ELSEVIER

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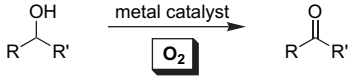
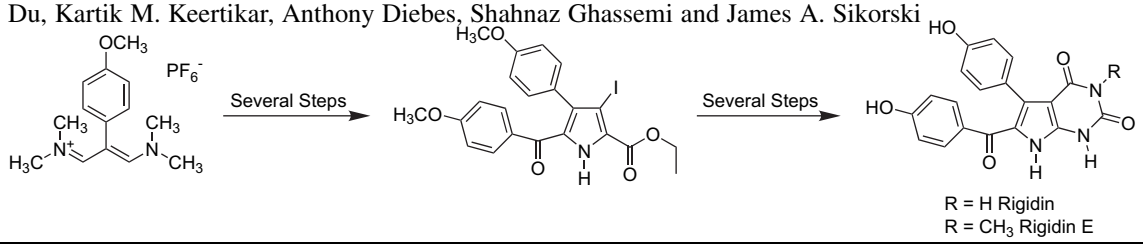
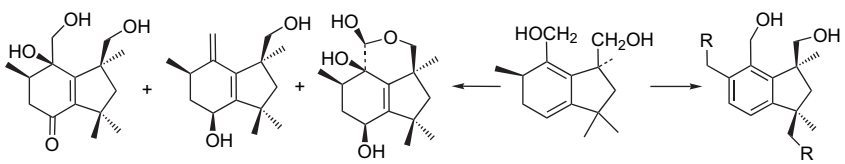
Contents

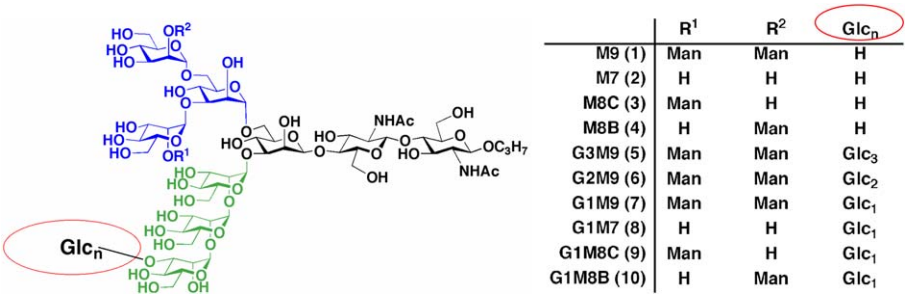
REPORT



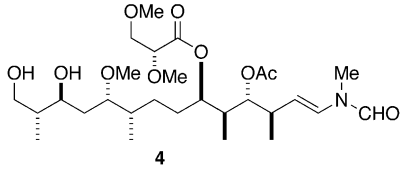
ARTICLES





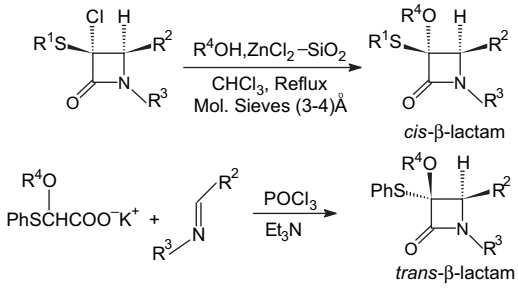


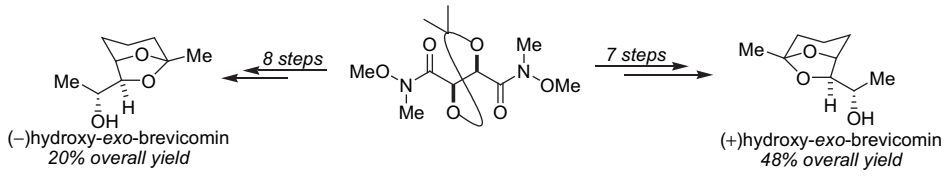
	R^1	R^2	Glc_n
M9 (1)	Man	Man	H
M7 (2)	H	H	H
M8C (3)	Man	H	H
M8B (4)	H	Man	H
G3M9 (5)	Man	Man	Glc_3
G2M9 (6)	Man	Man	Glc_2
G1M9 (7)	Man	Man	Glc_1
G1M7 (8)	H	H	Glc_1
G1M8C (9)	Man	H	Glc_1
G1M8B (10)	H	Man	Glc_1

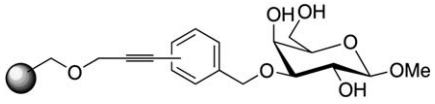


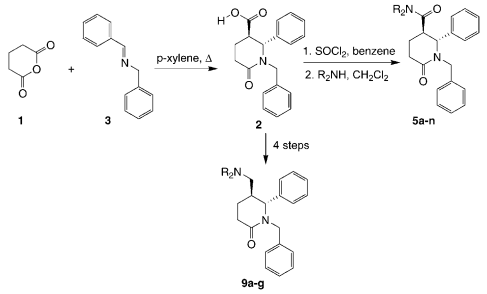
4

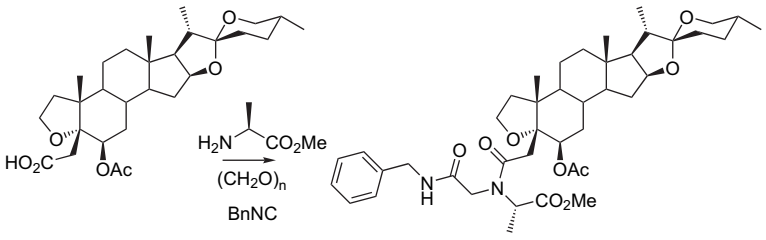
Mycalolide analog **4**, consisting only of the side chain of mycalolide B (**2**), was stereoselectively synthesized and was found to have strong actin-depolymerizing activity.

