Light Scattering (DLS) measurements. The resulting particles were purified by extensive ultrafiltration over a 10,000 Da (~3 nm) cut-off membrane to eliminate unreacted species and hydrolyzed monomers. No dye was detected in the filtrate, thus indicating the absence of leaking from the particles, at least in the short term. Colloid dimensions and morphology were investigated using Transmission

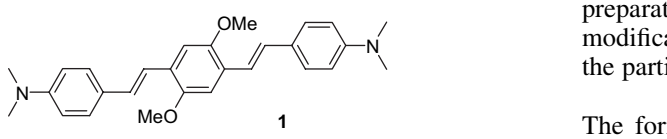
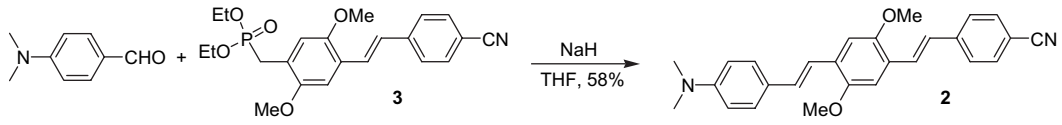


Chart 1.



Scheme 1. Synthesis of DSB derivative **2**.

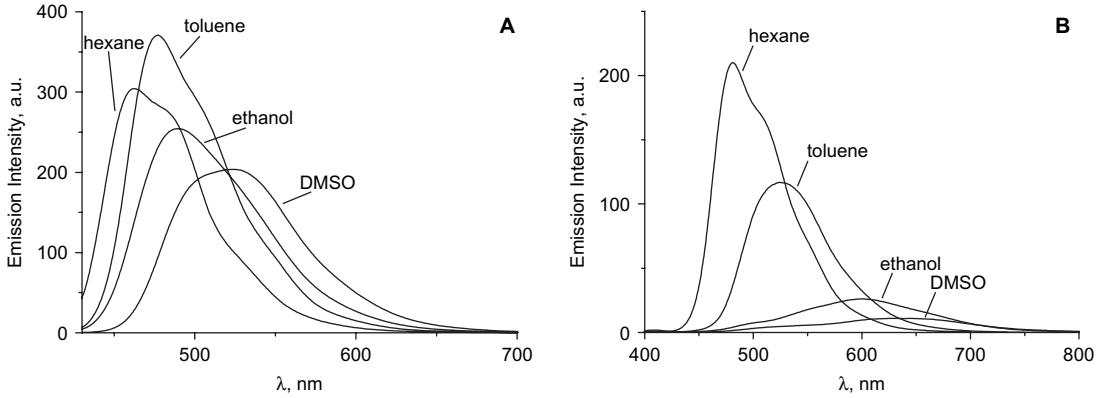


Figure 2. Emission spectra of dyes **1** (A) and **2** (B) in different solvents. Conditions: λ_{exc}=410 nm, [dye]=1.0×10^{-6} M, 25 °C.

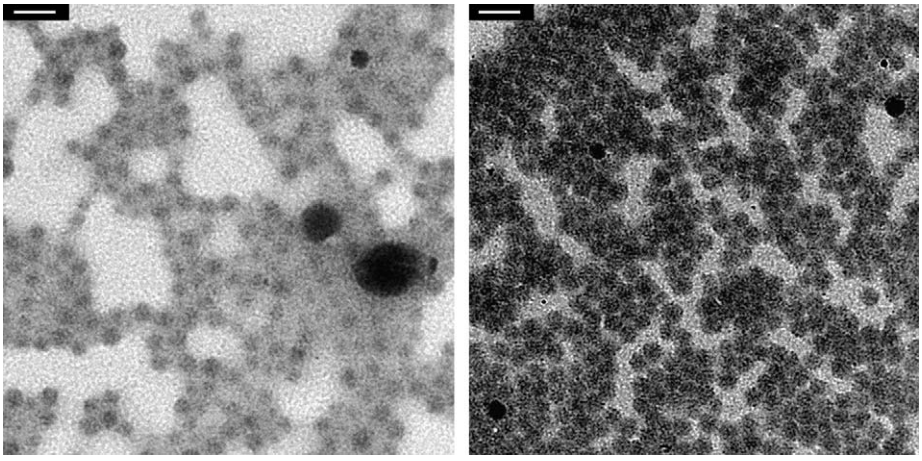


Figure 3. TEM images of dye-doped silica nanoparticles containing compounds **1** (left) and **2** (right), the bars (upper left corners) correspond to 100 nm.

Electron Microscopy (TEM, Fig. 3) and DLS (Table 1). The agreement between the hydrodynamic diameters obtained by DLS and the diameters obtained by electron microscopy is good. The particles appear spherical with a fairly low polydispersity.

The absorption spectra of DDNs in ethanol/water 9:1 are similar to those of the dyes in solution (Fig. 4). This allowed an estimation of the dye concentration in the DDN samples by absorbance measurements. On the other hand, nanoparticle concentrations (mg/mL) were determined by solvent evaporation. Typical DDN aqueous solutions, after purification, had 3.0–3.5 mg/mL nanoparticle concentration and 13–14 μM dye content.[16]

Interestingly, the spectrum of DDNs containing **1** (**1np**) in pure water shows some peculiarity (Fig. 4): the main band is blue-shifted at 392 nm and has a complex shape with several shoulders at 350, 425 and 475 nm. Such a solvent effect is not observed in the case of DDNs containing **2** (**2np**), which show similar features both in ethanol and in water (Fig. 4).

Fluorescence spectra of the DDNs in water are reported in Figure 5. Inclusion of the dyes within the particles is assessed by the high fluorescence anisotropy values recorded: 0.36 for **1np** and 0.33 for **2np**. Such values confirm the low mobility of the dyes due to grafting to the nanoparticles.

Table 1. Nanoparticles diameter (D, nm) and polydispersity (δ) of DDNs as obtained by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS)

DDNs	Dye	$D_{(TEM)}$, nm	δ, nm (%)	$D_{(DLS)}$, nm
1np	**1**	28.7	7.4 (18%)	31.4
2np	**2**	38.4	6.2 (16%)	36.6

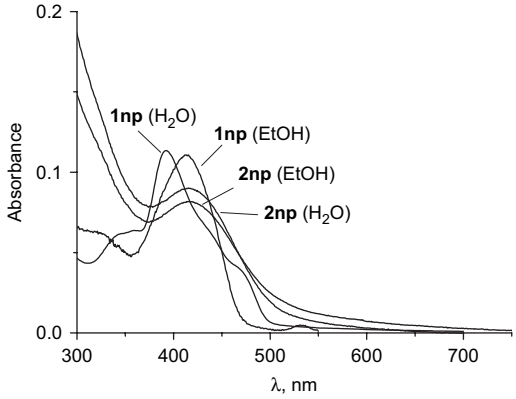


Figure 4. Absorption spectra of 0.2 mg/mL solutions of **1np** and **2np** in water and ethanol at 25 °C. Overall dye concentrations: [**1**]=1.2×10^{-6} M, [**2**]=1.0×10^{-6} M.

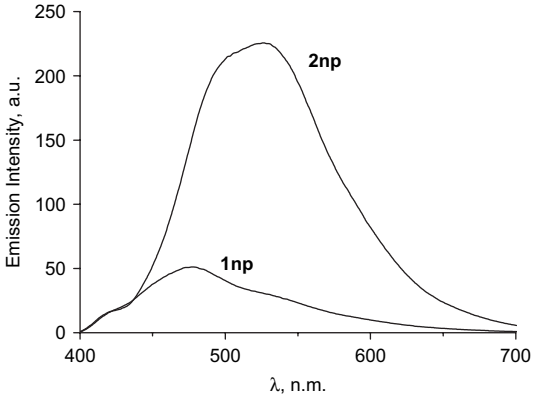


Figure 5. Emission spectra of 0.2 mg/mL ([dye]=1.0×10^{-6} M) solutions of **1np** and **2np** in water. Conditions: λ_{exc}=410 nm, 25 °C.

The emission bands are broad and the positions of the maxima are similar to those recorded for the free dyes in toluene. This indicates that the embedded dyes experience a moderately low polarity environment and that water is excluded by the particle core. Moreover, **2np** shows a much stronger emission (Φ=0.16) than **1np** (Φ=0.06). This result is quite unexpected, since embedding of the more solvent-sensitive dye **2** in the moderately polar nanoparticle environment should result in a lower emission efficiency in the case of **2np** (see Fig. 1). The low emission of **1np** could arise from concentration quenching, due to the formation of nanodomains of aggregated dyes. This phenomenon has been already reported in dye-doped silica nanoparticles:[17] the blue shift of the absorption spectra observed in water suggests that this could be the case also with **1np**.[18]

Besides the speculation on its origin, it is important to note that stronger emission by **2np** is observed also when the particles are excited at 780 nm (Fig. 6A), thus validating the DDNs as two-photon absorbing reporters. Profiles of fluorescence decay show a multiexponential behaviour where a fast component (0.45 ns for **2np** and 0.2 ns for **1np**) and a slower one (2.3 ns for **2np** and 1.6 ns for **1np**) dominate (Fig. 6B). Such complex behaviour is not unprecedented in dye-doped silica nanoparticles and probably arises from the lack of homogeneity of the systems due for instance, to different microenvironments experienced by the dye molecules.[19]

2.3. Cellular uptake

To determine the DDNs' cellular uptake, cells derived from a human oesophageal carcinoma (cell line KYSE-510) were incubated with a suspension of DDNs **1np** and **2np** in culture medium for 120 min and then observed at the epifluorescent microscope (Fig. 7). The DDNs appeared to be rapidly internalized by the cells at a concentration which was not cytotoxic (0.02 mg/mL), as resulted from the cytotoxicity test (see below). The fluorescence images of KYSE-510 cells show significant cellular staining with accumulation of the nanoparticles limited to the cytoplasm, as reported for other silica nanoparticles prepared with the same procedure.[2d]

The cytotoxicity of **1np** and **2np** was determined by using the MTT assay. The results are reported in Figure 8, which shows the amount of cell survival after 120 min of incubation with increasing concentrations of DDNs. Analysis of data reported in Figure 8 reveals that the presence of DDNs slightly affects cell viability up to a 0.02 mg/mL concentration.

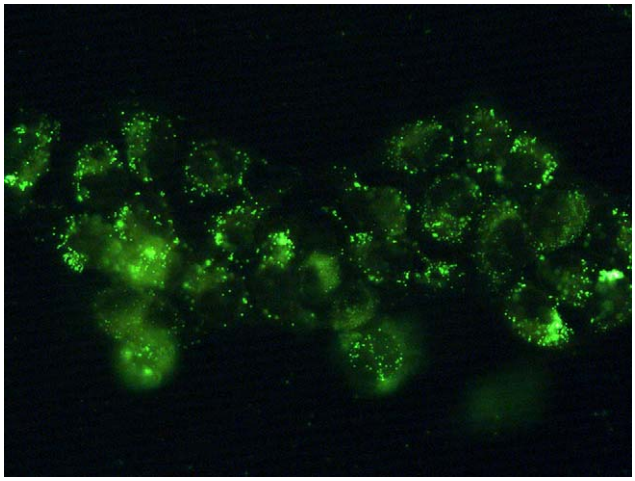


Figure 7. Epifluorescence microscopy showing uptake of **2np** by KYSE-510 cells. Conditions: nanoparticle concentration 0.02 mg/mL ([**2**]=0.1 μM), magnification 400x.

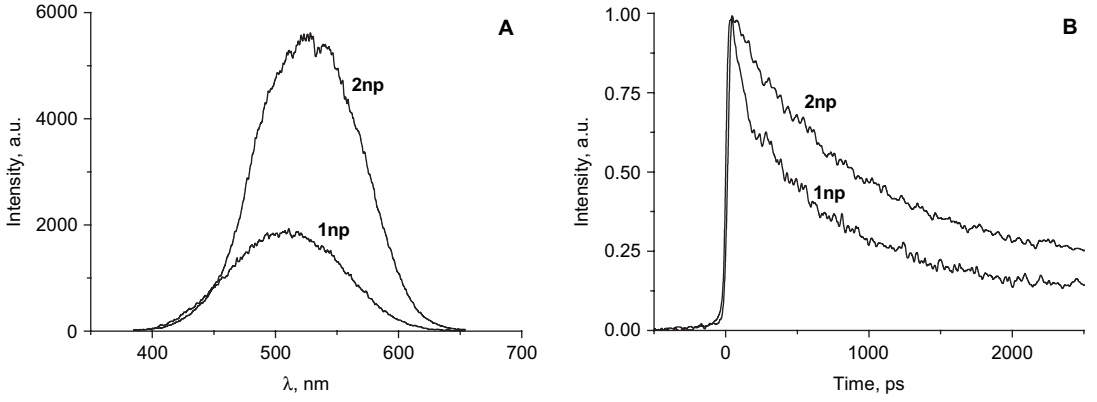


Figure 6. Emission spectra (A) and fluorescence decays (B) of 2.0 mg/mL solutions of dye-doped nanoparticles **1np** and **2np** in water upon two-photon excitation. Emission spectral shapes are affected by the use of an optical filter to cut the laser excitation. Both measurements were performed with the same filter so that the overall peak intensities are comparable. Overall dye concentrations: [**1**]=0.8×10^{-5} M, [**2**]=1.1×10^{-5} M. Conditions: λ_{exc}=780 nm, 25 °C.

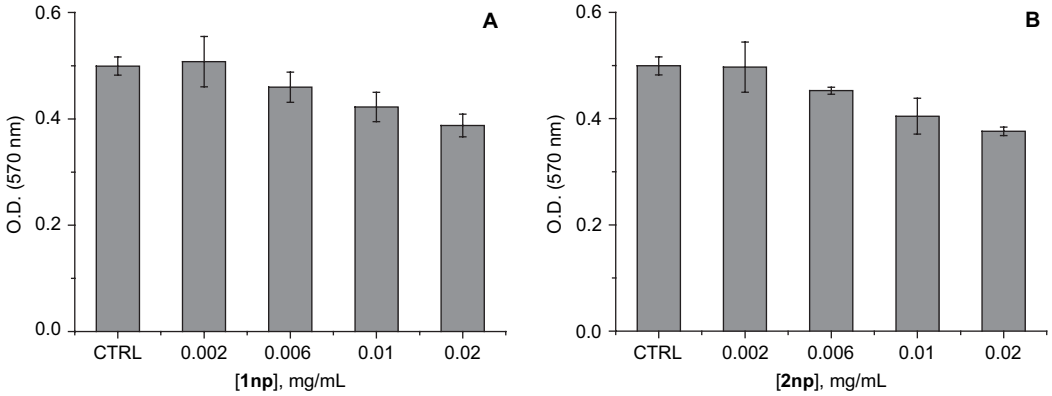


Figure 8. Viability of KISE-510 cells after incubation with **1np** (A) and **2np** (B) for 120 min, expressed as optical density (OD) at 570 nm after MTT treatment. The OD values at 570 nm are the mean of three replicates±standard deviation. CTRL=control.

3. Conclusions

The results obtained in this work indicate that it is possible to embed two-photon absorbing dyes into silica nanoparticles without any chemical modification of the dye structures. New insights have been obtained on the properties and behaviour of such DDNs. The particles are efficiently taken up by cells and localized in the cytoplasm. Fluorescence emission upon 780 nm excitation is observed and the decay profiles witness that the dye molecules embedded in the particles experience dishomogeneous environments. Nanoparticle inclusion has different effects on the emission properties of the embedded dyes, which cannot be easily deduced by their solution behaviour: DDNs doped with the less solvent-sensitive and more strongly emitting dye **1** show a quenched fluorescence emission in water, possibly due to aggregation of the dyes within the particles. To the contrary, **2np** shows a remarkably good emission quantum yield and, as a consequence, reveals to be strong emitting, water soluble, two-photon absorbing reporter that can easily penetrate and stain living cells.

4. Experimental

4.1. General

All commercially available solvents and reagents were used as received without further purification. Preparative column chromatography was carried out on glass columns packed with Macherey-Nagel 60 (70–230 mesh) silica gel. Compound **1**[14] and phosphonate **3**[20] were prepared as described.

Melting points were taken with a Buchi 510 apparatus and are uncorrected. NMR spectra were recorded with a Bruker AC 250F spectrometer. Chemical shifts are reported relative to tetramethylsilane as internal standard. Signals in NMR spectra are reported as follows: s=singlet, d=doublet, m=multiplet, br=broad. ESI-MS mass spectra were obtained with a Navigator ThermoQuest-Finningan mass spectrometer. Elemental analyses were performed by the Microanalysis Laboratory of the Department of Chemical Sciences of the University of Padova.

Transmission Electron Microscopy (TEM) experiments were performed at the Microscopy Facility of the Department of Biology of the University of Padova. TEM images of the particles were obtained with a Fei Tecnai 12 transmission electron microscope operating at 100 keV. Samples for TEM were prepared by spreading a drop of nanoparticle solution in water (~3 mg/mL) onto standard carbon-coated copper grids (200 mesh). Dimensional analysis of nanoparticles from TEM images was made with the *ImageJ* software.[21]

Dynamic Light Scattering measurements were obtained with a Particle Sizing Systems Nicomp Model 370 correlator equipped with a thermostated cell holder and a Spectra Physics Series 2016 Ar laser operating at 488 nm. Hydrodynamic particle diameters were obtained from cumulant fits of the autocorrelation functions at 90° scattering angle.

UV–vis absorption measurements were performed at 25 °C by means of Perkin–Elmer Lambda 16 e 45 spectrophotometers equipped with thermostated cell holders. Quartz cells with optical pathlength of 1 cm were used. Fluorescence spectra were recorded at 25 °C with a Perkin–Elmer LS-55 spectrofluorimeter equipped with a Hamamatsu R928 photomultiplier and thermostated cell holder, quartz cells with optical pathlength of 1 cm were used. Fluorescence quantum yields were determined using Coumarin 153 in ethanol (Φ=0.58) as standard.

Two-photon excitation was performed with a Ti:sapphire femtosecond laser (Spectra-Physics). A 2 mm Schott B18 filter was used to cut the laser excitation. Time resolved measurements were performed with a Hamamatsu streak camera.

4.2. Synthesis of 2

Phosphonate **3** (0.82 g, 2.0 mmol) was dissolved in 50 mL of anhydrous THF and the solution was cooled to 0 °C in an ice bath. 4-(Dimethylamino)benzaldehyde (0.29 g, 1.90 mmol) and NaH (0.13 g, 5.9 mmol) were added and the reaction mixture was stirred overnight under a nitrogen atmosphere (TLC, toluene/ethyl acetate 95:5). The solvent was evaporated and the crude product was purified by flash column chromatography (silica gel, eluent: toluene/ethyl acetate 95:5) and recrystallized from hexane/ethyl acetate to yield 0.46 g (58%) of an orange powder. Mp: 228–230 °C. IR (KBr): 3050–2800 (CH), 2200 (CN), 1607, 1522 (C=C),

1209, 1043, 956 cm^{-1} (CH). ^1H NMR (250 MHz, $CDCl_3$) δ: 7.61–7.56 (m, 5H), 7.47 (d, 2H, J=8.8 Hz), 7.30 (d, 1H, J_{trans}=17.0 Hz), 7.13–7.05 (m, 4H), 6.75 (br s, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.01 ppm (s, 6H). ^{13}C NMR (62.9 MHz, $CDCl_3$) δ: 151.95, 151.07, 148.72, 142.56, 132.38, 127.81, 127.12, 126.76, 126.16, 119.21, 109.99, 109.32, 108.44, 56.40, 56.19 ppm. Elemental analysis for $C_{27}H_{26}N_2O_2$ (410.51): calculated (%): C, 79.00; H, 6.38; N, 6.82. Found: C, 78.04; H, 5.98; N, 6.61.