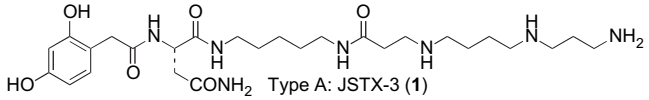


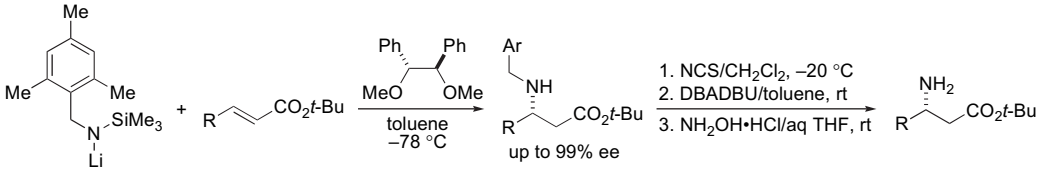


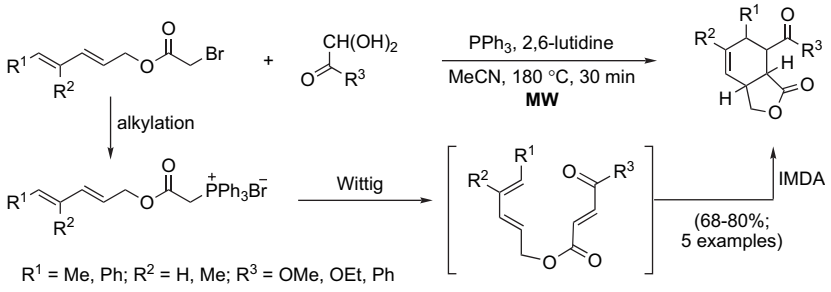


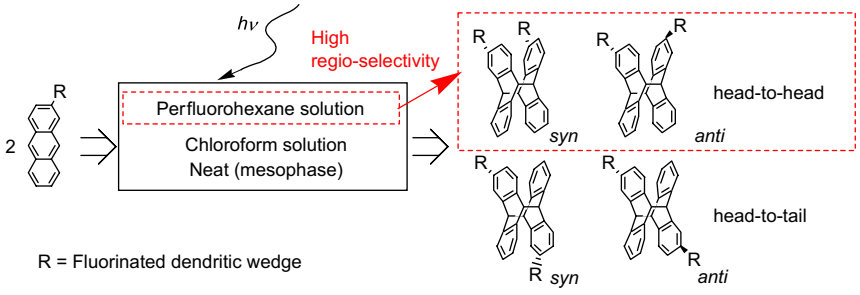


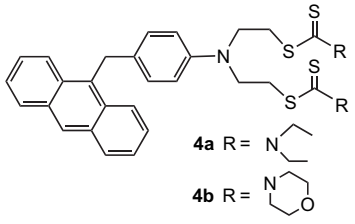




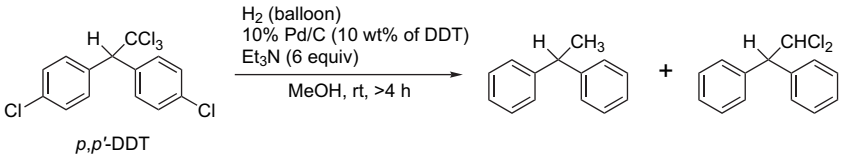


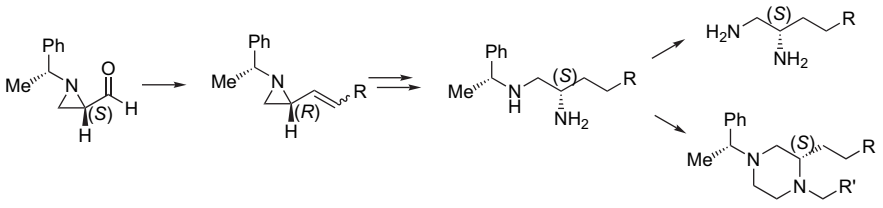


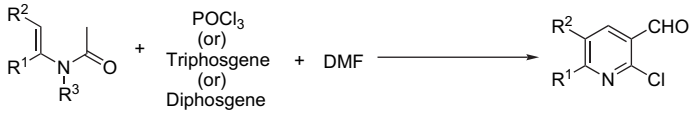


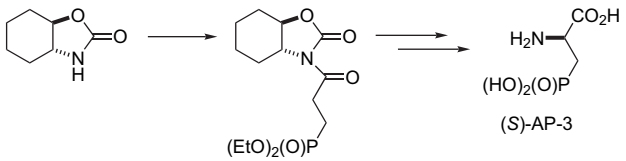


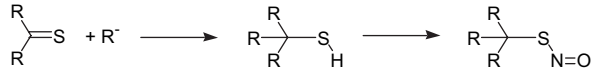
Compounds **4a** and **4b**, comprising an anthracene moiety as the fluorophore and a pair of dithiocarbamate functionalities as ligating groups, were designed as fluorescent chemosensors for Hg^{2+}.

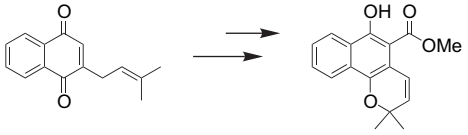


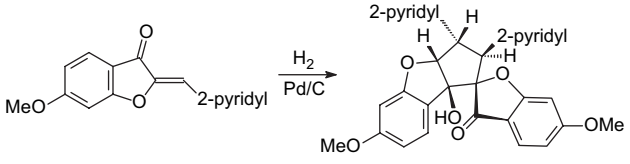












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ⓘ+ Supplementary data available via ScienceDirect

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Recent advances in homogeneous transition metal-catalyzed aerobic alcohol oxidations

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Contents

1. Introduction

The oxidation of an alcohol to the corresponding carbonyl product is a vital and common transformation in synthetic organic chemistry. Consequently, there are a vast number of diverse methods that accomplish this fundamental functional group manipulation. Unfortunately, many of the most common methods suffer from the use of forcing conditions and/or toxic stoichiometric oxidants. An emerging alternative process, which may address these issues, is the implementation of a catalyst in combination with molecular oxygen as the stoichiometric oxidant. The use of molecular oxygen as the stoichiometric oxidant should be advantageous because it is inexpensive, readily available, and ultimately produces benign byproducts such as H_2O. The attractive nature and potential of developing catalysts for aerobic alcohol oxidations have led to a significant increase in research effort as described in several recent reviews.[1–6] Therefore, this review will discuss the most recent developments and the key initial discoveries in the area of homogeneous,[7] metal-catalyzed aerobic oxidations of alcohols.

2. General challenges

Several challenges exist in the development of transition metal-catalyzed aerobic alcohol oxidations. Because numerous methods are available for the oxidation of alcohols, practicality plays an important role in any new method. This includes mild reaction temperatures, low pressures of O_2 especially in flammable organic solvents, low catalyst loading, and avoidance of costly/toxic additives. Other key challenges common to any new method are functional group tolerance and the ability to chemoselectively oxidize an alcohol in the presence of other groups susceptible to oxidation. Within

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this, an ultimate goal would be to develop catalysts that control the ability to oxidize one class of alcohols (i.e., secondary) in the presence of another (i.e., primary). Lastly, the development of diastereo- and/or enantioselective alcohol oxidations would provide another potentially useful tool for synthetic chemists.

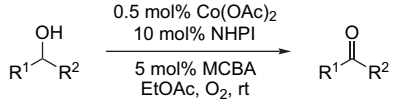
3. Catalyst systems

The subsequent sections describe in detail the different metal catalysts used in aerobic alcohol oxidation. Two key areas will be highlighted: synthetic potential and mechanistic considerations. It should be noted that this is not an exhaustive review of the topic and the examples are presented to illustrate the current state of the art and to describe the current mechanistic proposals.

3.1. Cobalt

In 1981, Tovrog and co-workers published the first Co-catalyzed aerobic oxidation of alcohols using Co–nitro complexes.[8] Following this early disclosure, several systems for Co-catalyzed aerobic alcohol oxidations have emerged. Ishii and co-workers have shown that combining *N*-hydroxyphthalimide (NHPI) with Co(III)-complexes, a variety of alcohols were successfully oxidized under aerobic conditions.[9,10] In their most recent report, addition of small amounts of benzoic acid increased the rate of alcohol oxidation, and the optimized procedure utilized 0.5 mol % $Co(OAc)_2$, 10 mol % NHPI, and 5 mol % *m*-chlorobenzoic acid (MCBA) under an oxygen atmosphere at room temperature (Table 1). This method was successful for the oxidation of secondary aliphatic, allylic, and benzylic alcohols (entries 1–4). Primary alcohols were oxidized to the corresponding carboxylic acids, and internal vicinal diols were converted to the corresponding diketones in modest yields (entries 5 and 6). In contrast, oxidation of terminal vicinal diols resulted in C–C bond cleavage and formation of the corresponding carboxylic acid.

Table 1. $Co(OAc)_2$/NHPI-catalyzed aerobic alcohol oxidation



Entry	Substrate	Time (h)	Yield (%)
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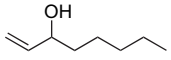
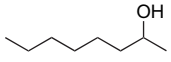
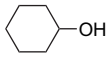
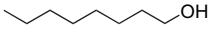
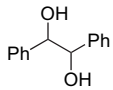
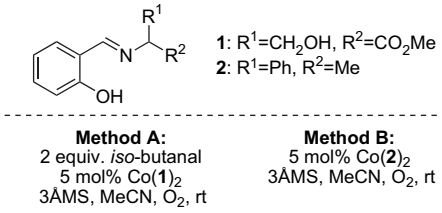
2		20	67
3		20	75
4		20	83
5[a]		20	78[b]
6[c,d]		20	84

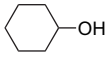
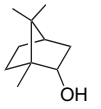
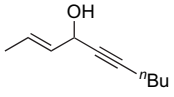
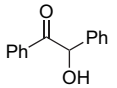
[a] *m*CPBA (1 mol %) substituted for MCBA.
[b] Product was corresponding acid.
[c] $Co(OAc)_2$ (1 mol %), no MCBA.
[d] Product was diketone.

In addition to Ishii's report, both Iqbal and Sain have disclosed similar examples of Co(II)-Schiff base catalyzed aerobic alcohol oxidations (Table 2, Method A and Method B, respectively).[11–14] These systems successfully oxidize both secondary aliphatic and benzylic alcohols (entries 1–6). Furthermore, Sain showed that α-hydroxyketones were oxidized using ligand **2**. The main difference between the two systems was the addition of *iso*-butanal in Method A. Adjusting the amount of added *iso*-butanal resulted in selective oxidation of benzyl alcohol to benzaldehyde as well as the oxidation of substrates containing olefins and/or alkynes without oxidation of the unsaturated bonds (entry 7). Mechanistically, Iqbal proposed the Co-complex with *iso*-butanal bound (**B**) was oxidized by molecular oxygen to form **C** (Scheme 1). **C** then oxidizes the aldehyde to the corresponding acid with concomitant formation of a Co(IV)-oxo species (**D**). **D** is responsible for the oxidation of alcohol to the corresponding carbonyl product with reduction to the Co(II)–Schiff base complex **A**.