4.3. Preparation of the dye-doped silica nanoparticles (DDNs)

Preparation of nanoparticles containing **1** is reported as an example of general procedure. Particles containing **2** were prepared by following the same procedure with the exception that 20 μL of a 15 mM dye solution in DMF was used.

A 3.0 mM solution of **1** (100 μL) in DMSO, followed by 200 μL of propyltriethoxysilane (PTES), was added to 20 mL of a stirred solution of AOT (0.44 g) and *n*-butanol (800 μM) in water. After 30 min, 10 μL of 3-aminopropyltriethoxysilane (APTES) were added. The reaction mixture was thermostated at 25 °C and vigorously stirred for 16 h. The resulting nanoparticle suspension was diluted to 70 mL with water and transferred into a 75 mL Amicon Ultrafiltration Cell, equipped with a 10-kDa regenerated cellulose membrane and an 800 mL solvent reservoir. The mixture was extensively ultra-filtered under a pressure of 4 bars (about 1600 mL of water were used). The solution obtained was finally filtrated through a 0.45 μm filter membrane. The dye concentrations in the resulting DDN solutions were obtained from their absorbances (**1**: λ_{max}=413 nm, ε=6.9×10^4 M^{-1} cm^{-1}; **2**: λ_{max}=425 nm, ε=7.3×10^4 M^{-1} cm^{-1}).

4.4. Nanoparticle uptake and imaging

The cells KYSE-510 (human oesophageal squamous cell carcinoma) were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated foetal bovine serum (FCS, Seromed) in a humidified incubator in an atmosphere of 5% CO_2 in air. For nanoparticle uptake and imaging, the cells were trypsinized, resuspended in fresh medium and seeded in 30-mm culture plates containing a glass cover slip (50×10^3 cells/plate). The next day, the cells were incubated with a suspension of DDNs **1np** and **2np** in RPMI without serum for 120 min and rinsed with PBS. After incubation in complete medium (RPMI 1640 plus FCS) for 1–2 h, the cells were observed by epifluorescent microscopy Leica DMR.

4.5. Cell viability assay

An in vitro MTT based assay kit (Sigma) was used to test the cytotoxicity of **1np** and **2np**. The MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide) method is designed for determining metabolically active cells on the basis of the reduction of MTT into formazan by mitochondrial dehydrogenases. The formazan crystals are dissolved in acidified isopropanol and the solution is spectrophotometrically measured at 570 nm. The cells were seeded in 96 multiwell-plates (13×10^3 cells/well) and incubated with a suspension of DDNs, as described above. After treatment, MTT solution was added and the cells were incubated following the supplier indications. The optical densities at 570 nm were used to determine the viability of cells after incubation with increasing nanoparticle concentrations.

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Intramolecular charge transfer dual fluorescent sensors from 4-(dialkylamino)benzanilides with metal binding site within electron acceptor

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Abstract—Three fluoroionophores (**2a–c**) were designed as the intramolecular charge transfer (CT) dual fluorescent sensors for metal cations with metal binding site within the electron acceptor. These sensors were derived from 4-dialkylaminobenzanilides (alkyl=methyl, ethyl, and *n*-butyl) with the amido phenyl ring being an arm of 15-crown-5 thus bearing binding site for alkaline and alkaline earth metal cations. Compounds **2a–c** were expected to have two possible CT channels of opposite direction. The absorption and fluorescence spectra of **2a–c** and their crown-ether free model molecules **3a–c** in a variety of solvents were recorded. Dual fluorescence was observed with **2a–c** and was assigned to the LE and the CT states, respectively. In nonpolar or less polar solvents the CT occurring with **2a–c** was identified as that occurred with benzanilides (BA) with the amido anilines being the electron donor (the BA-like CT), while in polar solvents such as acetonitrile (ACN), the CT was still mainly the BA-like. In the presence of alkali and alkaline earth metal cations in ACN, the CT dual fluorescence underwent substantial changes so as increased total quantum yield, red-shifted LE band and enhanced CT to LE intensity ratio. Binding of the metal cations at the 15-crown-5 moiety of **2a–c** was shown to turn the CT direction that the dialkylamino group in the binding complexes being the electron donor while the benzo-15-crown-5 moiety now being within the electron acceptor. The occurrence of this CT enhances metal cation binding to 15-crown-5 ether in **2a–c**, which was confirmed by the observed higher metal binding constants. Compounds **2a–c** as the CT dual fluorescent sensors were shown to operate under the mechanism of the metal cation binding induced switching of the CT character from the BA-like to that occurred with 4-(dimethylamino)benzamides (the DMABA-like). Compounds **2a–c** therefore represent successful examples for the CT dual fluorescent sensors for cations with the metal binding site within the electron acceptor and can be employed as sensitive ratiometric fluorescent sensors for metal cations of improved sensing performance.

1. Introduction

It is known that the excited-state intramolecular charge transfer (CT) and the accompanied dual fluorescence of the electron donor/acceptor substituted benzenes, such as 4-(dimethylamino)benzonitrile (DMABN), depend sensitively on the nature of the electron donor/acceptor.[1] Therefore it is possible to design fluorescent sensors for metal cation sensing. The dependence on the electron donor has been nicely supported by the effect of metal cation on the CT emission of DMABN derivatives with their amino nitrogen atom being incorporated in an aza-crown ether and employed to construct CT fluorescent sensors for metal cations.[2] Research, however, showed that there was a decoordination reaction in the excited metal–complexes of these DMABN derivatives as a consequence of the CT process in the DMABN derivatives, where a shift of the negative charge from the amino electron donor to the acceptor leads to an electrostatic repulsion between the metal cation and the positively charged donor.[3] The fluorescence emission, therefore, experiences relatively small changes in both its intensity and position because most of the fluorescence is emitted from the species in which the interaction between metal cation and the fluorescent sensor is much weaker or does not exist any more. It was hence expected that a similar strategy applied to the electron acceptor might lead to a better sensing performance since in this case the excited-state CT might enhance the binding of metal cations. Efforts have been made to prepare the derivatives of 4-(dimethylamino)-benzamide (DMABA) and 4-(dimethylamino)benzenesulfonamide (DMASA) of which the amido or sulfonamido nitrogen atoms in the electron acceptor are incorporated within a metal cation binding site.[4] It turned to be not straightforward to show the expected advantage, since metal-cation binding resulted in a breakdown of the electronic conjugation in DMABA derivatives of their amido

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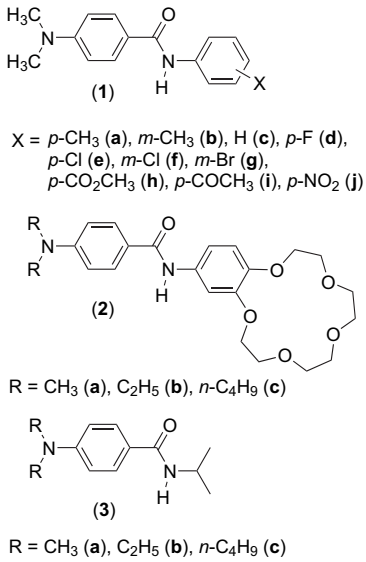


Chart 1. Molecular structures of 4-dimethylaminobenzalinides (**1**)[5] and related fluoroionophores (**2**) as metal cation sensors and their model molecules (**3**).

nitrogen with the benzoyl π-system due to steric reasons[4a] or deprotonation of the sulfonamido –NH proton.[4b] It hence remains a challenge to devise dual fluorescent CT sensors for metal cation with the electron acceptors bearing an ion binding site. The dual emission character makes these efforts worthwhile since ratiometric fluoroionophores might be constructed.

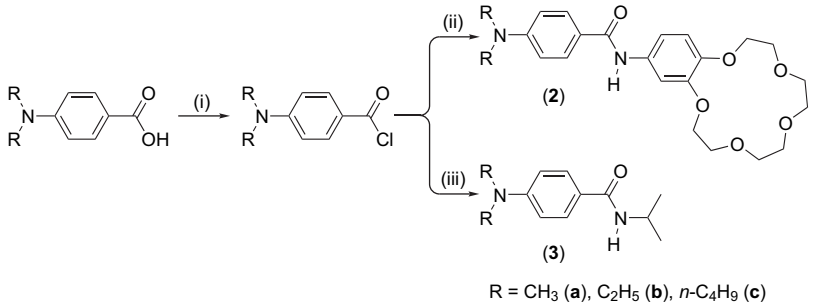
We recently investigated the intramolecular CT of a series of dual fluorescent 4-(dimethylamino)benzanilides bearing a *para*- or *meta*-substituent at the amido phenyl ring (**1**, Chart 1).[5] We showed that with **1** there existed two competitive CT channels, one from the 4-dimethylamino donor to the benzamide moiety as what was assigned for 4-(dimethylamino)benzamide[6] (the DMABA-like CT), and the other from the amido aniline to benzoyl as was shown with benzanilides[7] (the BA-like CT). It was found[5] that electron-withdrawing substituents at the amido phenyl ring in **1** could switch the excited-state CT from the BA-like to the DMABA-like. In the latter case the benzanilide moiety in **1** becomes electron acceptor. This might open up a new way of constructing CT dual fluorescent sensors for metal cations with the cation binding site within the electron acceptor while the steric influence previously encountered[4] can be efficiently avoided,[8] as now the metal binding site is away from the amido –C(O)NH– moiety while the metal binding message can be efficiently delivered via the amido phenyl π-moiety.

We report herein our proof-of-principle effort in regard to the three derivatives of 4-(dialkylamino)benzanilide with the amido phenyl ring being an arm of 15-crown-5, known as a good macrocycle for alkaline and alkaline earth metal cations, while alkyl is methyl, ethyl, and *n*-butyl, respectively (**2a–c**, Chart 1). Compounds **2a–c** and their crown-ether free model molecules **3a–c** were synthesized according to procedures depicted in Scheme 1. Compounds **2a**, **2b**, and **2c** have similar structure, differing only at the 4-amino alkyl substituent that was expected to tune the contribution of the DMABA-like CT in which the 4-dialkylamino group acts as the electron donor.[9] As 4-dialkylamine and amido aniline in **2a–c** can function as the electron donor of the DMABA- and BA-like CT, respectively, the present CT fluorescent sensors represent a new set of D–A–D′ molecular patterns.[10] Model molecules **3a–c** were designed to help understanding the CT character of **2a–c**, since they were assumed to have similar steric structure to that of **2a–c** at the amide conjunction, but have only the DMABA-like CT channel. It was expected that, upon cation complexation to the crown-ether group in **2a–c**, the 15-crown-5 ether moiety, taken as a substituent at the amido aniline moiety of **1** (Chart 1), might become less electron donating or even electron withdrawing. The DMABA-like CT reaction channel in **2a–c** would hence predominate over the BA-like CT, resulting in a detectable change in its dual fluorescence. In this article we will show that the CT with **2a–c** is switched from the BA-like to the DMABA-like when metal cation is bound to the crown-ether group, allowing hence for the CT dual fluorescent sensing of metal cations with the cation-binding site locating within the electron acceptor.

2. Results and discussion

2.1. 15-Crown-5 group as a 'substituent' in 2a–c

Previously we showed that substituent in **1** (Chart 1) at the *para*- or *meta*-position of the amido aniline did not affect the ground-state structure of **1**, in particular at the amide conjunction.[5] Actually a linear correlation was found between the ^{1}H NMR chemical shift of the amido –NH proton (δ_{-NH}, ppm) of **1** in DMSO-d_6 and the Hammett



Scheme 1. Syntheses of **2** and **3**. Reagents and conditions: (i) $SOCl_2$ (3 equiv), CH_2Cl_2, rt; (ii) 4-aminobenzo-15-crown-5 ether hydrochloride (1 equiv), NEt_3 (1.2 equiv), CH_2Cl_2, rt and (iii) *iso*-propylamine (1 equiv), CH_2Cl_2, rt.

substituent constant (σ_x), $\delta_{-NH}=0.66\sigma_x+9.84$. It is therefore possible to construct metal cation fluorescent sensors by incorporating a cation binding site, such as crown-ether, in the amido aniline at its *para*- and/or *meta*-positions. In this case metal binding would hardly introduce any steric hindrance to the amido conjunction. This was the basis for our design of **2a–c** as the metal cation sensors. Obviously the 15-crown-5 group in **2a–c**, a substituent at the amido aniline moiety in **1**, could be approximately taken as a *p*-OEt plus an *m*-OEt, and is therefore electron donating. On the basis of the chemical shift of the amido –NH proton of **2a** (9.722 ppm),[11] the 'substituent' constant of the 15-crown-5 group in **2a** was calculated from the aforementioned linear relationship to be −0.18, which is indeed close to the sum of the Hammett constants of *p*-OEt ($\sigma=-0.24$) and *m*-OEt ($\sigma=0.10$).[12] The 15-crown-5 group in **2a** was therefore confirmed as an electron donating 'substituent', similar to *p*-CH_3 that has a σ of −0.17.[12] Its CT behavior was hence expected to be similar to that of **1** dominated by the BA-like CT that the amido aniline acted as the electron donor.[5] Although the 15-crown-5 group in **2b** and **2c** would certainly be electron donating as a 'substituent' at the amido aniline, the CT behavior of **2b** and **2c** remains to be clarified because of the increased electron donor strength of the 4-diethylamino and 4-dibutylamino groups in **2b** and **2c**, respectively. The latter would enhance the DMABA-like CT in **2b** and **2c** as suggested from the observations made with the CT in a series of 4-(dialkylamino)benzonitriles of lengthening alkyl substituent.[9]

2.2. Intramolecular charge transfer with 2a–c

As the 15-crown-5 group in **2a** was identified as a 'substituent' similar to *p*-CH_3, the CT and emission of **2a** would be similar to those of the *p*-CH_3 derivative of **1**, **1a** (Chart 1).[5] In a nonpolar solvent such as cyclohexane (CHX) the CT in **2a** would therefore be the BA-like while in highly polar solvents such as acetonitrile (ACN) the BA-like CT is mixed with the DMABA-like, as reported for **1a**.[5] The electron donating ability of the 4-diethylamino and 4-dibutylamino groups in **2b** and **2c**, respectively, is stronger than that of the 4-dimethylamino group in **2a**, it was therefore not clear on the CT character of **2b** and **2c**. In order to confirm this hypothesis, fluorescence spectra of **2a–c** and their model molecules **3a–c** (Chart 1) in the following solvents, CHX, diethyl ether (DEE), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), and ACN, in an order of increasing polarity, were recorded and compared. Main spectral parameters are summarized in Table 1. Dual fluorescence was observed with **2a–c** in most cases. The fluorescence spectra of **2a** in a variety of solvents are shown in Figure 1 as an example. The long-wavelength emission of **2a** in CHX peaks at 515 nm. This emission in CHX can be reasonably assigned to the BA-like CT state in view of the emission of **1a** in CHX.[5] It is noticeable that the long-wavelength CT emission of **2a** observed in CHX does not undergo an expected monotonous red-shift when the solvent polarity is increased (Fig. 1 and Table 1). As shown in Figure 1, the long-wavelength

Table 1. Absorption and fluorescence spectroscopic parameters of **2a–c** and **3a–c** in organic solvents

	Solvent	Absorption		Fluorescence		
		λ_{abs} (nm)	ε (10^4 L mol^{-1} cm^{-1})[a]	λ_{LE} (nm)	λ_{CT} (nm)	Φ_F[b]
2a	CHX	307	2.27	334.4	515.0	—
	DEE	308	2.85	344.0	400.0	0.0013
	THF	311	3.09	344.0	432.0	0.0025
	CH_2Cl_2	318	2.73	345.0	451.0	0.0012
	ACN	314	2.78	346.0	495.0	0.0013
2b	CHX	312	2.70	370.0	510.0	0.0041
	DEE	313	3.63	344.0	408.0	0.0020
	THF	316	3.72	348.0	432.0	0.0022
	CH_2Cl_2	323	3.46	353.0	460.0	0.0021
	ACN	320	3.49	364.0	490.0	0.0016
2c	CHX	314	2.54	370.0	510.0	0.0045
	DEE	314	3.45	346.0	400.0	0.0029
	THF	317	3.48	348.0	429.0	0.0038
	CH_2Cl_2	325	3.30	353.0	451.0	0.0027
	ACN	321	3.06	356.0	480.0	0.0023
3a	CHX	284/295	0.68/0.59	335.0	—	0.1343
	DEE	285/297	1.53/1.28	342.0	—	0.1734
	THF	290	0.63	347.0	402.0	0.2301
	CH_2Cl_2	295/300	0.89/0.87	351.0	420.0	0.0801
	ACN	292	0.79	348.0	466.0	0.0806
3b	CHX	290/300	1.29/1.19	338.0	—	0.1053
	DEE	290/298	1.65/1.57	345.0	392.0	0.1250
	THF	294	1.18	350.0	412.0	0.0744
	CH_2Cl_2	296/307	1.13/1.18	355.0	422.0	0.0988
	ACN	300	0.93	351.0	464.0	0.0758
3c	CHX	288/299	1.79/1.66	342.0	—	0.1009
	DEE	290/300	1.44/1.37	354.0	392.0	0.1146
	THF	295	1.31	358.0	402.0	0.1540
	CH_2Cl_2	296/308	1.46/1.56	358.0	420.0	0.1603
	ACN	302	1.36	358.0	449.0	0.0944

[a] Mean of three measurements with standard deviation of less than 10%.
[b] Mean of three measurements with standard deviation of less than 30%.

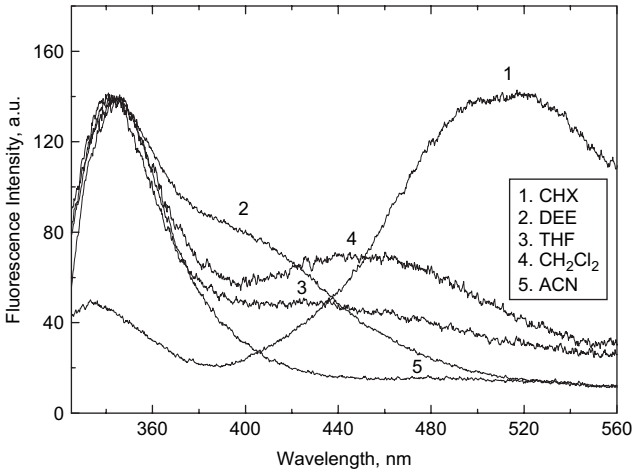


Figure 1. Fluorescence spectra of **2a** in solvents of varied polarity. Solvent: CHX, cyclohexane; DEE, diethyl ether; THF, tetrahydrofuran; CH_2Cl_2, dichloromethane; and ACN, acetonitrile. [**2a**]=1.00×10^{-5} mol L^{-1}. Excitation wavelength was 290 nm.

band of **2a** shifts dramatically to the blue from 510 nm in CHX to 400 nm in a weakly polar solvent DEE. Upon further increase in solvent polarity (from DEE to THF, CH_2Cl_2, and ACN), however, it shifts again to the red from 400 nm to 495 nm, indicating again the CT nature of the emissive state in these polar solvents. This apparently 'abnormal' solvatochromism, similar to what was observed with **1a**,[5] confirms that there are two CT channels of opposite direction with **2a** and the CT character changes from purely the BA-like CT in CHX to that mixed by the DMABA-like in polar solvents. Concentration dependent experiments were carried out with **2a** in ACN over 2.0×10^{-6}–1.2×10^{-4} mol L^{-1} and in CHX over 2.0×10^{-6}–1.2×10^{-5} mol L^{-1}. No dependence in the emission spectrum shape was observed. Aggregation that might lead to excimer or exciplex formation, another possible origin for the long-wavelength emission of **2a** particularly in nonpolar solvent CHX, could hence be ruled out. The same conclusion regarding to the CT character can be made with **2b** and **2c** because of the similar solvatochromism observed in their long-wavelength emission.

A comparison of the solvatochromism of the long-wavelength emission of **2a–c** with that of **3a–c**, that have only the DMABA-like CT, supported the conclusion made on the CT character of **2a–c**. Data given in Table 1 indicate that the long-wavelength emission of **3a–c** shifts, as expected for the DMABA-like CT fluorescence,[6] to the red with increasing solvent polarity. It is also observed that the long-wavelength emission of **2a–c** in polar solvent peaks at longer wavelength than that of the corresponding **3a–c**, which indicates the presence of the BA-like CT state of **2a–c**, since the emission from the BA-like CT state appears at longer wavelength in the same solvent than that from the DMABA-like CT state.[7] The fact that the fluorescence quantum yields of **2a–c** are much lower than those of **3a–c** in the same solvent over a large polarity range (Table 1) indicates that the BA-like CT is actually a predominant CT channel with **2a–c** even in highly polar solvents such as ACN. It is hence made clear that compounds **2a–c** have two competitive CT channels of opposite direction. With increasing solvent polarity from CHX to ACN, the CT character in **2** changes from the BA-like to that of the BA-like dominant with contribution of the DMABA-like CT.

2.3. Compounds 2a–c as the CT dual fluorescent sensors for metal cations

Fluoroionophores **2a–c** as the CT dual fluorescent sensors for alkali and alkaline earth metal cations such as Li^+, Na^+ K^+, Mg^{2+}, Ca^{2+}, and Ba^{2+} were examined in ACN by using both absorption and fluorescence spectroscopy. Detailed spectroscopic data of **2a–c** and their metal complexes in ACN are summarized in Table 2. 15-Crown-5 is known to bind alkali and alkaline earth metal cations. The binding was confirmed by means of NMR titration in **2** by taking Na^+/**2a** complex as an example. In the presence of excess amount of Na^+, the ^1H NMR chemical shifts in DMSO-d_6 of the $-CH_2$ protons of the 15-crown-5 group and the amido –NH proton in **2a** shifted to downfield significantly.[11] Meanwhile, the absorption and fluorescence spectra of **3a–c** (Chart 1), the crown-free model molecules of **2a–c**, showed practically no change in the presence of metal cations. This observation further supports that metal cations bind to the crown-ether group in **2a–c**. It is found in Table 2 that in general the alkaline earth metal cations induce more significant changes in the spectra of **2a–c** than the alkali metal cations. This agrees with the assignment of the binding of metal cation to the crown-ether group in **2a–c**. Figure 2 shows the absorption and fluorescence spectra of **2a–c** in ACN in the presence of Ca^{2+}. It is noted that the absorption spectra of **2a–c** undergo only a slight red-shift by ca. 4 nm in the presence of an excess amount of Ca^{2+} and isosbestic points can be located at 267 nm and 312 nm for **2a**, 271 nm and 319 nm for **2b**, and at 271 nm and 318 nm for **2c**. Similar but smaller red-shift in the absorption spectra of **2a–c** was observed when the other five metal cations were present (Table 2). The red-shifts in the absorption spectra in the presence of metal cations can be explained in terms of the decreased electron-donating character of the 15-crown-5 moiety when a metal cation binds to it.[2a,b] Despite the minor changes in the absorption spectra, the appearance of the isosbestic points in the spectral titration traces point to the formation of well defined complexes between the metal cations and **2a–c**. Job plots (not shown) point to the 1:1 binding stoichiometry.

In contrast, the originally weak fluorescent **2a–c** experience substantial changes in their dual emission when metal cations are introduced (see Fig. 2b for the case of Ca^{2+} and Table 2 for detailed data). It is noted in Figure 2b that, upon introduction of Ca^{2+}, the intensities of the LE and CT emissions are enhanced, despite to different extents, and the LE emission is substantially shifted to the red. Red-shifts of the CT emission bands can also be identified in the presence of metal cation, although they are not as significant as those of the LE emission (see data in Table 2). It is therefore made clear that substantial changes occur in the total fluorescence quantum yield, the CT to LE emission intensity ratio, and the LE band position of **2a–c** in ACN when metal cations bind to their 15-crown-5 moiety. This means that the CT dual fluorescence of **2a–c** can indeed be employed as the sensing parameters for metal cations.

Figures 3–5 display the response profiles against metal cation concentration of the CT emission intensity enhancement

Table 2. Absorption and fluorescence spectroscopic data of **2a–c** and their metal complexes in ACN at room temperature (298 K)

	Absorption		Fluorescence			$\Delta\nu_{ST}$ (cm^{-1})[b]	log K[c]
	λ_{abs} (nm)	ε (10^4 mol^{-1} L cm^{-1})	λ_{LE} (nm)	λ_{CT} (nm)	Φ_{Rel}[a]		
2a	314	3.48	346.0	495.0	1.00	2945.4	—
2a+Li^+	317	3.49	349.8	495.0	1.31	2957.9	4.09
2a+Na^+	315	3.48	353.8	495.0	1.38	3481.4	4.32
2a+K^+	315	3.73	349.0	495.0	1.15	3092.7	3.57
2a+Mg^{2+}	317	3.53	370.0	500.0	5.92	4518.7	5.38
2a+Ca^{2+}	318	3.61	371.0	500.0	6.46	4492.3	5.67
2a+Ba^{2+}	317	3.49	369.0	500.0	3.31	4445.4	5.92
2b	320	3.94	364.0	490.0	1.00	3777.5	—
2b+Li^+	321	3.81	367.0	490.0	2.43	3904.6	4.37
2b+Na^+	321	3.96	369.0	490.0	3.57	4052.3	4.17
2b+K^+	321	3.96	367.0	490.0	1.86	3904.6	3.56
2b+Mg^{2+}	324	4.02	372.0	497.0	22.0	3982.5	5.58
2b+Ca^{2+}	324	4.06	373.0	497.0	22.7	4054.5	5.54
2b+Ba^{2+}	322	3.99	373.0	498.0	12.4	4246.2	5.78
2c	321	3.97	356.0	480.0	1.00	3062.7	—
2c+Li^+	322	4.05	366.6	480.0	2.25	3778.2	4.32
2c+Na^+	322	3.98	370.0	480.0	3.19	4028.9	4.35
2c+K^+	321	3.94	365.6	480.0	2.00	3800.3	3.49
2c+Mg^{2+}	324	4.08	379.0	483.0	18.5	4479.0	5.33
2c+Ca^{2+}	324	4.09	378.0	482.0	19.6	4409.2	5.31
2c+Ba^{2+}	323	4.00	378.0	480.0	8.31	4504.8	5.90

[a] Quantum yield relative to that of sensor; metal cation concentration is 100 times that of the sensor's.
[b] Stokes shift of the LE emission, $\Delta\nu_{ST}=\nu_{abs}-\nu_{flu\ (LE)}$.
[c] Binding constant K in mol^{-1} L.

(I/I_0), the LE band position, and the CT to LE emission intensity ratio of **2a–c**. Note that all of the three parameters increase initially with metal cation concentration and level off at higher metal cation concentration. The corresponding leveled-off parameters can therefore attribute to those of the **2**-metal binding complexes.

Analysis of the variations of these parameters helps to understand the mechanism how the dual fluorescence of **2a–c** responds to the metal cations. It was shown[5] that with **1** the fluorescence quantum yield increased when the substituent X at the amido aniline phenyl ring became more electron-withdrawing. The quantum yields of **2a–c** increased in the presence of metal cations, in particular of Ca^{2+} with which enhancements of the quantum yields of **2a–c** amounted to 6, 23, and 20 folds (Table 2). The enhancements in the CT emission are more dramatic, being 11, 26, and 21 folds (Fig. 3). With other metal cations dramatic enhancements in the total quantum yields and the CT emission were also found (Fig. 3 and Table 2). Such significant fluorescence

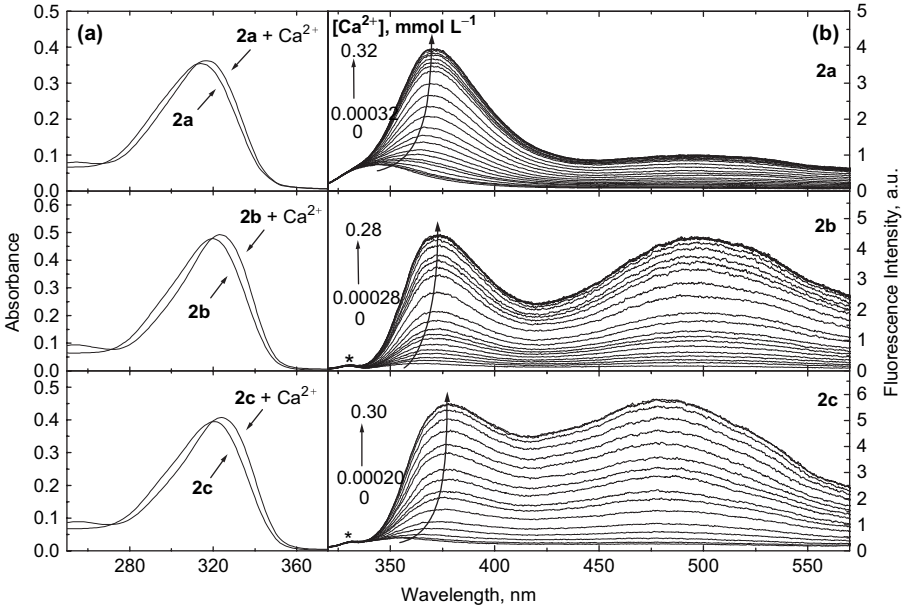


Figure 2. Absorption (a) and fluorescence spectra (b) of **2a** (1.00×10^{-5} mol L^{-1}), **2b** (1.22×10^{-5} mol L^{-1}), and **2c** (1.00×10^{-5} mol L^{-1}) in ACN in the absence and the presence of excess amount of Ca^{2+} (a) and of increasing concentration of Ca^{2+} (b). All metal cations existed in the form of perchlorate. Excitation wavelength was 300 nm. Asterisks in (b) refer to Raman scattering.

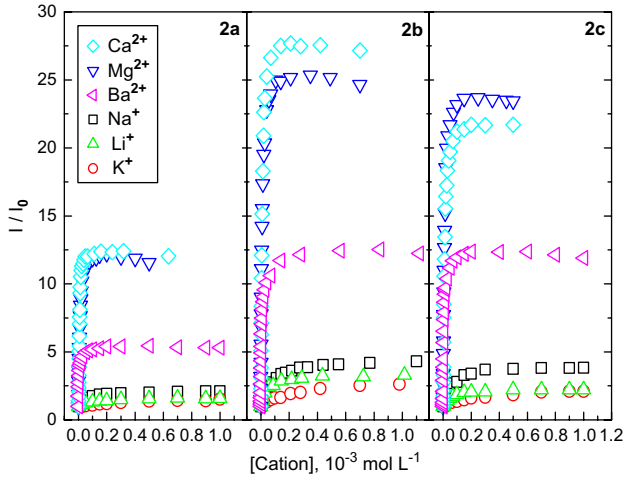


Figure 3. The CT fluorescence intensity enhancement (I/I_0) of **2a–c** as a function of cation concentration. Excitation wavelength was 300 nm. I and I_0 represent the CT emission intensities in the presence and absence of the metal cation, respectively.

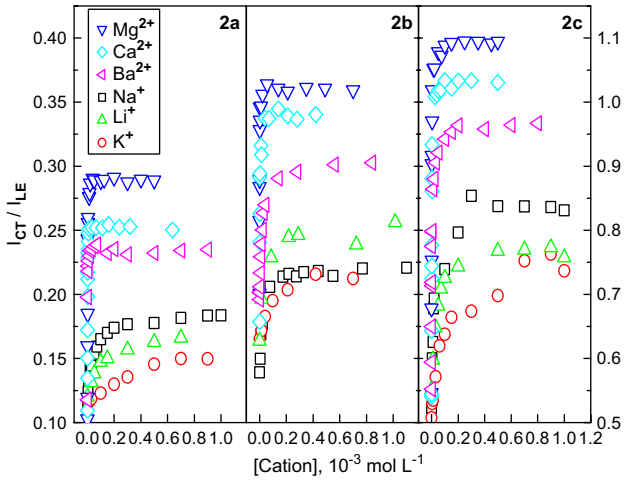


Figure 5. Plots of the CT to LE fluorescence intensity ratios of **2a–c** versus cation concentration. Plots of **2a** have the left coordinate and those of **2b** and **2c** have the same right coordinate.

enhancements have only been observed in some of the PET fluorescent sensing systems[13] and several well-designed ICT systems.[10]

The red-shift of the LE band of **2a–c** in ACN in the presence of metal cations is very helpful for understanding the emission enhancement. As shown in Figure 4, Mg^{2+}, Ca^{2+}, and Ba^{2+} induce substantial red-shifts in the LE emission (see also $\Delta\nu_{ST}$ in Table 2), whereas the monovalent alkali metal cations, Li^+, Na^+, and K^+ result in relatively less red-shifts. Upon comparing the LE band positions of the **2a**-metal complexes with those of **1** derivatives,[5] it was found that the LE band of **2a** that originally peaked at 346 nm was close to the LE band of a derivative of **1** bearing a highly electron-donating substituent. It shifted to 371 nm in its complex with Ca^{2+} that bears an analogy to a derivative of **1** with an electron-withdrawing substituent, *p*-Cl (Fig. 4). Obviously, cation binding changes the nature of the 15-crown-5 moiety in **2a**, considered as a substituent at the amido aniline of **1**,

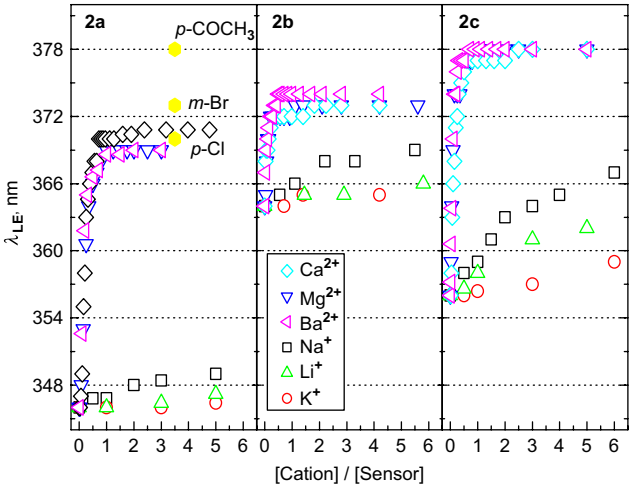


Figure 4. The LE band positions of **2a–c** in ACN in the presence of metal perchlorates. Excitation wavelength was 300 nm. Solid hexahedral symbols are the LE band position data taken from [5] for the derivatives of **1** bearing the indicated substituents at the amido aniline.

from electron donating to withdrawing. This means that **2a**, upon Ca^{2+} binding, changes its structural pattern from D–A–D′ to D–A–A′, the CT occurring in **2a**-Ca^{2+} complex might now be the DMABA-like that the 4-dimethylamino group is the electron donor. For the sake of clarity, part of the previously reported LE band positions of the derivatives of **1** in ACN are also given as solid hexahedrons in Figure 4. The red-shift in the LE band positions of **2b** and **2c** induced by cation binding behaves similar to that of **2a**. For example, the LE emission of **2b** shifts upon binding to Ca^{2+} from 364 to 373 nm and of **2c** from 356 nm to 378 nm.

The LE band positions of the **2b**-Ca^{2+} and **2c**-Ca^{2+} complexes can be compared to those of **1** bearing substituents *m*-Br and *p*-$COCH_3$, respectively, Figure 4. This means that, in the **2**-Ca^{2+} complexes, the consequence of lengthening the alkyl chain in the 4-dialkylamino group in **2** from methyl (**2a**) to ethyl (**2b**) and *n*-butyl (**2c**) is the same as in **1** with increasing electron-withdrawing ability of the substituent (X) at its amido aniline. It is therefore concluded that in the **2**-metal complexes, the excited-state CT occurs with the 4-dialkylamino group being the electron donor, i.e., the CT in the complexes is the DMABA-like. The 15-crown-5 moiety in **2**, the metal cation binding site, is therefore shown to be within the electron acceptor of their metal complexes. This means that metal cation binding to the crown-ether moiety in **2** results in the switching of the CT from mainly the BA-like with **2** to the DMABA-like with their metal complexes. During such a switching, the BA-like CT emission would be blue shifted while the DMABA-like CT emission is red shifted. This explains the observed small apparent red-shift of the CT emission of **2a–c** upon metal binding (Fig. 2 and Table 2), and in part the increase in the total fluorescence quantum yields of **2a–c** (Table 2).

Variations of the CT to LE emission intensity ratio of **2a–c** with metal cation concentration (Fig. 5) not only confirm that ratiometric fluorescent assays can be established, but they also provide further supports for the conclusion that metal cation binding induces switching of the CT direction in **2a–c**. For example, the observation that the CT to LE emission intensity ratio of the **2**-metal complexes increases

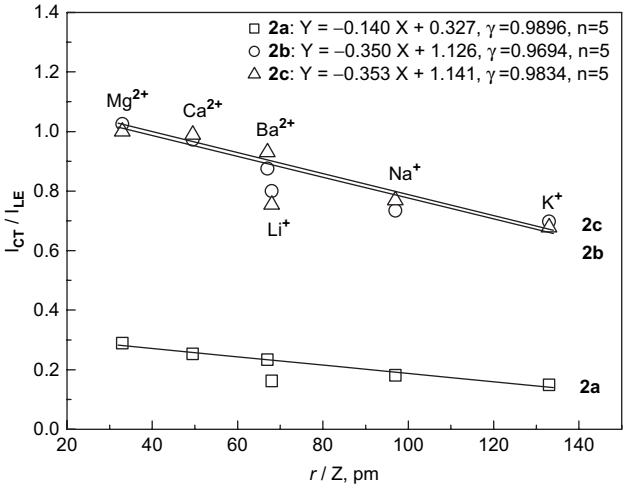


Figure 6. Linear correlation of the CT to LE intensity ratio of the **2**-metal complex with the radius to charge ratio of the metal cation. Data points for Li^+ were not included in the linear correlation.

from 0.26 (**2a**+Ca^{2+}) via 0.97 (**2b**+Ca^{2+}) to 0.99 (**2c**+Ca^{2+}) is similar to what was observed with the known CT fluorophores 4-(dialkylamino)benzonitriles of increasing alkyl chain in which the dialkylamino group was identified as the electron donor.[9] Significantly, the CT to LE emission intensity ratio of the metal complexes of **2a–c** was found to show a linear correlation with the charge density parameter (radius to charge ratio, *r*/*Z*) of the metal cation Na^+, K^+, Mg^{2+}, Ca^{2+}, and Ba^{2+},[14] with Li^+ being off line[15] (Fig. 6). This observation indicates that the electronic attractive interaction between the metal cation and the crown-ether moiety in **2a–c** promotes the total DMABA-like CT in the complex,[14c] confirming that the crown-ether moiety is within the electron acceptor of the complex. The observed linear slopes of the metal complexes of **2a–c** vary in the order of **2c**∼**2b**>**2a** of decreasing electron donating ability of the 4-dialkylamino group in **2** indicates that the metal cation influence is stronger in case where more DMABA-like CT is expected. This suggests that in the metal complexes of **2a–c** the occurrence of the DMABA-like CT enhances the metal cation binding to **2a–c**.