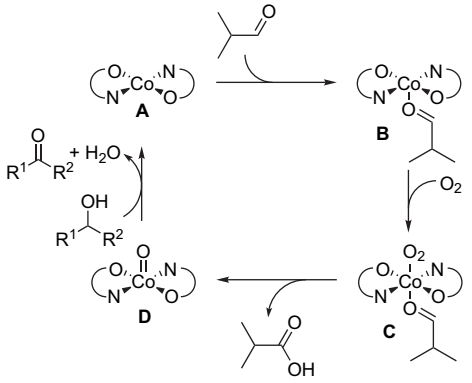
Sain and co-workers have also shown that a Co–phthalocyanine complex catalyzes the aerobic oxidation of alcohols.[15,16] This procedure utilized 5 mol % catalyst and 1 equiv of KOH in xylenes at reflux under an O_2 atmosphere for the oxidation of secondary benzylic, aliphatic, and propargylic alcohols (Table 3). In addition, the Co-catalyst was

Table 2. Co-Schiff base catalyzed aerobic alcohol oxidations



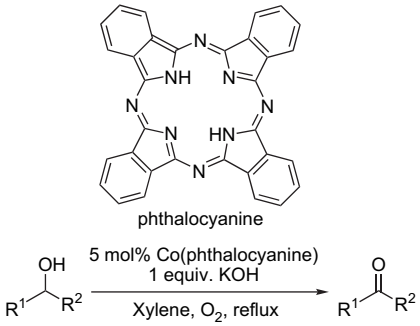
Entry	Substrate	Method	Time (h)	Yield (%)
1		B	5.5	96
2[a]		A	16	78
3		A	16	79
4		B	15	45
5		A	16	70
6		B	20	40
7		A	16	55
8		B	2.5	95

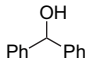
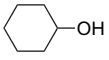
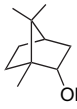
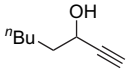
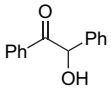
[a] *iso*-Butanal (1 equiv).



Scheme 1. Proposed mechanism for the Co-Schiff base catalyzed aerobic alcohol oxidation.

Table 3. Co-phthalocyanine catalyzed aerobic alcohol oxidation



Entry	Substrate	Time (h)	Yield (%)
1		1	94
2		3	95
3		5.5	75
4		7	92
5		7	70
6		0.2	96

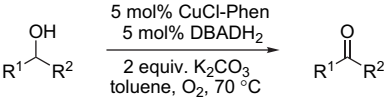
applied to the oxidation of a variety of α-hydroxyketones (entry 6).

While multiple systems have been developed for the Co-catalyzed aerobic alcohol oxidation, several limitations remain. Most secondary alcohols are converted to ketones, but primary alcohols are often oxidized to the carboxylic acid. To date, Ishii's system is the most synthetically useful due to the low catalyst loadings and mild conditions. Unfortunately, few mechanistic studies have been reported to assist in the development of improved catalysts.

3.2. Copper

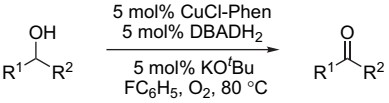
In 1984, Semmelhack reported the first practical Cu-catalyzed aerobic oxidation of alcohols. In this early disclosure, 10 mol % CuCl and 10 mol % TEMPO were used to oxidize primary benzylic, allylic, and aliphatic alcohols in DMF under an O_2 atmosphere at room temperature.[17] Using these standard conditions, secondary alcohols were oxidized but with significantly slower rates compared to primary alcohols. Since Semmelhack's early work, several reports on Cu-catalyzed aerobic alcohol oxidations have appeared and the topic has been thoroughly reviewed.[2,3] Much of the catalyst development has been pioneered by Markó and co-workers.[18–22] In Markó's initial report, an assortment of alcohols were oxidized using 5 mol % CuCl, 5 mol % phenanthroline, 5 mol % di-*tert*-butyl hydrazine-1,2-dicarboxylate ($DBADH_2$), and 2 equiv of K_2CO_3 (Eq. 1).[22] Unfortunately, this initial system required 2 equiv of a strong base and was not effective for the oxidation of primary aliphatic alcohols. More recently, it was shown that a change of the solvent from toluene to fluorobenzene allowed the use of catalytic K_2CO_3 or KO*t*-Bu instead of 2 equiv of K_2CO_3 (Eq. 2). Under catalytic base conditions, alcohols with α-chiral centers were oxidized with no observed racemization of the chiral center. However, the procedure continued to be inconsistent for the oxidation of primary aliphatic alcohols. Further additive evaluation led to the discovery that addition of catalytic *N*-methylimidazole (NMI) provided an efficient catalyst system for the oxidation of primary aliphatic alcohols (Eq. 3).

Generation 1



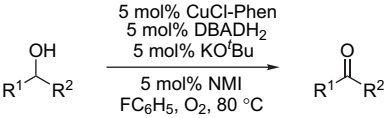
(1)

Generation 2



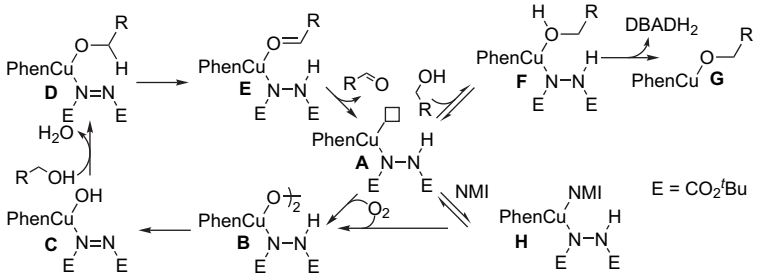
(2)

Generation 3



(3)

A number of mechanistic experiments and possible roles of the additives have led to a proposed mechanism (Scheme 2). First, the active catalyst **A** is oxidized by molecular oxygen to form a Cu(II)-hydrazide derivative **B**. Following homolytic cleavage of **B** and intramolecular hydrogen abstraction, **C** is formed. This is followed by ligand substitution with the alcohol to form the Cu(I)-alkoxide **D**. Intramolecular hydride transfer to DBAD followed by dissociation of the aldehyde reforms the active Cu(I)-catalyst **A**. To support this mechanism, **A** was prepared independently and exposed to alcohol under anaerobic conditions. This resulted in no alcohol oxidation; however, when oxygen was added, rapid conversion to the product was observed, thus implying preliminary oxidation of **A** with O_2 prior to alcohol oxidation.