2.4. Binding constants of 2a–c with metal cations

Binding constants of **2a–c** with the investigated metal cations in ACN were then evaluated by nonlinear regressions[16] from the fluorescence titration data. The data can be nicely fitted assuming a 1:1 binding stoichiometry, which is confirmed by the Job plots (not shown). The log *K* values of **2a–c** differ not much with the same cation, while for the same sensor they vary in the order of Mg^{2+}∼Ca^{2+}∼Ba^{2+}>Na^+∼Li^+>K^+ (Table 2). The latter order is again consistent with the electronic attractive nature for the interaction between metal cation and the crown-ether moiety in **2**. The binding constants of the metal cations with **2** were found indeed higher than those with ionophores bearing benzo-15-crown-5 binding moiety but without the occurrence of the excited-state CT[13b,17] and, in particular, higher than those with the CT dual fluorescent sensors with the aza-15-crwon-5 binding site being within the electron donor.[2a–d,2h,3b,10b,c,15] The high binding constants for alkaline metal cations at 10^5 mol^{-1} L orders of magnitude (Table 2) make highly sensitive ratiometric fluorescent assays possible at sub-micromolar level (Figs. 2 and 5).

3. Conclusions

A series of the CT dual fluorescent sensors **2a–c** for alkali and alkaline earth metal cations with cation binding site within the electron acceptor were developed. These sensors were designed on the basis of the structural framework of 4-(dimethylamino)benzanilides (**1**) in which two CT channels of opposite direction were identified by varying the substituents at the amido aniline phenyl ring. 15-Crown-5 was incorporated in but separated from the amide moiety by a rigid phenyl ring, thereby avoiding any steric consequence when the metal cation binds to the 15-crown-5 moiety. Indeed, **2a–c** underwent substantial changes in their CT photophysics and the accompanied dual emission upon metal cation binding in ACN, i.e., increased total quantum yield and the CT to LE emission intensity ratio, and the red-shifted LE emission. These changes were shown to result from the switching of the CT character of mainly the BA-like in the absence of the metal cation to the DMABA-like when metal cation bound to the 15-crown-5 moiety. We therefore succeeded in developing the CT dual fluorescent sensors for cations with binding site within the electron acceptor and without obvious steric interference caused by cation binding. The expected CT-facilitated cation binding was confirmed by the higher cation binding constants. Substantial increase in the CT to LE emission intensity ratio upon cation binding also allows for highly sensitive ratiometric assays for cations, at for example sub-micromolar level for the alkaline metal cations. As a wealth of knowledge is now available with the related CT photophysics,[1] and the ease of incorporating other metal binding groups in the amido aniline phenyl ring, extensions of the current proof-of-principle structural framework are promising in constructing CT dual fluorescent sensors for metals of better and more practical applications.

4. Experimental

4.1. Instruments

Steady-state fluorescence spectra were recorded with a Hitachi F-4500 fluorescence spectrophotometer using excitation and emission slits of 5 nm. Fluorescence quantum yields were measured using quinine sulfate as a standard (Φ_F= 0.546 in 0.5 mol L^{-1}).[18] Absorption spectra were taken on a Varian Cary 300 absorption spectrophotometer using a 1-cm quartz cell. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data were acquired in $CDCl_3$ on a Varian Unity$^+$ 500 MHz NMR spectrometer using TMS as an internal reference. HRMS were obtained on a Micromass LCT spectrometer. Elemental analyses were carried out by using CARLO ERBA1500 element analyzer.

4.2. Materials

4-(Dimethylamino)benzoic acid was synthesized from the reaction of 4-aminobenzoic acid with dimethylsulfate in alkaline aqueous solution.[19] 4-(Diethylamino)benzoic acid was purchased from Aldrich. 4-(Di(*n*-butyl)amino)benzoic

acid was synthesized according to a reported method.[20] Alkali and alkaline earth metal perchlorates were purchased from Shanghai Chemicals Company (Shanghai, China) and were kept anhydrous over P_2O_5 in a desiccator. Solvents for spectroscopic measurements were purified before use and checked to have no fluorescent impurity at the employed excitation wavelengths.

4.3. General procedures for the synthesis of 2a–c and 3a–c

As shown in Scheme 1, **2a–c** were synthesized by addition of 4-(dialkylamino)benzoyl chloride into CH_2Cl_2 solution of 4-aminobenzo-15-crown-5 hydrochloride and triethylamine, followed by stirring at room temperature for 6 h. The crude products were purified by column chromatography on silica gel by using CH_2Cl_2, ethyl acetate, and methanol mixture (8:1:1, v/v/v) as the eluent. The model molecules **3a–c** were synthesized in a similar manner and the crude products were easily obtained by removing the solvent and by washing with dilute aqueous NaOH solution. The obtained white precipitates were purified by repeated recrystallizations from acetone.

4.3.1. 4-Dimethylamino-*N*-(6,7,9,10,12,13,15,16-octahydro-5,8,11,14,17-pentaoxabenzocyclopentadecen-2-yl)-benzamide (2a). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.03 (s, 6H), 3.75 (d, J=4.5 Hz, 8H), 3.88–3.91 (m, 4H), 4.12 (t, J=4 Hz, 2H), 4.15 (t, J=4 Hz, 2H), 6.71 (d, J=8.5 Hz, 2H), 6.83 (d, J=8.5 Hz, 1H), 6.95 (d, J=8.5 Hz, 1H), 7.52 (s, 1H), 7.77 (d, J=8.5 Hz, 2H), 7.82 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ 40.27 (2C), 68.69 (1C), 69.44 (1C), 69.69 (2C), 70.39 (1C), 70.58 (1C), 70.92 (1C), 71.01 (1C), 107.18 (1C), 111.43 (2C), 112.41 (1C), 114.89 (1C), 121.81 (1C), 128.56 (2C), 132.88 (1C), 145.44 (1C), 149.35 (1C), 152.27 (1C), 165.46 (O═C) ppm. HRMS (ESI) for $C_{23}H_{31}N_2O_6$ (M+H$^+$) calcd 431.2182, found 431.2183 (M+H$^+$), 453.2003 (M+Na$^+$). Anal. Calcd for $C_{23}H_{30}N_2O_6$: C, 64.17; H, 7.02; N, 6.51. Found: C, 64.04; H, 6.95; N, 6.30.

4.3.2. 4-Diethylamino-*N*-(6,7,9,10,12,13,15,16-octahydro-5,8,11,14,17-pentaoxabenzocyclopentadecen-2-yl)benzamide (2b). ^{1}H NMR ($CDCl_3$, 500 MHz): 1.19 (t, J= 7 Hz, 6H), 3.38–3.42 (m, 4H), 3.75 (d, J=4 Hz, 8H), 3.87–3.90 (m, 4H), 4.12 (t, J=4 Hz, 2H), 4.15 (t, J=4 Hz, 2H), 6.65 (s, 2H), 6.82 (d, J=8.5 Hz, 1H), 6.94 (d, J=7 Hz, 1H), 7.52 (s, 1H), 7.75 (d, J=8 Hz, 2H), 7.80 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): 12.41 (2C), 44.40 (2C), 68.66 (1C), 69.43 (1C), 69.68 (2C), 70.38 (1C), 70.57 (1C), 70.91 (1C), 71.01 (1C), 107.05 (1C), 110.47 (2C), 112.27 (1C), 114.89 (1C), 120.27 (1C), 128.79 (2C), 132.91 (1C), 145.35 (1C), 149.33 (1C), 150.16 (1C), 165.39 (O═C) ppm. HRMS (ESI) for $C_{25}H_{35}N_2O_6$ calcd 459.2495 (M+H$^+$), found 459.2495 (M+H$^+$), 481.2351 (M+Na$^+$). Anal. Calcd for $C_{25}H_{34}N_2O_6$: C, 65.48; H, 7.47; N, 6.11. Found: C, 65.66; H, 7.81; N, 5.86.

4.3.3. 4-Dibutylamino-*N*-(6,7,9,10,12,13,15,16-octahydro-5,8,11,14,17-pentaoxabenzocyclopentadecen-2-yl)benzamide (2c). ^{1}H NMR ($CDCl_3$, 500 MHz): 0.96 (t, J=7 Hz, 6H), 1.34–1.39 (m, 4H), 1.58 (s, 4H), 3.31 (t, J=7.5 Hz, 4H), 3.75 (d, J=4 Hz, 8H), 3.89 (s, 4H), 4.11 (t, J=4 Hz, 2H), 4.15 (t, J=4 Hz, 2H), 6.62 (s, 2H), 6.83 (d, J=8.5 Hz, 1H), 6.91 (d, J=7.5 Hz, 1H), 7.52 (s, 1H), 7.72 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): 13.91 (2C), 20.22 (2C), 29.22 (2C), 50.68 (2C), 68.66 (1C), 69.43 (1C), 69.70 (2C), 70.38 (1C), 70.55 (1C), 70.91 (1C), 70.99 (1C), 107.03 (1C), 110.57 (2C), 112.22 (1C), 114.91 (1C), 120.15 (1C), 128.69 (2C), 132.93 (1C), 145.33 (1C), 149.35 (1C), 150.57 (1C), 165.39 (O═C) ppm. HRMS (ESI) for $C_{29}H_{43}N_2O_6$ calcd 515.3121 (M+H$^+$), found 515.3116 (M+H$^+$), 537.2929 (M+Na$^+$). Anal. Calcd for $C_{29}H_{42}N_2O_6$: C, 67.68; H, 8.23; N, 5.44. Found: C, 67.36; H, 8.12; N, 5.28.

4.3.4. 4-Dimethylamino-*N*-(*iso*-propyl)benzamide (3a). ^{1}H NMR ($CDCl_3$, 500 MHz): 1.24 (d, J=6.5 Hz, 6H), 3.00 (s, 6H), 4.23–4.32 (m, 1H), 5.85 (s, 1H), 6.66 (d, J=8 Hz, 2H), 7.67 (d, J=9 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): 22.95 (2C), 40.14 (2C), 41.48 (1C), 111.09 (2C), 121.83 (1C), 128.19 (2C), 152.22 (1C), 166.56 (O═C) ppm. HRMS (ESI) for $C_{12}H_{19}N_2O$ calcd 207.1497 (M+H$^+$), found 207.1495. Anal. Calcd for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.80; N, 13.58. Found: C, 69.68; H, 9.00; N, 13.72.

4.3.5. 4-Diethylamino-*N*-(*iso*-propyl)benzamide (3b). ^{1}H NMR ($CDCl_3$, 500 MHz): 1.17 (t, J=7 Hz, 6H), 1.23 (d, J=6.5 Hz, 6H), 3.36–3.40 (m, 4H), 4.23–4.32 (m, 1H), 5.80 (s, 1H), 6.62 (d, J=8.5 Hz, 2H), 7.64 (d, J=9 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): 12.43 (2C), 22.99 (2C), 41.42 (1C), 44.38 (2C), 110.42 (2C), 120.77 (1C), 128.47 (2C), 149.82 (1C), 166.56 (O═C) ppm. HRMS (ESI) for $C_{14}H_{23}N_2O$ calcd 235.1810 (M+H$^+$), found 235.1808. Anal. Calcd for $C_{14}H_{22}N_2O$: C, 71.76; H, 9.46; N, 11.95. Found: C, 71.90; H, 9.38; N, 12.19.

4.3.6. 4-Dibutylamino-*N*-(*iso*-propyl)benzamide (3c). ^{1}H NMR ($CDCl_3$, 500 MHz): 0.95 (t, J=7.5 Hz, 6H), 1.23 (d, J=6.5 Hz, 6H), 1.31–1.39 (m, 4H), 1.53–1.59 (m, 4H), 3.29 (t, J=7.5 Hz, 4H), 4.22–4.32 (m, 1H), 5.80 (s, 1H), 6.58 (d, J=8.5 Hz, 2H), 7.63 (d, J=8.5 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): 13.89 (2C), 20.20 (2C), 22.97 (2C), 29.21 (2C), 41.36 (1C), 50.63 (2C), 110.44 (2C), 120.58 (1C), 128.35 (2C), 150.19 (1C), 166.54 (O═C) ppm. HRMS (ESI) for $C_{18}H_{31}N_2O$ calcd 291.2436 (M+H$^+$), found 291.2441. Anal. Calcd for $C_{18}H_{30}N_2O$: C, 74.44; H, 10.41; N, 9.65. Found: C, 73.96; H, 10.38; N, 9.24.

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11. ^{1}H NMR data (in ppm) of **2a** in DMSO-d_6 in the absence and the presence of saturated $NaClO_4$: (a) **2a** in the absence of $NaClO_4$, 2.995 (s, 6H), 3.617 (s, 8H), 3.759–3.788 (m, 4H), 4.023 (s, 4H), 6.749 (d, J=9 Hz, 2H), 6.898 (d, J=8.5 Hz, 1H), 7.279 (d, J=8.5 Hz, 1H), 7.462 (s, 1H), 7.845 (d, J=8.5 Hz, 2H), and 9.722 (s, 1H); (b) **2a** in the presence of saturated $NaClO_4$, the data becoming 2.996 (s, 6H), 3.635 (s, 8H), 3.777–3.810 (m, 4H), 4.076 (s, 4H), 6.751 (d, J=9 Hz, 2H), 6.976 (d, J=8.5 Hz, 1H), 7.306 (d, J=8.5 Hz, 1H), 7.541 (s, 1H), 7.847 (d, J=8.5 Hz, 2H), and 9.760 (s, 1H).
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Stereoselective iodocyclisation of 3-acylamino-2-methylene alkanoates: a computational insight

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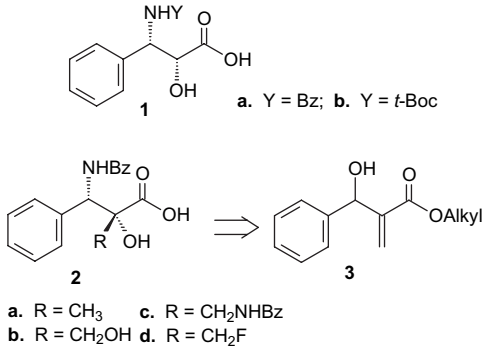
Abstract—In order to explain the high stereocontrol occurring in the iodocyclisation of 3-acylamino-2-methylenealkanoates, either the conformational space of the starting products or the cyclisation reaction potential energy surface (PES) was explored at DFT level of theory, the polarised continuum formalism (PCM) for chloroform being used in order to consider the solvent effect. The observed stereoselection was ascribed to both the near attack conformations (NACs) distribution and the energy differences between the two possible and competitive cyclisation pathways leading to cis- and trans-diastereomers.

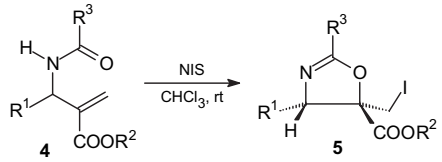
1. Introduction

3-Amino-2-hydroxy acids are compounds of particular interest owing to their biological activity. For example, *N*-benzoyl-*syn*-phenylisoserine **1a**[1] and *N*-*tert*-butoxycarbonyl-*syn*-phenylisoserine **1b**[2] occur as the C-13 side chain of paclitaxel (Taxol®)[3] and docetaxel (Taxotere®),[4] respectively. Within a research project directed to induce conformational changes in bioactive oligopeptides by means of conformationally restricted β-amino acids, starting from the Baylis–Hillman adduct **3** the racemic compounds **2a–d** were prepared, analogues of *N*-benzoyl-*syn*-phenylisoserine **1a** (Scheme 1).[5]



Scheme 1.

In the key step of the synthetic route, 3-acylamino-2-methylene-3-arylpropanoates **4** underwent iodocyclisation with NIS in chloroform[6] to give the corresponding dihydro-1,3-oxazoles **5** in high yield and with total diastereoselection (Scheme 2).[5] Thus, a computational study was initiated in order to rationalise the outcome of the cyclisation reaction.[7]



a. R^1 = Ph, R^2 = Et, R^3 = Ph
b. R^1 = Ph, R^2 = Et, R^3 = *t*-Bu
c. R^1 = 4-$CH_3OC_6H_4$, R^2 = Et, R^3 = Ph
d. R^1 = Ph, R^2 = *t*-Bu, R^3 = Ph
e. R^1 = Ph, R^2 = *t*-Bu, R^3 = Bn
f. R^1 = Ph, R^2 = *t*-Bu, R^3 = CH_2Cl
g. R^1 = Ph, R^2 = *t*-Bu, R^3 = CCl_3
h. R^1 = Me, R^2 = *t*-Bu, R^3 = CCl_3
i. R^1 = 4-NO_2-C_6H_5, R^2 = Me, R^3 = P
j. R^1 = 4-Cl-C_6H_5, R^2 = Et, R^3 = Ph
k. R^1 = Ph, R^2 = Me, R^3 = Ph

Scheme 2.

2. Computational methods

All calculations were carried out on SGI Octane2 IRIX 6.5 workstations. Molecular mechanics calculation were performed using the implementation of AMBER force field (AMBER*)[8] within the framework of Macromodel version 5.5.[9] The torsional space of each molecule was randomly varied with the usage-directed Monte Carlo conformational search.[10] For each search, at least 1000 starting structures for each variable torsion angle was generated and minimised until the gradient was less than 0.01 kcal/Åmol. Duplicate

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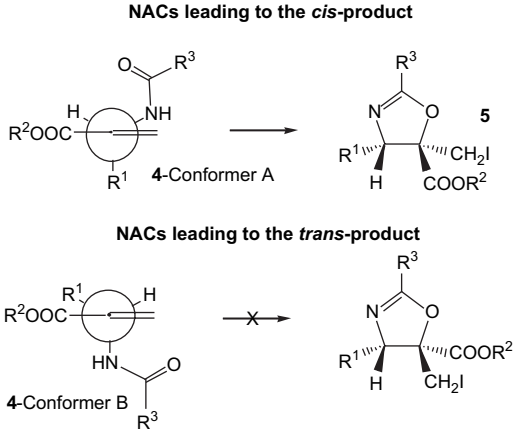
conformations and those having energy in excess of 6 kcal/mol above the global minimum were discarded. The solvent effect was included by using the implicit chloroform GB/SA solvation method of Still et al.[11]

All DFT calculations were carried out using the standard tools available in the *Gaussian 98* package,[12] with Becke's three parameter hybrid functional having the Lee–Yang–Parr correlation term (B3LYP),[13] with 6-311G** basis set for iodine and 6-31G* for all the other atoms. These functional and basis sets have been shown to properly describe the considered systems and polarisation functions are required in such calculation to obtain correct results. The transition states were searched by using the synchronous transit-guided quasi-Newton method. Frequency analyses were performed in order to obtain both energetic information about the reaction pathways and to fully characterise the nature of the stationary points onto the reaction potential energy surface (PES). The only imaginary frequency of the TS structures really corresponds to the correct reaction coordinate. Solvent effects were modelled using the polarised continuum (overlapping spheres) formalism (PCM).[14] The PCM method models the solvent as a continuum of uniform dielectric constant, and the solute is placed into a cavity within the solvent. The cavity is constructed by placing a sphere around each solute heavy atom. Hydrogen atoms are always enclosed within the sphere of the atom to which they are bonded. For the atomic radii, the Bondi approximation was used. In this method the effects of solvation are folded into the interactive SCF procedure. The dielectric constant of the solvent used was ε=4.9 (for chloroform).