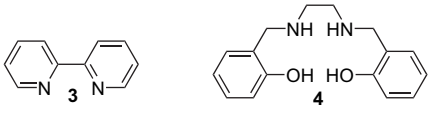
Scheme 2. Mechanism for Cu-DBADH$_2$ catalyzed aerobic oxidation of alcohols.

After discovering additives such as NMI improved the oxidation of primary aliphatic alcohols, an addition to the mechanistic proposal was made. It was shown that Cu(Phen)-alkoxides derived from primary aliphatic alcohols (such as **G**) do not undergo oxidation and thus the addition of NMI was believed to competitively bind to **A** preventing catalyst deactivation through this process.

In addition to Markó's developments, two groups have recently reported chemoselective oxidations of primary alcohols using Cu. After evaluating a variety of Cu-sources and ligands, Sheldon and co-workers demonstrated that the use of 2,2′-bypyridine as a ligand for $CuBr_2$ in combination with TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) resulted in oxidation of primary benzylic, allylic, and aliphatic alcohols to the corresponding aldehydes with no overoxidation to the acid observed (Table 4, Method A).[23,24] Secondary alcohols were not oxidized, and when mixtures of primary and secondary alcohols were exposed to the reaction conditions, only the primary alcohol was converted. In a related system, Punniyamurthy and co-workers recently disclosed an oxidation that employed a salen-type ligand on Cu that also chemoselectively oxidized primary alcohols (Table 4, Method B).[25] While this system had a similar scope to Sheldon's, it required a pure O_2 atmosphere as well as elevated temperatures (100 °C). Additionally, the authors showed that the catalyst could be recycled up to three times with no loss in activity by using an aqueous workup.

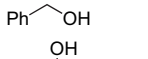
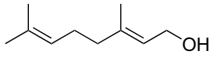
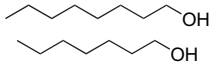
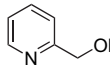
Both groups proposed a mechanism based on galactose oxidase-catalyzed oxidation of alcohols.[26] In these proposals, Cu(II) undergoes ligand exchange to form a Cu-alkoxide **B** that binds with TEMPO (Scheme 3). The Cu-TEMPO intermediate **C** can proceed to the aldehyde via hydrogen atom abstraction by TEMPO with concomitant formation of **D**. Molecular oxygen is proposed to reoxidize TEMPOH to TEMPO followed by reoxidation of Cu(I) by TEMPO. The authors proposed that the key to the chemoselectivity is the formation of intermediate **C** wherein secondary alcohols cannot arrange in a manner to undergo hydrogen transfer due to the steric interactions.

Table 4. Cu-TEMPO catalyzed aerobic alcohol oxidations

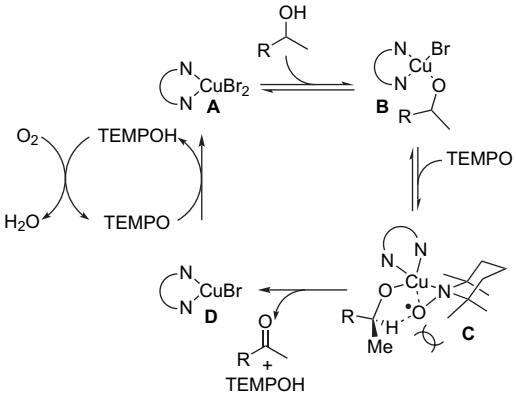


Method A:
5 mol% Cu(**3**)Br_2
5 mol% TEMPO, 5 mol% KO*t*Bu
MeCN:H_2O (2:1), air, rt

Method B:
5 mol% Cu(**4**)
5-7 mol% TEMPO
toluene, O_2, 100 °C

Entry	Substrate	Method	Time (h)	Yield (%)
1		A	2.5	>99
2		B	10	99
3		A	5	N.R.
4		B	12	2
5		A	5	>99
6		B	23	79
7[a]		A	24	95
8		B	21	90
9		B	26	92

[a] Reaction performed at 40 °C.

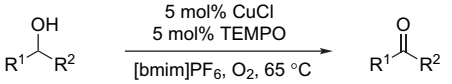


Scheme 3. Cu-TEMPO catalyzed aerobic alcohol oxidation.

The application of nontraditional organic solvents, which can allow simple product purification and catalyst recycling, presents an appealing alternative to the use of traditional organic solvents in this chemistry. In 2002, Ansari and Gree reported a CuCl-TEMPO catalyzed aerobic alcohol oxidation that succeeded in the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6) (Eq. 4).[27] This method was successful for the oxidation of primary and secondary benzylic and allylic alcohols using 5 mol % CuCl and 5 mol % TEMPO at 65 °C. Aliphatic alcohols were also successfully oxidized under these conditions, but with significantly slower rates and often with incomplete conversion. While the ionic liquid could be recycled up to eight times with little decrease in the efficiency, the authors were not able to recycle the catalyst. More recently, Jiang and Ragauskas used a pyridyl based ionic liquid, 1-butyl-4-methylpryridinium hexafluorophosphate ([bmpy]PF_6),

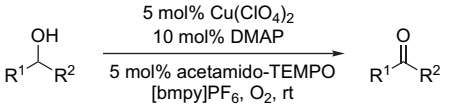
along with acetamido-TEMPO (a TEMPO source that can be recycled in ionic liquids) and DMAP for the Cu-catalyzed aerobic alcohol oxidation (Eq. 5).[28] The reactions were successful at room temperature for the chemoselective oxidation of a broad range of primary benzylic, allylic, and aliphatic alcohols with no oxidation of secondary alcohols observed. In contrast to Ansari and Gree's system, the catalyst/ionic liquid could be recycled up to five times with only a slight lowering of catalyst activity.