3. Results and discussion

At first, in order to definitively ascertain the mechanism of the iodocyclisation reaction taking into account the experimental evidence, we fully explorated the conformational space of the starting 3-acylamino derivatives **4a–k**. This step was carried out with the aim to localise the near attack conformations (NACs)[15,16] leading to the *cis*- and *trans*-dihydro-1,3-oxazoles through the NIS mediated iodocyclisation reaction (Scheme 3).



Scheme 3. Geometry of NACs conformers of compound **4** leading to *cis*- and *trans*-dihydro-1,3-oxazoles.

Table 1. MC-search results for conformers **A** and **B** of compounds **4a–k**, obtained with 1000 step/torsional, GB/SA, $CHCl_3$, AMBER* and ΔE=6.0 kcal/mol

Compound **4**	Conformers ΔE=6.0 kcal/mol	Lowest energy conformer type A (kcal/mol)	Lowest energy conformer type B (kcal/mol)
a	52	0.0	2.60
b	44	0.0	2.69
c	132	0.0	2.38
d	23	0.0	2.25
e	41	0.0	2.27
f	18	0.0	2.23
g	13	0.0	2.08
h	11	0.0	2.97
i	28	0.0	1.71
j	35	0.0	1.60
k	24	0.0	2.62

Once the NAC has been defined, the problem can be viewed as consisting of two parts: (a) identifying ground state conformational equilibria, including formation of NACs, and (b) locating the transition state of the NAC to product. Using the energy of the conformations in a weighed Boltzmann distribution, we calculated the percentage fraction of conformers present as NACs for each compound (Table 2). Since there is a direct linear free-energy relationship between the log of the fraction of this kind of conformations and $\Delta G^{\ddagger}$, the rate constants for the cyclisation can be related to the mole fraction of the ground state of each amide present as NACs.

From the data collected, we found out two main groups of conformations, namely conformer A and B, whose structure is shown in Scheme 3. In conformer A, the iodocyclisation reaction can take place with attack of the amidic oxygen to the *re*-face of the allylic carbon, leading to the *cis*-dihydro-1,3-oxazole, while in conformer B the attack takes place on the *si*-face forming the trans-product. Since the reaction proceeds with total diastereoselection to give the cis-diastereomer, exclusively, we expect that a larger number of the NACs like A must be present and at lower energy with respect to conformations like B. Indeed, from the energetic point of view, the calculations strongly agree with the expectations (Tables 1 and 2).

Then, the geometry of each structure obtained from the conformational analysis was analysed and all the stable

Table 2. Populations of all conformers lying within 3.6 kcal/mol for compounds **4a–k**

Compounds	Population NACs cis %	Population NACs trans %	Population not-NACs
a	97.59	0.92	1.49
b	94.55	2.13	3.32
c	93.6	1.40	4.99
d	93.83	4.35	1.84
e	86.19	3.15	10.66
f	92.85	4.33	2.82
g	86.01	2.07	11.92
h	99.89	0.11	0.0
i	76.51	18.70	4.79
j	87.29	5.49	7.22
k	92.85	0.88	6.27

conformations, which lie in energy gap of 3.6 kcal/mol were classified into three categories: NACs for cis-cyclisation, NACs for trans-cyclisation and non-NACs. In Table 2, the global population of each group is summarised at T=298 K.

It was found that for all the compounds examined, the conformation type A (NACs for the observed *re*-face attack) are more populated than the corresponding type B (NACs for the not observed *si*-face attack). Since higher the population of NACs the lower is the activation energy of reaction, $\Delta G^{\ddagger}$,[16] we could immediately argue that the iodocyclisation leading to a cis-isomer is strongly favoured with respect to the iodocyclisation leading to a trans-isomer, since the molar fraction of the corresponding NACs is much larger for cis than for trans.

However, even if these data support our findings, this result alone does not completely explain the total stereoselection of the reaction, in particular for compound **4i** whose conformer type B at lower energy is significantly populated (Fig. 1).

This result prompted us to further investigate the mechanism of the iodocyclisation reaction localising the reaction paths by means of DFT quantum mechanical calculations starting from a representative compound of the amides **4a–k** having a simpler structure. Particularly, we choose the model amide **6** having R^1=*i*-Pr, R^2=*t*-Bu and R^3=CH_3, i.e., substituent groups smaller than those considered in the reported reaction (Scheme 2), which keep however the similar stereoelectronic properties. This allowed us to perform higher level ab initio calculation saving CPU time.

There are three possible reaction mechanisms, which can give rise to a cyclic product: (a) the formation of the NIS-substrate complex followed by iodonium ion formation and cyclisation (type 1); (b) the formation of the NIS-substrate complex followed by cyclisation (concerted mechanism) (type 2) and (c) the formation of the iodine cation (I^+)-substrate complex, followed by the cyclisation reaction (type 3) (Schemes 4 and 5). In order to validate or to exclude each one of these possible pathways, we localised the reaction paths onto the PES minimising the energy of all the molecular species involved (Scheme 6).

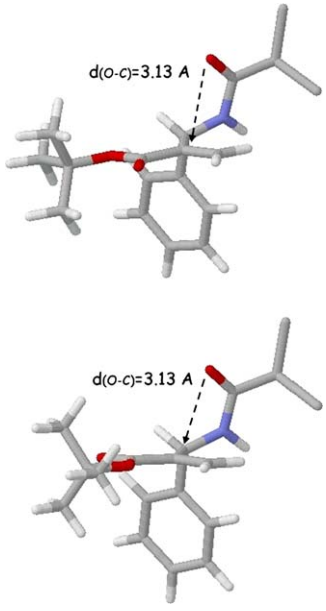
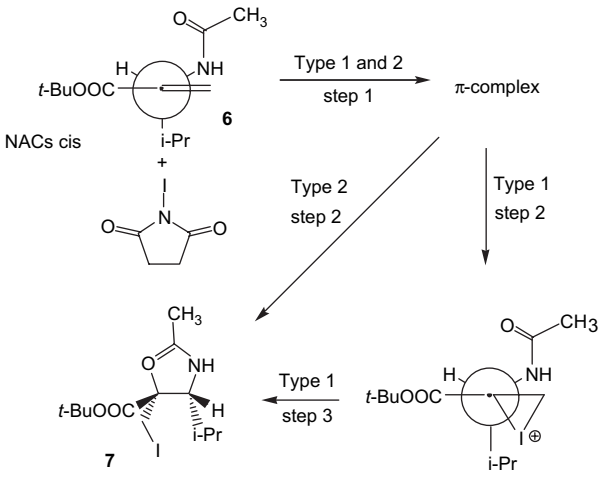


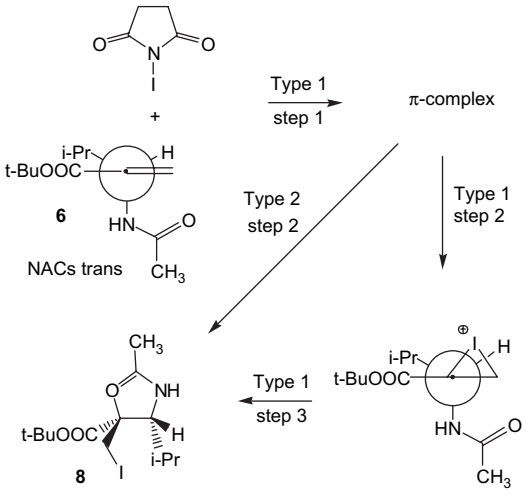
Figure 1. Lowest energy conformers (NACs for cis) for compound **4g** (type A).



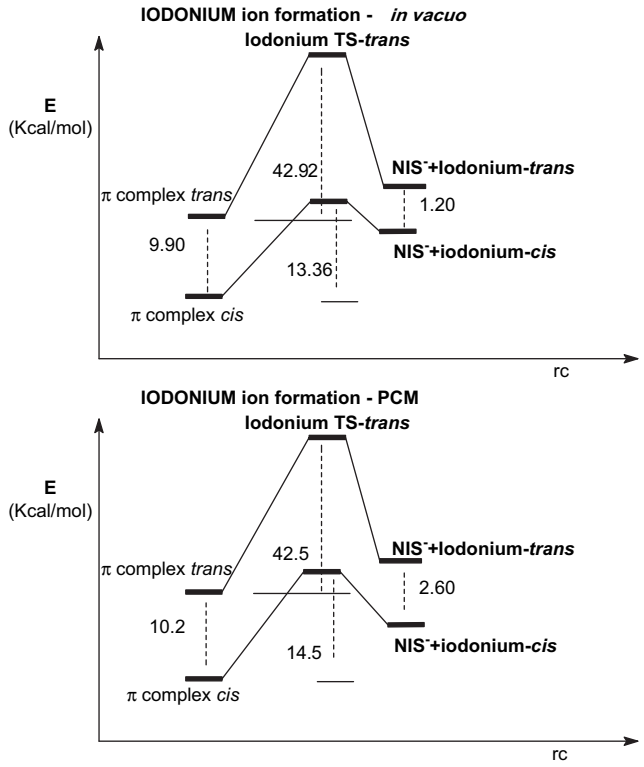
Scheme 4. Type 1 and type 2 mechanisms for iodocyclisation of **6** leading to the cis-product **7**.

Type 1 mechanism was analysed first beginning from reaction steps 1 and 2 relative to the π-complex formation for both cis- and trans-iodocyclisation. Since a complex formation is generally a very fast process, so that it cannot be involved in the stereoselectivity control, the reaction step 1 was not explicitly considered being furthermore structurally and thermodynamically very similar for both iodocyclisation reactions (namely cis and trans).

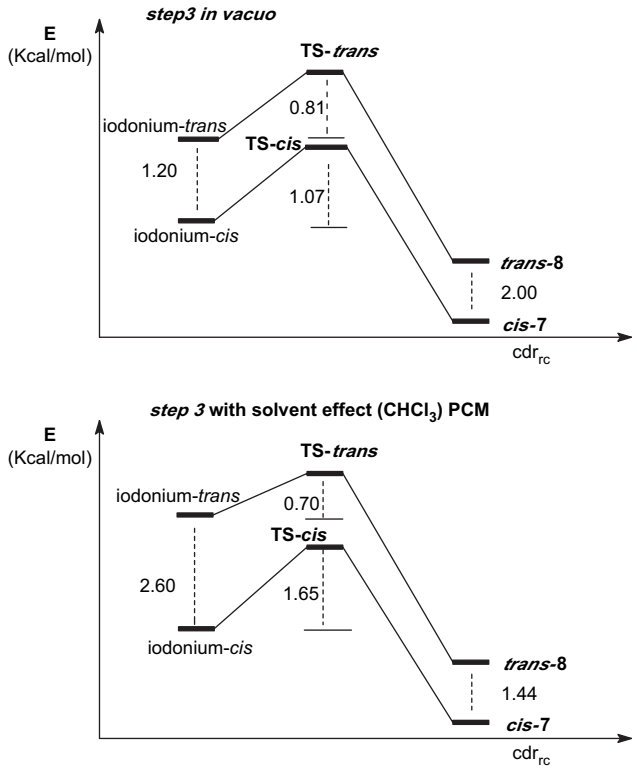
The existence of the π-complex was confirmed localising it as a stationary point onto the PES surface and also the transition state (TS) leading to the iodonium ion was found both for the cis- and trans-cyclisation (Fig. 2). All these calculations were performed in vacuo and in order to obtain more



Scheme 5. Type 1 and type 2 mechanisms for iodocyclisation of **6** leading to the trans-product **8**.



Scheme 6. Energy profiles for cis and trans-iodocyclisation step 2 (type 1 cyclisation) both in vacuo and in chloroform (PCM).



Scheme 7. Energy profiles for iodocyclisation step 3 (type 1 cyclisation) leading to either *cis*-**7** or *trans*-**8** both in vacuo and in chloroform (PCM).

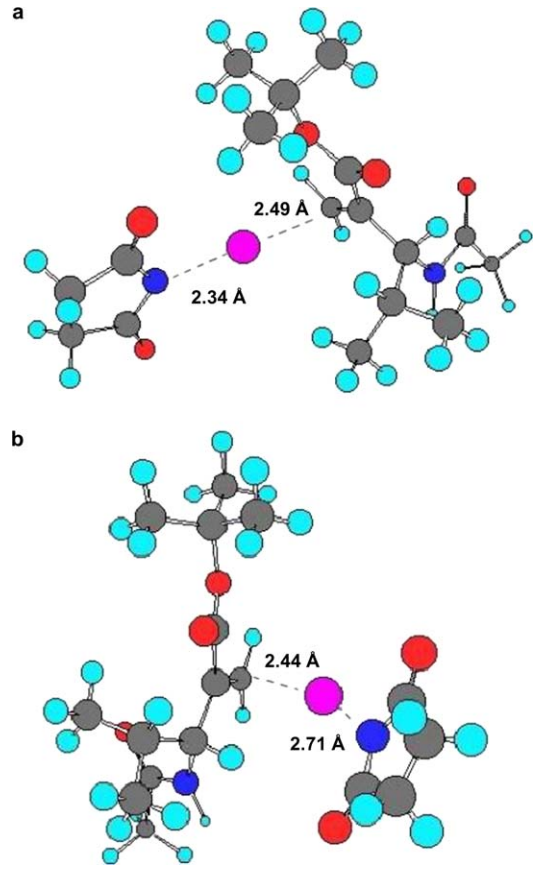


Figure 2. Transition structures of 'cis' (a) and 'trans' (b) TS of the iodonium ion formation starting from the NIS–amide–NACS π-complex (step 2).

accurate results, we included the solvent effect implicitly by using the PCM model of solvation in chloroform. When comparing the results of these two different approaches, we observed in general a change in energy but not in the relative stability of all the molecular species involved in the process.

Furthermore, for step 2 (type 1 cyclisation) it was shown that the solvent does not change the stability of the π-complex significantly or the activation energy of this step but induce a further stabilisation of the final product, *cis*-iodonium ion with respect to the trans cation.

The activation energy of this process was found to be high for the trans path and small for the *cis*-one (Scheme 7). In addition, the energy of the iodonium 'cis' is lower than the corresponding 'trans', suggesting that the cis-process is favoured over the *trans*-one by both kinetic and thermodynamic control.

When we subsequently considered step 3, the reaction pathway shows it to be fast owing to very low activation energy for both cis and trans-cyclisation processes, with the geometry of TS close to that of reagents according to the Hammond postulate (Fig. 3). Anyway, even if this step cannot affect stereoselectivity as it is too fast, the iodocylisation leading to the cis-isomer is again favoured, having a lower activation energy and forming the most stable product. From all these results, we could deduce that step 2, i.e., the formation of the π-complex, is the steady state of the overall reaction and is responsible for the stereoselection observed, according to the mechanism type 1.

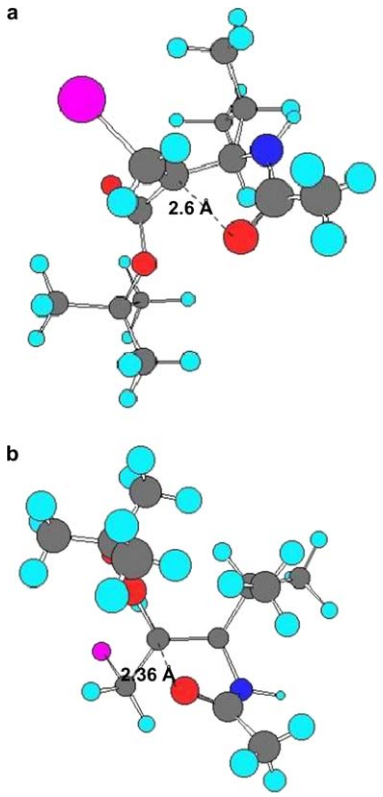


Figure 3. TS structures for type 1 mechanism step 3 leading to (a) *cis*-**7**, (b) *trans*-**8**.

These calculations were also performed both with and without considering the solvent effect, and the major changes were observed in the energy of the two iodonium cations, which have a high energy gap in relative stability (1.13 kcal/mol in vacuo, 2.57 kcal/mol in solvent) thus suggesting that stereoselection is strongly related to the formation of these two species. This result, together with the minor population of the NACs conformers leading to the trans-product, is in full agreement with the observed diastereoselection.

Eventually, although the type 1 mechanism allows explanation of the experimental data, the other mechanisms proposed must be considered, too, since they can interfere and superimpose with this latter. In particular the mechanism type 2 can compete with mechanism type 1 [i.e., iodocyclisation directly through the π-complex without formation of iodonium ion (concerted mechanism)]. However, the π-complex NIS-amide 'cis' is more stable than the NIS-amide 'trans' one, and the same is observed for the corresponding cyclic products.

Then, according to the Hammond postulate, it can be deduced that the same trend takes place for transition states observed since they are similar to the starting products. Thus, we expect that the relative activation energy has the same trend, suggesting that the observed cis-cyclisation is the more favoured process both kinetically and thermodynamically. Unfortunately the corresponding TS structures arising from these π-complexes and leading to the cyclisation product does not exist as stationary point onto the PES, automatically excluding this iodocyclisation mechanism.

At last, although intuitively it was less probable, mechanism type 3 was considered, but as expected, we were unable to find stationary points onto the PES surface corresponding to a possible π-complex between I^+ and the double bond of the substrate. In fact, all the optimisation strategies directly converge to either the iodonium ion or the cyclisation product.

4. Conclusions

From the computational results it can be strongly concluded that the stereochemical outcome of the iodocyclisation reaction of compound **3** relies on two different but concurrent aspects. On one side there is the conformational behaviour of the starting compounds, which strongly prefer to adopt a NAC arrangement leading to 'cis' iodocyclisation. On the other there is energetics of the possible reaction pathways. From the data obtained we could exclude the two competing mechanisms involving either the direct formation of the iodine cation (I^+)-substrate complex (type 3) or the π-complex (type 2) both leading to the cyclized product without involving a iodonium intermediate. Instead, we identify an exclusive reaction pathway for iodocyclisation (namely type 1), which include the formation of an iodonium cation intermediate. Furthermore from the energetics of this path, it is found that the formation of the cis-product is due to both kinetic and thermodynamic control, and thus it is the only formed product. This is in agreement with the observed stereoselectivity. Finally, even if the solvent effect is important since ionic species are involved, we must remember that the reaction is conducted in a low polarity environment (chloroform). In fact, as we can argue, we found out that this affects only the energy of the species involved and not their relative stability, thus being not able to change the nature of the mechanism itself.