Ansari and Gree



(4)

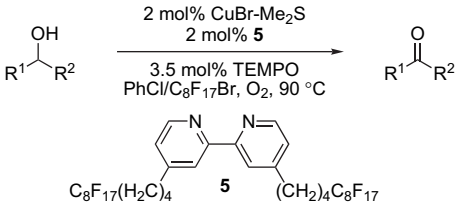
Jiang and Ragauskas



(5)

A biphasic solvent system has also been employed by Knochel and co-workers for Cu-catalyzed aerobic alcohol oxidations to enhance catalyst recyclability.[29] This was accomplished using a bipyridine ligand containing fluorinated 'ponytails' in combination with a chlorobenzene/perfluorooctyl bromide solvent mixture (Eq. 6). The oxidation was successful for a variety of primary and secondary benzylic, allylic, and aliphatic alcohols. As seen previously, primary alcohols generally oxidized more rapidly than secondary alcohols. Additionally, the authors demonstrated that the fluorous layer containing the catalyst could be reused up to eight times with little loss of catalyst activity.

Knochel and Coworkers



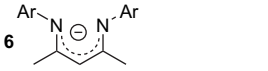
(6)

Overall, several useful systems have been developed for Cu-catalyzed aerobic alcohol oxidations. Markó and co-workers have developed multiple generations of catalysts for the oxidation of a broad scope of alcohols under mild conditions. Several groups have also developed catalyst systems that employ TEMPO in combination with Cu both in traditional and nontraditional solvents. Additionally, use of ligands in these oxidations produces a sterically encumbered environment at the Cu-center, thus resulting in a chemoselective oxidation of primary alcohols.

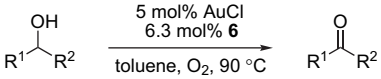
3.3. Gold

While gold salts have emerged as viable catalysts for several synthetic transformations, only one example of a homogeneous Au-catalyzed aerobic oxidation of alcohols has been reported.[30] In early evaluations, Shi and co-workers revealed several stoichiometric oxidants (O_2, H_2O_2, TBHP) in combination with catalytic AuCl and a monoanionic bidentate ligand **6** produced active systems for the oxidation of benzyl alcohol. Optimization of an aerobic oxidation led to the use of 5 mol % AuCl and 6.3 mol % **6** in toluene at 90 °C under an atmosphere of oxygen for the oxidation of primary and secondary benzylic and allylic alcohols (Table 5). Of particular note, the catalyst loading could be lowered to 1 mol % (entry 1) or the oxygen atmosphere was replaced with an air atmosphere with extended reaction times. Cyclic secondary aliphatic alcohols oxidized well under the standard conditions, but no examples of straight chain secondary aliphatic alcohols were reported (entry 6). A limitation of this system was that the primary aliphatic alcohols had slower oxidation rates and formed aldol byproducts (entry 7).

Table 5. Au-catalyzed aerobic alcohol oxidation



Ar = 2,6-di-*iso*-PrPhenyl



Entry	Alcohol	Time (h)	Yield[a] (%)
1[b]	Ph, OH	24	96
2	*p*-MeO-Ph, OH	10	99
3	*p*-F-Ph, OH	24	99
4	*p*-NO_2-Ph, OH	36	92
5	Ph, OH	24	96
6	OH	24	99
7	OH	48	68

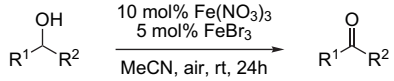
[a] GC-yield.
[b] AuCl (1 mol %), **6** (1.2 mol %).

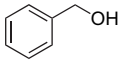
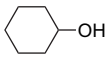
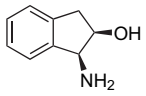
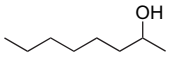
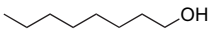
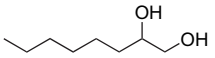
While very little work on the Au-catalyzed aerobic oxidation has been reported, Shi's report is very encouraging for future development. This system does require forcing conditions, but further mechanistic studies, ligand variation, and scope evaluation could result in an excellent system for the aerobic oxidation of alcohols using gold catalysts.

3.4. Iron

Recently, iron salts have been employed as catalysts for the aerobic oxidation of alcohols. In 2002, Martin and Suárez reported the first Fe-catalyzed aerobic alcohol oxidation that used a combination of $Fe(NO_3)_3$ and $FeBr_3$ to oxidize alcohols to the corresponding aldehydes and ketones under an ambient air atmosphere at room temperature (Table 6).[31] While the scope of this oxidation was not explored in depth, it was shown that secondary aliphatic and primary benzylic alcohols were readily oxidized (entries 1–4). However, primary aliphatic alcohols were not oxidized (entry 5) and no examples of allylic alcohols were reported. Additionally,

Table 6. Fe-catalyzed aerobic alcohol oxidation



Entry	Substrate	Yield (%)
1		80
2		80
3		85
4		75
5		N.R.
6		74[a]

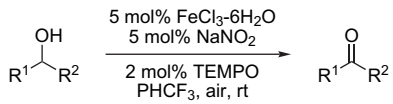
[a] Product corresponds to α-hydroxyketone.

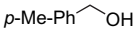
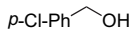
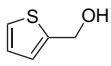
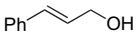
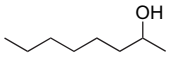
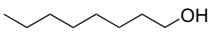
when a terminal 1,2-diol was exposed to the reaction conditions, the corresponding α-hydroxyketone was isolated in 74% yield with no oxidation of the primary alcohol observed. In contrast to the previously described Cu-TEMPO catalyzed oxidation, this represented an effective chemoselective oxidation of secondary aliphatic alcohols (entry 6).

More recently, a Fe-catalyzed aerobic alcohol oxidation was disclosed by Liang and co-workers that utilized $NaNO_2$/TEMPO to oxidize a variety of alcohols.[32] The optimized conditions for this system employed 5 mol % $FeCl_3$, 5 mol % $NaNO_2$, and 2 mol % TEMPO in trifluorotoluene at room temperature under ambient air pressure (Table 7). The scope of this oxidation system included primary and secondary benzylic alcohols along with secondary aliphatic alcohols and cinnamyl alcohol. It is noteworthy that thioethers were not oxidized under the reaction conditions (entry 2). Unfortunately, oxidation of primary aliphatic alcohols resulted in modest selectivity for aldehyde formation with both acid and ester byproducts observed (entry 8). The authors proposed a mechanism analogous to Cu-TEMPO oxidation that included two separate cycles (Scheme 4). Oxidation of the alcohol by a Fe(III)-TEMPO species is proposed to form the desired carbonyl product and a reduced Fe(II)-species. The Fe(III)-catalyst is proposed to be regenerated by the oxidation of Fe(II) with NO_2 wherein NO_2 is formed via rapid oxidation of NO with O_2.

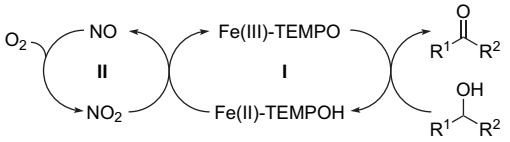
These two catalytic methods are performed under relatively mild conditions for an aerobic alcohol oxidation (room temperature and air atmosphere). However, relatively high catalyst loadings are employed. The Fe-TEMPO system has been explored for a slightly wider scope of alcohols while the $FeBr_3$/$Fe(NO)_3$ system provided a chemoselective oxidation of secondary aliphatic alcohols. These initial studies illustrate the potential of using Fe-based systems for aerobic alcohol oxidation but significant work remains in development of scope and mechanistic elucidation.

Table 7. Fe/TEMPO/$NaNO_2$-catalyzed aerobic alcohol oxidation



Entry	Substrate	Time (h)	Conversion[a] (%)
1		8	>99
2		16	>99[b]
3		8	>99
4		12	>99
5[c]		12	>99
6[c]		12	>99
7[c]		8	>99
8		6	>99[d]

[a] Conversion measured by GC.
[b] No oxidation of the sulfur observed.
[c] $NaNO_2$ (8 mol %) and TEMPO (5 mol %).
[d] Selectivity, 71%.



Scheme 4. Proposed mechanism for Fe/TEMPO/$NaNO_2$-catalyzed aerobic alcohol oxidation.

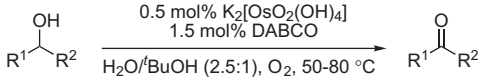
3.5. Osmium

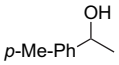
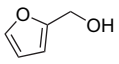
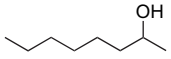
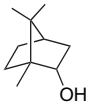
While Os has long been used for the dihydroxylation of olefins,[33] Beller and co-workers recently disclosed the first osmium-catalyzed aerobic alcohol oxidation.[34] This system operates using low catalyst loadings (≤0.5 mol %) along with catalytic DABCO for the oxidation of primary and secondary benzylic alcohols and secondary aliphatic alcohols under an oxygen atmosphere (Table 8). Additionally, by increasing the pressure of air to 40 bar, the catalyst loading could be lowered to 0.005 mol % for the oxidation of *sec*-phenethyl alcohol and benzyl alcohol with turnover numbers (TON) of up to 16,600 (entry 1). Similar to Fe and Au much work remains for the Os-catalyzed aerobic alcohol oxidations. While TON of 16,600 are especially impressive, the reaction requires forcing conditions and the scope and mechanism has not been thoroughly explored to date.

3.6. Palladium

Among the metal complexes explored for the aerobic oxidation of alcohols, palladium has arguably received the most recent attention. In 1977, Schwartz and Blackburn published the first synthetically viable Pd-catalyzed aerobic alcohol oxidation using $Pd(OAc)_2$ in combination with NaOAc.[35]

Table 8. Os-catalyzed aerobic alcohol oxidation



Entry	Substrate	Time (h)	Yield (%)
1		24	83[a]
2		12	93
3		24	85
4		24	59[b]
5		20	98

[a] $K_2[OsO_2(OH)_4]$ (0.005 mol %), DABCO (0.015 mol %), and 40 bar air.
[b] $K_2[OsO_2(OH)_4]$ (1 mol %) and DABCO (3 mol %).