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15. With the term near attack conformation (NAC) we define the required conformation for juxtaposed reactants to enter a transition state (TS). The greater is the mole fraction of reactant conformations that are present as NACs, the greater is the rate constant.
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Synthesis of novel poly(ethylene glycol) supported benzazepines: the crucial role of PEG on the selectivity of an intramolecular Heck reaction

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Abstract—Poly(ethylene glycol) (PEG 3400) was used as a soluble polymeric support for the synthesis of a series of novel benzazepines. The key step for the preparation of these heterocycles was a phosphine-free palladium-catalyzed Heck reaction. Palladium nanoparticles formed during the course of the reaction were characterized. The presence of PEG 3400 influenced the outcome of the reaction in terms of selectivity.

1. Introduction

For economical, environmental, and practical reasons, organic synthesis relies more and more on catalysis.[1] Organometallic catalysis has proven to be a major field in carbon–carbon and carbon–heteroatom bond formations, key to the efficient building of more complex molecules. Major breakthrough has been performed in the field of homogeneous catalysis by the design and preparation of new ligands leading to efficient catalytic systems.[2] A complementary approach was the discovery that finely divided and stabilized metallic particles are also active in catalytic transformations.[3–6] Nanoparticles are generally generated in the presence of a protector providing either an electrostatic or steric stabilization.[3] Several types of macromolecules have been described for their ability of stabilizing metallic particles by steric shielding (polymers, cyclodextrins, surfactants…)[4] including poly(ethylene glycol) (PEG), a polymer widely used for biomedical applications[7] and in supported synthesis.[8] Among the different metals employed in catalysis, palladium has found a special place due to the number of transformations, which can be mediated by this metal.[9]

We now report that a soluble PEG 3400 polymer supporting a substrate involved in a Pd-catalyzed Heck cyclization acts also as stabilizer for in situ generated nanoparticles. To the best of our knowledge, this is the first reaction performed on the end group of a PEG polymer with simultaneous formation of nanoparticles, which have been unambiguously characterized by Transmission Electronic Microscopy and Light Scattering experiments. Furthermore, the efficiency and selectivity induced by the presence of PEG 3400 were demonstrated when using PEG 3400-OH as a solvent in a non-supported version of the reaction. Interestingly smaller liquid PEGs such as PEG 300 do not exert the same influence. This reaction was applied to the synthesis of a series of original benzazepines.

2. Results and discussion

Bifunctional poly(ethylene glycol) with an average molecular weight of 3400 was used as a polymeric support for the preparation of α-methylene β-aminoesters **1a–g** by an aza-Baylis–Hillman reaction involving PEG-SES-NH_2, an aldehyde, and methyl acrylate in the presence of DABCO (Scheme 1).[10–12] Compound **2a**, obtained by alkylation of **1a** with 2-bromobenzyl bromide, was subjected to different Heck reaction conditions using $Pd(OAc)_2$ as catalyst in the presence of a base in DMF. After 12 h at 80 °C, **2a** was fully converted to the cyclized benzazepine **3a** using either K_2CO_3 or Oct_3N as a base. The reaction time was shortened to 6 h in the absence of solvent. The reaction was very selective since only **3a** was obtained among the different products, which could be formed during the reaction.[13] Since

Keywords: Polymer supported chemistry; Heterocycles; Heck reaction; Nanoparticles; Immobilization; Palladium; Solvent effects.
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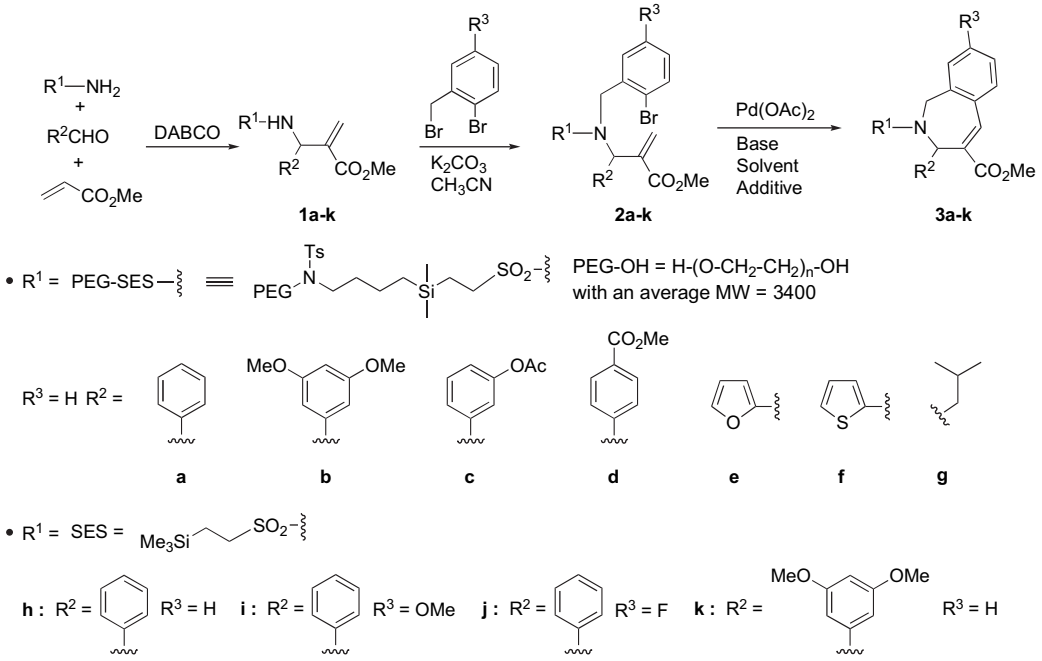
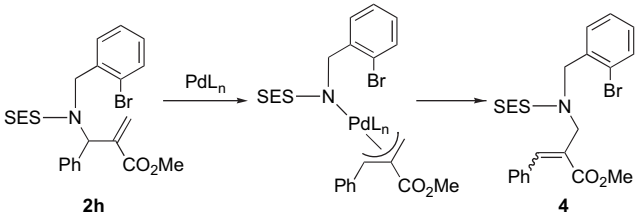


Scheme 1.

the reaction product was isolated by precipitation in Et_2O and filtration, the use of lipophilic Oct_3N was preferred because it was readily eliminated during this operation.[14]

In a non-supported version of the reaction,[12] substrate **2h**, which is not linked to PEG, was reacted in the Pd-catalyzed cyclization using similar conditions as for the transformation of **2a** in **3a** (Table 1). In sharp contrast with the results previously obtained, we could not reach the same results in terms of rate, selectivity, and reproducibility using 'classical' Heck reaction conditions.

Using Et_3N, the reaction was slower and a side product **4** arising from a competing Tsuji–Trost rearrangement was formed (entry 1 and Eq. 1).[15–17] Using Oct_3N, the reaction was complete but the formation of side product **4** increased (entry 2). Jeffery's conditions[18] were tried but resulted in a slower reaction and the exclusive formation of **4** (obtained in this case as an *E*/*Z* mixture with a 4/1 diastereomeric ratio) (entry 3). Since the presence of PEG seemed to be of prime significance in the catalytic system, we explored the improvement of the results by adding PEG 3400-OH to the reaction mixture, in the same weight amount as in the case of the polymer supported substrate. The reaction was not complete within 12 h but only formation of the cyclized product **3h** was observed (entry 4). Finally, PEG 3400-OH, solid at room temperature but liquid at 80 °C, was used as the solvent (entry 5). In this case, excellent results were obtained, similar to those obtained in the supported reaction, with exclusive formation of **3h**. Interestingly, replacing solid PEG 3400-OH by liquid PEG 300-OH with either Oct_3N or K_2CO_3 yielded only a sluggish mixture of products. Furthermore, the presence of the hydroxyl groups on PEG is necessary, since the use of PEG 3400-OMe instead of PEG-OH as a solvent did not give the cyclized product (entry 6).



(1)

Various substrates were used in the reaction described within this study. Structures and the corresponding yield of the alkylated aminoesters **2a**–**k** are presented in Figure 1. Aminoesters **2a**–**k** were cyclized to benzazepines **3a**–**k**, which are presented in Figure 2. In all cases, complete conversion of the starting material to the expected product was obtained.

Table 1. Conversion of **2h** under Pd catalysis[a]

Entry	Base	Solvent	Additive	Reaction time (h)	**2h** (%)	**3h** (%)	**4** (%)
1	Et_3N	DMF		36	10	68	22
2	Oct_3N	DMF		12	0	50	50
3	K_2CO_3	DMF	*n*-Bu_4NCl	48	0	0	100
4	K_2CO_3	DMF	PEG 3400-OH	12	54	46	0
5	K_2CO_3	PEG 3400-OH		12	0	100	0
6	K_2CO_3	PEG 3400-OMe		12	100	0	0

[a] Reaction conditions: **2h** (1 equiv), $Pd(OAc)_2$ (0.1 equiv), K_2CO_3 (3 equiv), and PEG 3400-OH (100 mg/mg of $Pd(OAc)_2$) were stirred during the indicated time at 80 °C.

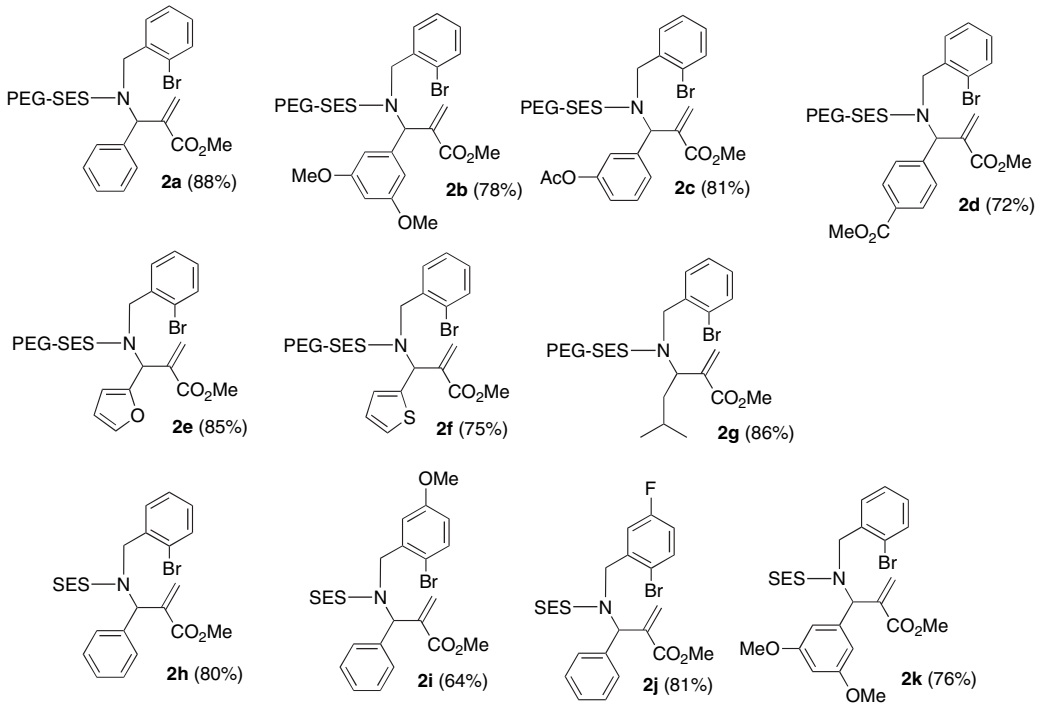


Figure 1.

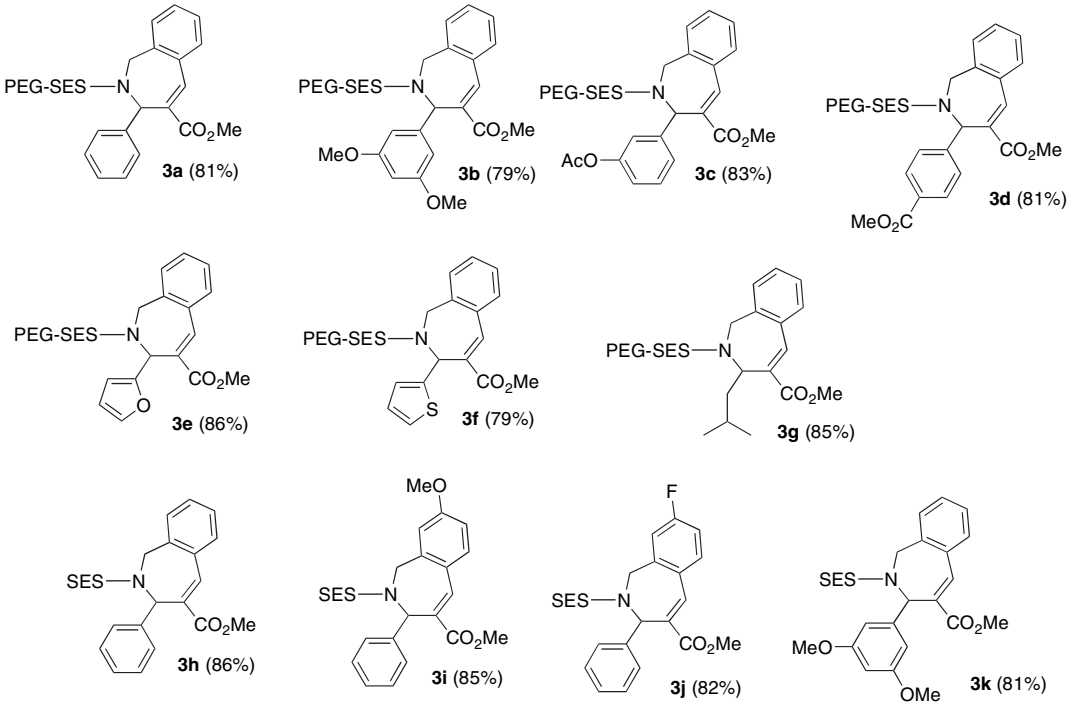


Figure 2.

Purity of the PEG-supported molecule was further ascertained by ^{1}H NMR and HPLC.[19] The diversity of cyclic structures in the supported version of the reaction originated from the aldehydes employed in the aza-Baylis–Hillman reaction (Scheme 1 and Fig. 1). In the case of the non-supported synthesis of benzazepines, we have shown that further diversity could be obtained by adding a substituent on the aromatic ring of the alkylating agent (Scheme 1 and Fig. 2).

The catalytic systems described above for the supported and non-supported reactions are quite simple. No phosphine was necessary to achieve good conversion and selectivity and the presence of ammonium salts did not improve the results. Furthermore, direct observation of a mixture of $Pd(OAc)_2$, Oct_3N, and DMF in the presence of PEG 3400-OH showed a dramatic difference in the homogeneity of the mixture (Fig. 3).

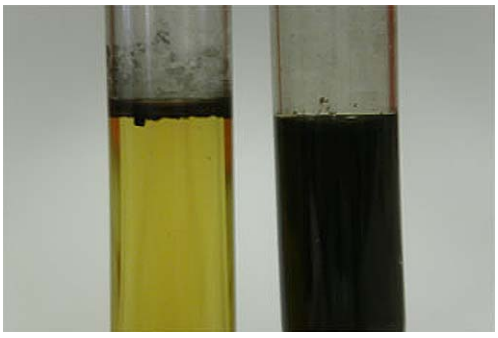


Figure 3. Left: mixture of $Pd(OAc)_2$, Oct_3N, DMF; right: mixture of $Pd(OAc)_2$, Oct_3N, DMF, PEG 3400-OH.

Since PEG has been recently described as nanoparticle stabilizer,[20–22] we investigated the possible presence of nanoparticles by analyzing the reaction mixture after 12 h of **2a** with $Pd(OAc)_2$ and Oct_3N in DMF, by Transmission Electronic Microscopy (TEM) either directly after evaporation of the solvent or through a layer of carbon. The images resulting from both techniques revealed the presence of homogeneously dispersed nanoparticles of palladium of 5–10 nm diameter (Fig. 4). This was further confirmed by light scattering experiments. Nanoparticles were also obtained when a mixture of PEG 3400-OH, $Pd(OAc)_2$, and K_2CO_3 was heated at 80 °C.

The most striking results were obtained when larger PEGs were used as solvent for unsupported reactions. The use of PEG as the sole solvent has a strong influence on the outcome of the reaction in terms of stereo- and regioselectivity. The use of co-solvents generated the formation of side products or slowed down the reaction. One plausible hypothesis is that the presence of palladium species stabilized by PEG may play a role on the kinetics of the different steps of the reaction and orients the outcome of the process to one pathway (mainly Heck cyclization) versus another (mainly Tsuji–Trost allylation). It has indeed been shown in the literature that ionic liquid-stabilized nanoparticles can accelerate such steps.[23] Furthermore, smaller PEGs, liquids at room temperature, have also been used as solvent in conjunction with palladium catalysts providing an easy medium for catalyst recycling.[24–26] However, a larger polymer is more prone to stabilize nanoparticles[20] while in the case of smaller PEGs additional stabilizers[26] are needed to induce these nanostructures. As we have shown in this study, the absence of selectivity with smaller PEGs is indeed not comparable with the results obtained with larger PEGs. Additionally, it has been reported that stabilization by ammonium salts (Jeffery's conditions) can also yield nanoparticles that differ in size and probably in structure, explaining the discrepancy with the results presented herein.[27] The importance of the size effect of larger PEGs in stabilizing nanoparticles can be correlated to similar adsorption effects, which have been reported either on proteins,[28] on silica particles,[29] or on surfaces.[30,31] Nevertheless, the question of the actual catalytic species is still pending, since some literature data advocate that nanoparticles may serve as a reservoir for small quantities of leaching active palladium species.[32–35] In the present case, PEG 3400 would then influence the nature of this reservoir.

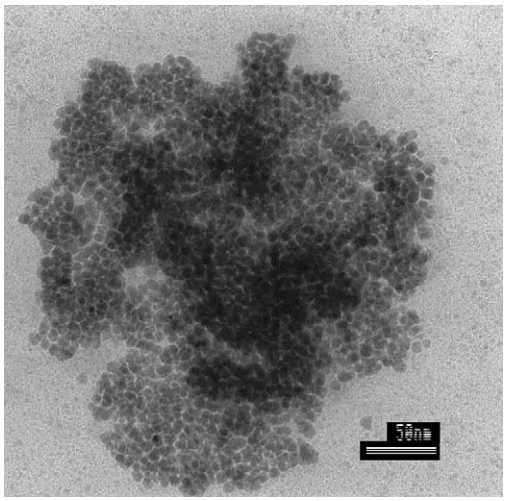


Figure 4. TEM of reaction mixture of **2a** with $Pd(OAc)_2$ and $OctN_3$ in DMF deposited on a copper-grid.

Modified PEGs were previously used as support for Pd catalysts.[36–39] These PEGs are usually terminated by a ligand such as a phosphine to generate a soluble supported complex of palladium that have been in some cases characterized by solution NMR. Nonetheless, one cannot rule out in these examples the additional stabilizing effect exerted by the polymer on the metallic centers and probably the concomitant existence of two forms of palladium. Most probably, the involvement of in situ generated Pd nanoparticles must be also considered in these cases.[40]

3. Conclusion

In conclusion, we have shown that a PEG polymer supporting an organic substrate can also stabilize a Pd catalyst. This phenomenon leads to a very efficient and selective Heck cyclization of the supported organic molecule for the preparation of novel heterocyclic structures. These results seem to be general in PEG-supported chemistry since our group[41,42] and others[43] have reported few years ago the positive effect of larger PEG polymeric support on a Heck reaction. Additionally, following the recent results concerning the use of larger PEG/Pd as catalytic medium for intermolecular Heck reaction,[20,22,44] we have shown herein that this system could be used in its intramolecular version to elaborate more complex and heterocyclic structures. Further studies to assess recycling of this catalytic system and to broaden its scope to other Pd-catalyzed reactions are underway in our laboratories.

4. Experimental

4.1. General remarks

All reagents were purchased from Aldrich Chemical Co. and used without further purification. ^1H and ^{13}C NMR analyses were performed with a Bruker AM 300 MHz spectrometer, and calibrated using residual undeuterated solvents as an internal reference. Mass spectra (electrospray ionization mode, ESIMS) were recorded on a Platform II (Micromass,

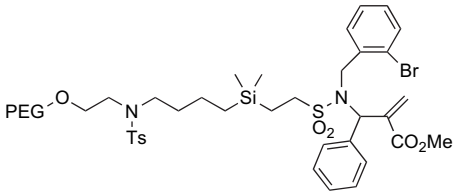
Manchester, U.K.) quadrupole mass spectrometer fitted with an electrospray interface. The mass spectrometer was calibrated in the positive- and negative-ion ESI mode. The sample was dissolved in H_2O/CH_3CN (50/50 v/v). FAB mass spectra and HRMS (High-Resolution Mass Spectrum) were recorded on a JEOL JMS DX300-SX 102 in positive mode using NBA (3-nitrobenzylalcohol) as matrix.

The preparation of unsaturated β-aminoesters **1a–g** and **1k** has been previously described.[10–12]

4.2. General procedure for the alkylation of PEG 3400-supported β-aminoesters

A mixture of PEG 3400-supported sulfonamide (1 equiv), 2-bromobenzyl bromide (8 equiv), and K_2CO_3 (10 equiv) in CH_3CN (5 mL/100 mg de PEG) was heated to reflux for 6 h. After concentration, the residue was diluted in CH_2Cl_2, filtered over Celite, and precipitated in ether. After filtration, the solid was dried in vacuo to yield the corresponding *N*-2-bromobenzyl-β-aminoester.

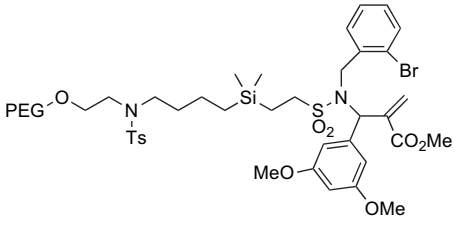
4.2.1. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(phenyl)methyl)acrylate (2a).



Prepared according to the general procedure with **1a** (900 mg, 0.20 mmol), 2-bromobenzyl bromide (800 mg, 3.2 mmol), and K_2CO_3 (550 mg, 4 mmol) to afford 850 mg (88%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.17 (s, 6H), 0.32–0.42 (m, 2H), 0.86–0.94 (m, 2H), 1.06–1.22 (m, 2H), 1.44–1.55 (m, 2H), 2.37 (s, 3H), 2.49–2.65 (m, 2H), 3.08 (t, *J*=7.0 Hz, 2H), 3.20–3.27 (m, 2H), 3.50–3.70 (large s, 154H), 3.53 (s, 3H), 4.71 (s, 2H), 5.82 (s, 1H), 6.10 (s, 1H), 6.39 (s, 1H), 6.98 (dt, *J*=1.5, 7.7 Hz, 1H), 7.09–7.37 (m, 10H), 7.65 (d, *J*=8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.18, 9.11, 14.78, 21.02, 21.89, 32.67, 47.96, 49.40, 50.51, 50.86, 52.47, 62.28, 70.37–70.86, 122.96, 127.45, 127.50, 128.60, 128.98, 129.05, 129.09, 129.88, 130.03, 132.74, 136.68, 137.18, 137.70, 139.28, 143.52, 166.42.

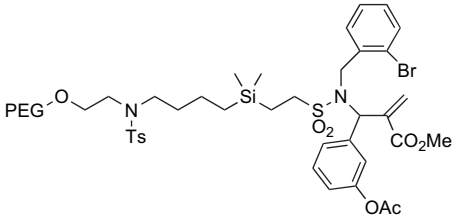
4.2.2. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(3,5-dimethoxyphenyl)methyl)acrylate (2b).



Prepared according to the general procedure with **1b** (900 mg, 0.20 mmol) to afford 750 mg (78%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.08 (s, 6H), 0.41–0.49 (m, 2H), 0.96–1.05 (m, 2H), 1.14–1.30 (m, 2H), 1.50–1.64 (m, 2H), 2.44 (s, 3H), 2.65–2.82 (m, 2H), 3.14 (t, *J*=7.0 Hz, 2H), 3.30 (t, *J*=7.0 Hz, 2H), 3.50–3.70 (large s, 154H), 3.61 (s, 3H), 3.75 (s, 6H), 4.76 (s, 2H), 5.89 (s, 1H), 6.12 (s, 1H), 6.30 (s, 1H), 6.43 (s, 1H), 6.49 (s, 2H), 7.01–7.08 (m, 1H), 7.11–7.34 (m, 3H), 7.41 (d, *J*=8.9 Hz, 1H), 7.47 (d, *J*=8.0 Hz), 7.71 (d, *J*=7.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.77, 8.84, 14.41, 20.65, 21.47, 32.31, 47.57, 49.00, 50.02, 50.37, 52.10, 55.32, 61.56, 70.08–70.47, 99.90, 106.72, 122.46, 122.46, 126.96, 127.12, 128.61, 129.40, 129.62, 130.17, 132.25, 136.37, 136.85, 138.76, 139.60, 143.10, 160.85, 165.98.

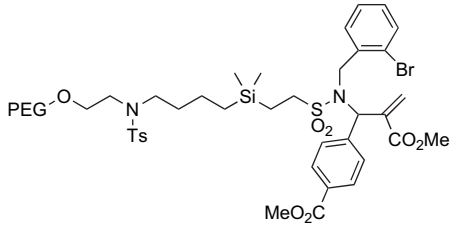
4.2.3. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(3-acyloxyphenyl)methyl)acrylate (2c).



Prepared according to the general procedure with **1c** (600 mg, 0.13 mmol) to afford 520 mg (81%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.12 (s, 6H), 0.36–0.48 (m, 2H), 0.90–0.99 (m, 2H), 1.10–1.25 (m, 2H), 1.46–1.58 (m, 2H), 2.26 (s, 3H), 2.39 (s, 3H), 2.55–2.78 (m, 2H), 3.10 (t, *J*=7.5 Hz, 2H), 3.26 (t, *J*=7.5 Hz, 2H), 3.50–3.70 (large s, 154H), 3.57 (s, 3H), 4.72 (s, 2H), 5.87 (s, 2H), 6.11 (s, 1H), 6.44 (s, 1H), 6.93–7.20 (m, 5H), 7.24–7.42 (m, 5H), 7.66 (d, *J*=8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.77, 8.73, 14.36, 20.64, 21.13, 21.48, 32.30, 47.55, 49.01, 50.29, 52.14, 61.61, 70.06–71.00, 121.30, 122.10, 122.62, 125.65, 127.11, 127.20, 128.78, 129.56, 129.63, 129.91, 130.18, 132.43, 136.05, 136.29, 136.81, 138.53, 138.83, 139.09, 143.03, 150.80, 165.96, 168.93.

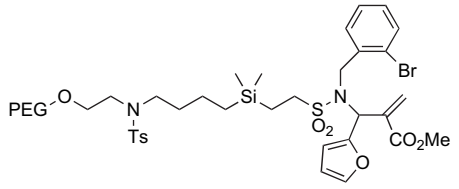
4.2.4. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(4-methyloxycarbonyl-phenyl)methyl)acrylate (2d).



Prepared according to the general procedure with **1d** (900 mg, 0.20 mmol) to afford 700 mg (72%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.11 (s, 6H), 0.34–0.54 (m, 2H), 0.89–1.04 (m, 2H), 1.10–1.26 (m, 2H), 1.42–1.64 (m, 2H), 2.40 (s, 3H), 2.62–2.82 (m, 2H), 3.11 (t, J=7.7 Hz, 2H), 3.22–3.33 (m, 2H), 3.50–3.75 (large s, 154H), 3.87 (s, 3H), 4.73 (s, 2H), 5.84 (s, 1H), 6.16 (s, 1H), 6.48 (s, 1H), 7.00 (dt, J=1.7, 7.5 Hz, 1H), 7.15 (dt, J=1.3, 7.4 Hz, 1H), 7.23–7.53 (m, 6H), 7.67 (d, J=8.2 Hz, 2H), 7.91 (d, J=8.2 Hz, 2H).

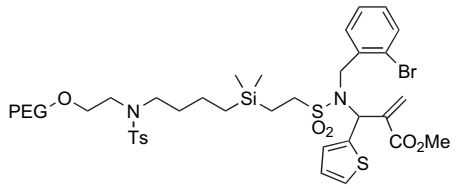
4.2.5. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(furan-2-yl)methyl)acrylate (2e).



Prepared according to the general procedure with **1e** (500 mg, 0.11 mmol) to afford 460 mg (85%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.10 (s, 6H), 0.39–0.49 (m, 2H), 0.86–1.01 (m, 2H), 1.12–1.28 (m, 2H), 1.48–1.60 (m, 2H), 2.41 (s, 3H), 2.62–2.79 (m, 2H), 3.12 (t, J=7.0 Hz, 2H), 3.23–3.30 (m, 2H), 3.50–3.75 (large s, 154H), 3.57 (s, 3H), 4.58 (d, J=17.2 Hz, 1H), 4.86 (d, J=17.2 Hz, 1H), 5.86 (s, 1H), 6.20 (s, 1H), 6.26 (s, 1H), 6.33–6.38 (m, 2H), 7.06 (dt, J=1.5, 7.4 Hz, 1H), 7.20–7.56 (m, 6H), 7.68 (d, J=8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.82, 8.38, 14.42, 20.65, 21.47, 32.33, 47.59, 48.45, 49.04, 49.49, 52.09, 54.91, 70.10–70.47, 109.84, 110.78, 122.43, 127.07, 128.61, 129.63, 129.75, 130.04, 130.12, 132.32, 136.71, 136.81, 136.94, 142.83, 143.12, 151.12, 165.31.

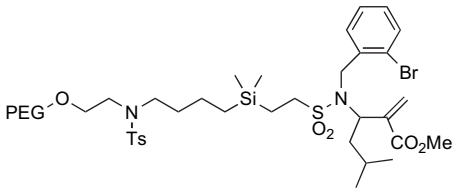
4.2.6. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(thiofuran-2-yl)methyl)acrylate (2f).



Prepared according to the general procedure with **1f** (900 mg, 0.20 mmol) to afford 730 mg (75%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.16 (s, 6H), 0.31–0.49 (m, 2H), 0.83–0.95 (m, 2H), 1.06–1.26 (m, 2H), 1.42–1.60 (m, 2H), 2.39 (s, 3H), 2.47–2.73 (m, 2H), 3.04–3.14 (m, 2H), 3.21–3.29 (m, 2H), 3.50–3.75 (large s, 154H), 3.54 (s, 3H), 4.61 (d, J=17.5 Hz, 1H), 4.74 (d, J=17.5 Hz, 1H), 5.94 (s, 1H), 6.28 (s, 1H), 6.26 (s, 1H), 6.42 (s, 2H), 6.93–7.54 (m, 9H), 7.66 (d, J=8.2 Hz, 2H).

4.2.7. Methyl 2-(*N*-2-bromobenzyl-(PEG 3400-SES-amido)(2-isobutyl)methyl)acrylate (2g).



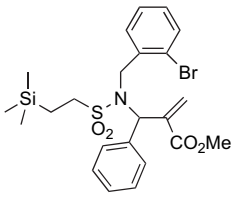
Prepared according to the general procedure with **1g** (900 mg, 0.21 mmol) to afford 830 mg (86%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ 0.00 (s, 3H), 0.01 (s, 3H), 0.42–0.57 (m, 2H), 0.76 (d, J=6.1 Hz, 3H), 0.84 (d, J=6.1 Hz, 3H), 0.88–1.07 (m, 2H), 1.15–1.34 (m, 4H), 1.39–1.80 (m, 3H), 2.43 (s, 3H), 2.72–3.04 (m, 2H), 3.15 (t, J=7.4 Hz, 2H), 3.25–3.35 (m, 2H), 3.50–3.80 (large s, 154H), 3.79 (s, 3H), 4.47–4.65 (m, 3H), 4.89–4.99 (m, 1H), 5.83 (s, 1H), 6.52 (s, 1H), 7.13 (dt, J=1.7, 7.4 Hz, 1H), 7.27–7.37 (m, 3H), 7.50 (dd, J=1.3, 8.0 Hz, 1H), 7.63–7.74 (m, 3H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.79, 8.79, 14.51, 20.70, 21.37, 21.46, 23.17, 24.92, 32.32, 41.12, 47.54, 49.00, 49.36, 52.23, 55.43, 70.07–70.44, 122.55, 127.09, 127.33, 128.85, 129.45, 129.61, 130.78, 132.49, 136.84, 137.07, 137.13, 143.08, 166.99.

4.3. General procedure for the alkylation of non-supported β-aminoesters

A mixture of sulfonamide (1 equiv), 2-bromobenzyl bromide or a related reagent (1.2 equiv), and K_2CO_3 (10 equiv) in CH_3CN was heated to reflux for 6 h. After concentration, the residue was diluted in ether, washed successively with a solution of 5% $KHSO_4$ and brine, dried over $MgSO_4$, and evaporated. Purification by silica gel chromatography (hexane/Et_2O) afforded the corresponding *N*-2-bromobenzyl-β-aminoester.

4.3.1. Methyl 2-((*N*-2-bromobenzyl-2-(trimethylsilyl)-ethan-3-ylsulfonamido)(phenyl)methyl)acrylate (2h).

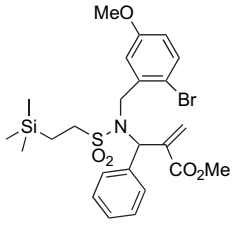


Prepared according to the general procedure with **1h** (100 mg, 0.28 mmol), 2-bromobenzyl bromide (85 mg, 0.34 mmol), and K_2CO_3 (390 mg, 2.83 mmol) to afford 118 mg (80%) of the title compound after silica gel chromatography (hexane/Et_2O, 8/2).

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.05 (s, 9H), 0.95–1.05 (m, 2H), 2.55–2.77 (m, 2H), 3.61 (s, 3H), 4.78 (s, 2H), 5.90 (d,

J=1.48 Hz, 1H), 6.17 (s, 1H), 6.46 (s, 1H), 7.04 (dt, J=1.48, 7.6 Hz, 1H), 7.16–7.45 (m, 7H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.05, 10.20, 50.35, 50.62, 52.04, 62.07, 122.63, 127.08, 128.19, 128.64, 128.68, 129.33, 130.29, 132.38, 136.34, 137.34, 139.02, 166.08; HPLC t_R=14.50 min; ESIMS m/z 524.2 $(M+H)^+$ (monoisotopic); HRMS calcd for $C_{23}H_{31}BrNO_4SSi$ 524.0926 (monoisotopic), found 524.0952; R_f (hexane/Et_2O, 8/2) 0.31.

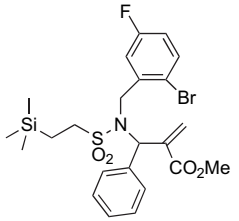
4.3.2. Methyl 2-((*N*-2-bromo-5-methoxy-benzyl-2-(trimethylsilyl)ethan-3-ylsulfonamido)(phenyl)methyl)acrylate (2i).



Prepared according to the general procedure with **1i** (75 mg, 0.21 mmol) and 5-methoxy-2-bromobenzyl bromide (71 mg, 0.25 mmol) to afford 75 mg (64%) of the title compound after silica gel chromatography (hexane/Et_2O, 85/15).

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.05 (s, 9H), 0.96–1.04 (m, 2H), 2.56–2.80 (m, 2H), 3.61 (s, 3H), 3.75 (s, 3H), 4.74 (s, 2H), 5.87 (d, J=1.5 Hz, 1H), 6.19 (s, 1H), 6.45 (s, 1H), 6.61 (dd, J=3.0, 8.9 Hz, 1H), 6.97 (d, J=3.0 Hz, 1H), 7.25–7.40 (m, 6H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −1.98, 10.18, 50.29, 50.47, 52.04, 55.38, 61.98, 112.17, 115.02, 115.38, 128.22, 128.57, 128.72, 129.58, 132.91, 137.32, 137.53, 139.03, 158.81, 166.14; HPLC t_R=14.56 min; ESIMS m/z 554.3 $(M+H)^+$ (monoisotopic); HRMS calcd for $C_{24}H_{33}BrNO_5SSi$ 554.1032 (monoisotopic), found 554.1039; R_f (hexane/Et_2O, 7/3) 0.33.

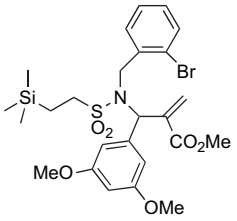
4.3.3. Methyl 2-((*N*-2-bromo-5-fluoro-benzyl-2-(trimethylsilyl)ethan-3-ylsulfonamido)(phenyl)methyl)acrylate (2j).



Prepared according to the general procedure with **1j** (75 mg, 0.21 mmol) and 5-fluoro-2-bromobenzyl bromide (68 mg, 0.25 mmol) to afford 93 mg (81%) of the title compound after silica gel chromatography (hexane/Et_2O, 85/15).

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.03 (s, 9H), 0.98–1.06 (m, 2H), 2.61–2.86 (m, 2H), 3.65 (s, 3H), 4.74 (s, 2H), 5.83 (d, J=1.5 Hz, 1H), 6.22 (s, 1H), 6.46 (s, 1H), 6.75 (dt, J=3.0, 7.7 Hz, 1H), 7.13 (dd, J=3.0, 7.7 Hz, 1H), 7.21–7.39 (m, 6H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.06, 10.22, 50.23, 52.18, 61.75, 115.79 (J=22.8 Hz), 116.07 (J=3.1 Hz), 116.98 (J=24.8 Hz), 128.34, 128.42, 128.78, 129.39, 133.40 (J=7.9 Hz), 138.95 (J=7.4 Hz), 139.10, 161.85 (J=246.6 Hz), 166.00; HPLC t_R=14.58 min; ESIMS m/z 541.9 $(M+H)^+$ (monoisotopic); HRMS calcd for $C_{23}H_{30}BrFNO_4SSi$ 542.0832 (monoisotopic), found 542.0825; R_f (hexane/Et_2O, 8/2) 0.39.

4.3.4. Methyl 2-((*N*-2-bromobenzyl-2-(trimethylsilyl)-ethan-3-ylsulfonamido)(3,5-dimethoxyphenyl)methyl)-acrylate (2k).



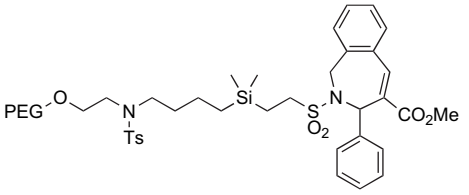
Prepared according to the general procedure with **1k** (75 mg, 0.18 mmol) and 2-bromobenzyl bromide (55 mg, 0.22 mmol) to afford 80 mg (76%) of the title compound after silica gel chromatography (hexane/Et_2O, 8/2).

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.04 (s, 9H), 0.98–1.08 (m, 2H), 2.64–2.85 (m, 2H), 3.61 (s, 3H), 3.75 (s, 6H), 4.77 (s, 2H), 5.90 (s, 1H), 6.12 (s, 1H), 6.30 (t, J=2.2 Hz, 1H), 6.43 (s, 1H), 6.50 (d, J=2.2 Hz, 2H), 7.04 (dt, J=1.5, 8.0 Hz, 1H), 7.21 (t, J=7.7 Hz, 1H), 7.41 (d, J=8.0 Hz, 1H), 7.48 (d, J=8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.10, 10.29, 50.19, 50.46, 52.11, 55.32, 61.61, 99.92, 106.92, 122.49, 127.00, 128.62, 129.38, 130.22, 132.27, 136.40, 138.81, 139.65, 160.87, 166.00; HPLC t_R=14.39 min; ESIMS m/z 584.1 $(M+H)^+$ (monoisotopic); HRMS calcd for $C_{25}H_{34}BrNO_6SSi$ 583.1059 (monoisotopic), found 583.1066; R_f (hexane/Et_2O, 7/3) 0.22.

4.4. General procedure for the Heck reaction on PEG 3400-supported substrate

To a mixture of PEG 3400-supported *N*-2-bromobenzyl-β-aminoester (1 equiv) and $Pd(OAc)_2$ (0.1 equiv) in DMF (1 mL/50 mg de PEG) was added (n-octyl)$_3$N (3 equiv). The solution was stirred at room temperature for few minutes under nitrogen. The mixture was then heated at 80 °C for 6 h, filtered over Celite, and precipitated in ether. After filtration, the solid was dried in vacuo to yield the corresponding benzazepine.

4.4.1. 2-(PEG 3400-SES)-3-phenyl-2,3-dihydro-1*H*-benzo-[*c*]azepine-4-carboxylic acid methyl ester (3a).

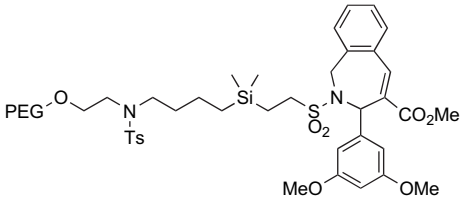


Prepared according to the general procedure with **2a** (500 mg, 0.10 mmol), $Pd(OAc)_2$ (5 mg, 0.022 mmol), and

(n-octyl)$_3$N (222 mg, 0.63 mmol) to afford 410 mg (81%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.26 (s, 3H), −0.25 (s, 3H), 0.24–0.32 (m, 2H), 0.68–0.76 (m, 2H), 1.00–1.14 (m, 2H), 1.46–1.58 (m, 2H), 2.44 (s, 3H), 2.27–2.56 (m, 2H), 3.11 (t, J=7.9 Hz, 2H), 3.27–3.33 (m, 2H), 3.55–3.75 (large s, 154H), 3.70 (s, 3H), 4.14 (d, J=16.8 Hz, 1H), 4.46 (d, J=16.8 Hz, 1H), 6.43 (s, 1H), 7.22–7.44 (m, 10H), 7.54–7.60 (m, 1H), 7.72 (d, J=8.3 Hz, 2H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −4.07, 8.35, 14.32, 20.59, 21.50, 32.27, 47.37, 47.56, 49.06, 49.83, 52.57, 61.02, 70.09–71.03, 127.12, 128.04, 128.29, 128.63, 128.69, 129.64, 130.30, 132.02, 132.05, 135.27, 136.81, 140.40, 140.67, 140.83, 143.12, 167.26.

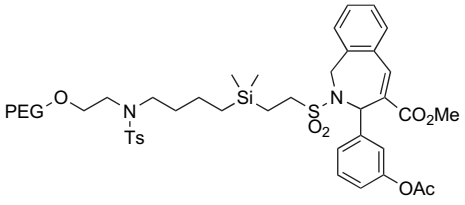
4.4.2. 2-(PEG 3400-SES)-3-(3,5-dimethoxyphenyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3b).



Prepared according to the general procedure with **2b** (500 mg, 0.10 mmol) to afford 380 mg (79%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.29 (s, 3H), −0.28 (s, 3H), 0.21–0.29 (m, 2H), 0.64–0.74 (m, 2H), 0.97–1.09 (m, 2H), 1.43–1.61 (m, 2H), 2.23–2.40 (m, 2H), 2.44 (s, 3H), 3.05–3.13 (m, 2H), 3.25–3.31 (m, 2H), 3.55–3.75 (large s, 154H), 3.71 (s, 3H), 3.71 (s, 6H), 4.20 (d, J=17.4 Hz, 1H), 4.45 (d, J=17.4 Hz, 1H), 6.32 (s, 1H), 6.38 (t, J=2 Hz, 1H), 6.53 (d, J=2.0 Hz, 2H), 7.19–7.56 (m, 6H), 7.69 (d, J=8.0 Hz, 2H), 7.95 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −4.09, 8.36, 14.33, 20.60, 21.49, 32.36, 47.45, 47.57, 49.07, 49.83, 52.61, 55.37, 60.96, 70.10–71.07, 99.81, 106.97, 127.14, 128.25, 128.65, 129.58, 129.65, 130.31, 131.85, 132.05, 135.27, 136.86, 140.39, 140.55, 143.14, 160.89, 167.24.

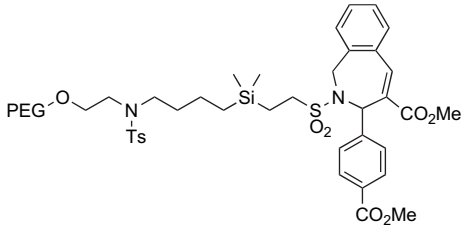
4.4.3. 2-(PEG 3400-SES)-3-(3-acyloxyphenyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3c).



Prepared according to the general procedure with **2c** (500 mg, 0.10 mmol) to afford 400 mg (83%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.27 (s, 3H), −0.26 (s, 3H), 0.24–0.40 (m, 2H), 0.68–0.77 (m, 2H), 0.96–1.14 (m, 2H), 1.43–1.60 (m, 2H), 2.29 (s, 3H), 2.44 (s, 3H), 2.27–2.56 (m, 2H), 3.10–3.22 (m, 2H), 3.25–3.35 (m, 2H), 3.55–3.75 (large s, 154H), 4.12 (d, J=17.4 Hz, 1H), 4.47 (d, J=17.4 Hz, 1H), 6.41 (s, 1H), 7.05–7.55 (m, 10H), 7.71 (d, J=8.2 Hz, 2H), 8.02 (s, 1H).

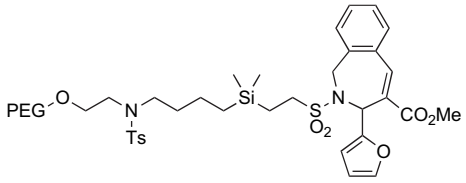
4.4.4. 2-(PEG 3400-SES)-3-(4-methyloxycarbonylphenyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3d).



Prepared according to the general procedure with **2d** (500 mg, 0.10 mmol) to afford 370 mg (81%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.30 (s, 6H), 0.19–0.29 (m, 2H), 0.62–0.76 (m, 2H), 0.94–1.12 (m, 2H), 1.40–1.58 (m, 2H), 2.39 (s, 3H), 2.24–2.51 (m, 2H), 3.01–3.15 (m, 2H), 3.21–3.29 (m, 2H), 3.50–3.80 (large s, 154H), 3.88 (s, 3H), 4.03 (d, J=17.4 Hz, 1H), 4.41 (d, J=17.4 Hz, 1H), 6.40 (s, 1H), 7.16–7.59 (m, 8H), 7.66 (d, J=7.4 Hz, 2H), 7.96–8.04 (m, 3H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.77, 8.34, 14.27, 20.55, 21.47, 32.30, 46.91, 47.44, 47.55, 49.01, 49.90, 52.18, 52.61, 60.73, 70.05–70.98, 127.10, 128.46, 128.59, 128.73, 129.63, 129.81, 129.96, 130.53, 131.31, 135.27, 136.82, 140.05, 141.21, 143.12, 145.76, 166.59, 166.98.

4.4.5. 2-(PEG 3400-SES)-3-furan-2-yl-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3e).

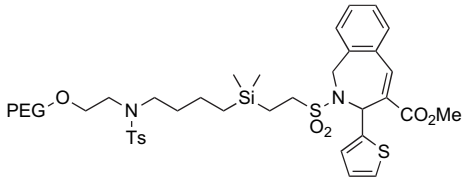


Prepared according to the general procedure with **2e** (300 mg, 0.063 mmol) to afford 250 mg (86%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.15 (s, 3H), −0.14 (s, 3H), 0.30–0.53 (m, 2H), 0.80–0.96 (m, 2H), 1.10–1.30 (m, 2H), 1.46–1.60 (m, 2H), 2.44 (s, 3H), 2.65–2.80 (m, 2H), 3.10–3.30 (m, 4H), 3.27–3.33 (m, 2H), 3.50–3.80 (large s, 154H), 3.76 (s, 3H), 4.28 (d, J=16.7 Hz, 1H), 4.57 (d, J=16.7 Hz, 1H), 6.22 (d, J=4.2 Hz, 1H), 6.34–6.40 (m, 2H), 7.25–7.60 (m, 7H), 7.72 (d, J=8.3 Hz, 2H), 7.91 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −3.94, 8.56, 14.38, 20.61, 21.47, 32.24, 47.02, 47.58, 49.03, 49.84, 52.55, 55.74, 70.08–71.00, 110.53, 110.88, 127.11, 128.09,

128.23, 129.63, 129.75, 130.34, 131.60, 135.37, 136.83, 140.47, 140.72, 143.12, 152.56, 166.97.

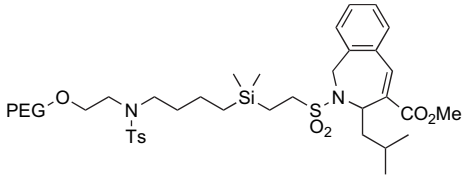
4.4.6. 2-(PEG 3400-SES)-3-thiofuran-2-yl-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3f).



Prepared according to the general procedure with **2f** (500 mg, 0.10 mmol) to afford 380 mg (79%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.24 (s, 6H), 0.19–0.29 (m, 2H), 0.62–0.76 (m, 2H), 0.94–1.12 (m, 2H), 1.40–1.58 (m, 2H), 2.42 (s, 3H), 2.43–2.57 (m, 2H), 3.01–3.15 (m, 2H), 3.21–3.29 (m, 2H), 3.50–3.80 (large s, 154H), 3.88 (s, 3H), 4.32 (d, J=17.2 Hz, 1H), 4.52 (d, J=17.2 Hz, 1H), 6.56 (s, 1H), 6.87–6.97 (m, 2H), 7.18–7.54 (m, 7H), 7.68 (d, J=7.5 Hz, 2H), 7.89 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −4.17, 8.30, 14.18, 20.44, 21.33, 32.16, 47.34, 47.42, 48.88, 49.89, 52.48, 56.64, 69.92–70.32, 126.59, 126.64, 126.96, 127.82, 128.04, 128.30, 129.49, 130.23, 131.61, 131.81, 135.18, 136.67, 142.98, 144.11, 166.96.

4.4.7. 2-(PEG 3400-SES)-3-(2-isobutyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3g).



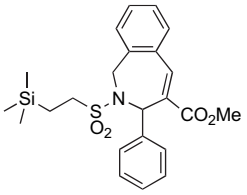
Prepared according to the general procedure with **2g** (500 mg, 0.10 mmol) to afford 410 mg (85%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.31 (s, 3H), −0.29 (s, 3H), 0.20–0.27 (m, 2H), 0.64–0.76 (m, 2H), 0.95 (d, J=6.5 Hz, 3H), 1.06 (d, J=6.5 Hz, 3H), 1.13–1.30 (m, 2H), 1.40–1.72 (m, 4H), 2.09–2.23 (m, 1H), 2.42 (s, 3H), 2.25–2.47 (m, 2H), 3.06–3.20 (m, 2H), 3.24–3.32 (m, 2H), 3.50–3.75 (large s, 154H), 3.84 (s, 3H), 4.45 (d, J=17.0 Hz, 1H), 4.69 (d, J=17.0 Hz, 1H), 5.16–5.22 (m, 1H), 7.10–7.56 (m, 6H), 7.64 (s, 1H), 7.69 (d, J=8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −4.09, 8.22, 14.31, 20.59, 21.48, 23.61, 23.77, 32.34, 43.79, 46.56, 47.55, 49.05, 49.22, 52.44, 56.28, 70.09–71.04, 127.13, 128.29, 128.62, 128.69, 129.64, 130.08, 132.52, 134.75, 135.35, 136.85, 138.08, 139.61, 143.12, 167.55.

4.5. General procedure for the Heck reaction with PEG as solvent

To *N*-2-bromobenzyl-β-aminoester (1 equiv) and $Pd(OAc)_2$ (0.1 equiv) was added a mixture of PEG 3400-OH (100 mg/mg de $Pd(OAc)_2$) and finely powdered K_2CO_3 (3 equiv). The resulting mixture was heated at 80 °C with strong stirring for 12 h. After cooling, the crude is solubilized in CH_2Cl_2 and precipitated in ether. After filtration, the filtrate was concentrated to yield the corresponding benzazepine.

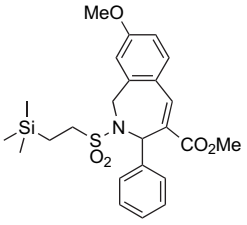
4.5.1. 3-Phenyl-2-(2-(trimethylsilanyl)ethylsulfonyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3h).



Prepared according to the general procedure with **2h** (29 mg, 0.055 mmol), $Pd(OAc)_2$ (1.2 mg, 5.5 μmol), PEG-OH (120 mg), and K_2CO_3 (25 mg, 0.18 mmol) to afford 21 mg (86%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.20 (s, 9H), 0.70–0.80 (m, 2H), 2.32–2.58 (m, 2H), 3.71 (s, 3H), 4.15 (d, J=17.0 Hz, 1H), 4.45 (dd, J=17.0, 1.5 Hz, 1H), 6.45 (s, 1H), 7.23–7.44 (m, 8H), 7.55–7.59 (m, 1H), 8.02 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.97, 9.07, 46.70, 49.32, 51.88, 60.35, 127.35, 127.53, 127.97, 128.00, 131.37, 131.41, 134.54, 139.80, 140.02, 140.20, 166.66; HPLC t_R=13.92 min; ESIMS m/z 444.2 $(M+H)^+$, 887.5 $(2M+H)^+$; HRMS calcd for $C_{23}H_{30}NO_4SSi$ 444.1665, found 444.1677.

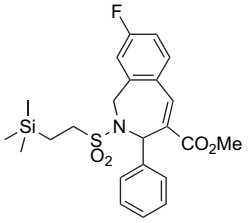
4.5.2. 8-Methoxy-3-phenyl-2-(2-(trimethylsilanyl)ethylsulfonyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3i).



Prepared according to the general procedure with **2i** (40 mg, 0.072 mmol) to afford 29 mg (85%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.16 (s, 9H), 0.71–0.85 (m, 2H), 2.35–2.70 (m, 2H), 3.70 (s, 3H), 3.85 (s, 3H), 4.09 (d, J=17.3 Hz, 1H), 4.41 (dd, J=17.3, 1.7 Hz, 1H), 6.43 (s, 1H), 6.77 (d, J=2.6 Hz, 1H), 6.90 (dd, J=2.6, 8.3 Hz, 1H), 7.27–7.44 (m, 5H), 7.50 (d, J=8.5 Hz, 1H), 7.99 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.25, 9.83, 47.54, 50.01, 52.39, 55.53, 60.92, 113.14, 114.36, 124.88, 127.99, 128.67, 128.72, 137.31, 140.54, 140.87, 142.53, 161.13, 167.54; HPLC t_R=13.53 min; ESIMS m/z 474.1 $(M+H)^+$, 947.6 $(2M+H)^+$; HRMS calcd for $C_{24}H_{32}NO_5SSi$ 474.1770, found 474.1757.

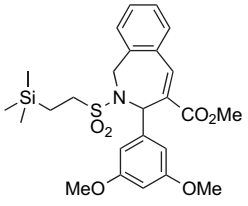
4.5.3. 8-Fluoro-3-phenyl-2-(2-(trimethylsilanyl)ethylsulfonyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3j).



Prepared according to the general procedure with **2j** (20 mg, 0.036 mmol) to afford 14 mg (82%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.15 (s, 9H), 0.70–0.88 (m, 2H), 2.41–2.67 (m, 2H), 3.71 (s, 3H), 4.11 (d, J=17.2 Hz, 1H), 4.42 (dd, J=17.2, 1.8 Hz, 1H), 6.42 (s, 1H), 6.98 (dd, J=2.7, 8.6 Hz, 1H), 7.08 (dt, J=2.7, 8.0 Hz, 1H), 7.28–7.41 (m, 5H), 7.55 (dd, J=5.6, 8.6 Hz, 1H), 7.98 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.24, 9.90, 47.15, 50.13, 52.59, 61.05, 115.03 (d, J=21.5 Hz), 115.89 (d, J=22.2 Hz), 128.20, 128.48 (d, J=3.6 Hz), 128.62, 128.79, 131.35, 131.33 (d, J=2.9 Hz), 137.39 (d, J=8.7 Hz), 139.53, 140.53, 143.45 (d, J=7.3 Hz), 163.27 (d, J=255.1 Hz), 167.20; HPLC t_R=14.08 min; ESIMS m/z 462.2 $(M+H)^+$; HRMS calcd for $C_{23}H_{29}FNO_4SSi$ 462.1571, found 462.1611.

4.5.4. 3-(3,5-Dimethoxy-phenyl)-2-(2-(trimethylsilanyl)-ethylsulfonyl)-2,3-dihydro-1*H*-benzo[*c*]azepine-4-carboxylic acid methyl ester (3k).



Prepared according to the general procedure with **2k** (20 mg, 0.034 mmol) to afford 14 mg (81%) of the title compound.

^{1}H NMR ($CDCl_3$, Me_4Si) δ −0.20 (s, 9H), 0.67–0.80 (m, 2H), 2.25–2.59 (m, 2H), 3.74 (s, 3H), 3.80 (s, 6H), 4.23 (d, J=17.0 Hz, 1H), 4.49 (dd, J=1.7, 17.0 Hz, 1H), 6.34 (s, 1H), 6.41 (t, J=2.2 Hz, 1H), 6.56 (d, J=2.2 Hz, 2H), 7.22–7.46 (m, 3H), 7.51–7.59 (m, 1H), 7.99 (s, 1H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ −2.29, 9.77, 47.46, 50.00, 52.59, 55.38, 60.98, 99.85, 106.99, 128.18, 128.66, 130.25, 131.90, 132.09, 135.22, 140.47, 140.57, 143.21, 160.91, 167.29; HPLC t_R=13.56 min; ESIMS m/z 504.2 $(M+H)^+$; HRMS calcd for $C_{25}H_{34}NO_6SSi$ 504.1876, found 504.1855.

4.6. General procedure for the Heck reaction with liquid PEG as solvent

To a mixture of *N*-2-bromobenzyl-β-aminoester **2h** (1 equiv) and $Pd(OAc)_2$ (0.1 equiv) in PEG 300-OH (100 mg/mg de $Pd(OAc)_2$) was added a base (K_2CO_3 or Oct_3N (3 equiv)). The resulting mixture was heated at 80 °C with strong stirring for 12 h. The mixture was diluted with AcOEt, and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated to give the crude product, which was then analyzed by ^{1}H NMR.

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