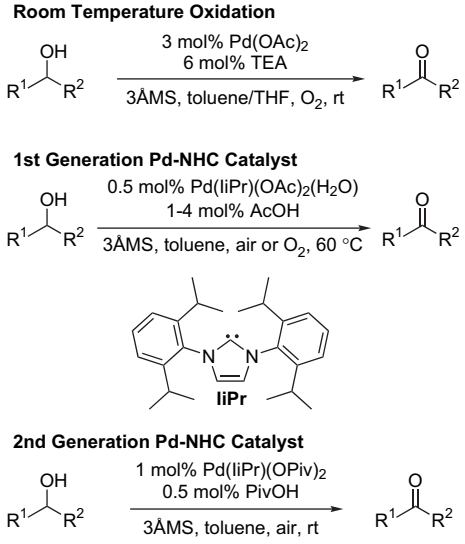
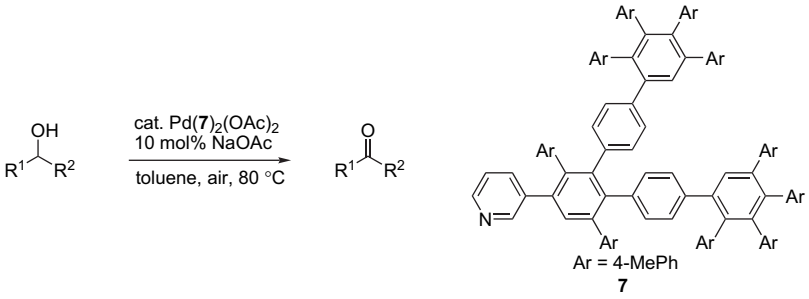


Figure 1. Sigman's Pd-catalyzed aerobic alcohol oxidations.

Following this disclosure, 20 years passed with few developments in this field until the late 1990s, when several improved catalyst systems were reported by the Uemura,[36–41] Larock,[42] and Sheldon groups.[43–46] This has led to the development of various catalyst systems and a number of thorough mechanistic studies for Pd-catalyzed aerobic alcohol oxidations including the oxidative kinetic resolution (OKR) of secondary alcohols, which has been extensively reviewed.[1,5,47,48] For the purpose of this review, only the most recent disclosures on Pd-catalyzed aerobic alcohol oxidations will be highlighted.

In 2004, Tsuji and co-workers reported that substituted pyridines prevent formation of Pd-black, and therefore, allowed the Pd-catalyzed aerobic oxidation of alcohols under an air atmosphere using low catalyst loadings with TON up to 1480 (Eq. 7).[49] Use of the sterically encumbered ligand **7** allowed primary and secondary benzylic as well as secondary aliphatic alcohols to be oxidized to the corresponding carbonyl products in fair yields with no Pd-black formation observed.

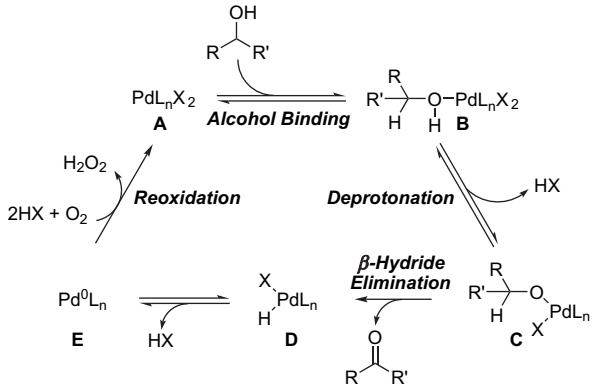


(7)

More recently, Sigman and co-workers have reported a comparison study of three catalyst systems developed within their group (Fig. 1).[50] In this report, a second generation Pd-*N*-heterocyclic carbene catalyst was used to oxidize alcohols at room temperature under an air atmosphere using 1 mol % catalyst. This represented one of the mildest aerobic alcohol oxidations reported to date. The scope of this catalyst was compared with the previously reported $Pd(OAc)_2$/TEA[51] and $Pd(IiPr)(OAc)_2$[52] catalyst systems. While the $Pd(IiPr)(OPiv)_2$ system represented a mild oxidation, the scope was limited to the primary aliphatic alcohols and sterically encumbered alcohols were not oxidized well. In addition to reporting a new catalyst, the substrate scope of the three systems was evaluated for more complex substrates including 1,2- and 1,3-mono-protected diols as well as amino alcohols. Overall, the authors demonstrated that both the $Pd(OAc)_2$/TEA and $Pd(IiPr)(OAc)_2$ systems performed well for a broad scope of alcohols, and the $Pd(OAc)_2$/TEA system represented the most convenient of the three catalyst systems developed.

Over the past five years, a considerable number of detailed mechanistic studies have led to a generally accepted mechanism for Pd-catalyzed aerobic alcohol oxidations (Scheme 5).[5,48,53–57] The catalytic cycle begins with binding of the alcohol to the Pd(II)-catalyst to form alcohol bound intermediate **B**. Deprotonation of **B** then occurs to form a Pd-alkoxide (**C**), which undergoes β-hydride elimination to liberate the carbonyl product and form a Pd-hydride species (**D**). The Pd-hydride can reductively eliminate an equivalent of acid to form Pd(0), which is then reoxidized by molecular oxygen and 2 equiv of acid. For majority of these catalyst systems, β-hydride elimination has been proposed as the rate-limiting step. There are two exceptions to this: the $Pd[(-)\text{-sparteine}]Cl_2$-catalyzed aerobic oxidative kinetic resolution at low [base][58] and $Pd(OAc)_2$/TEA[59] in which

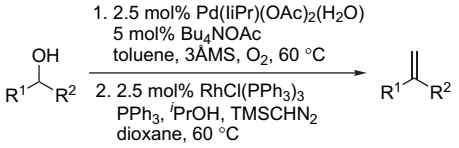
deprotonation is proposed as the rate-limiting step. The mechanistic studies have also led to a better understanding of the role(s) of ligands in the oxidation. Ligands are required to support Pd(0) and prevent metal aggregation and catalyst deactivation; however, excess ligands can also inhibit the oxidation by preventing substrate binding and/or β-hydride elimination.



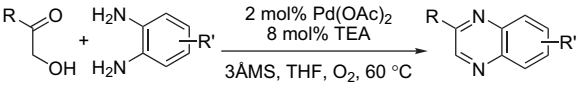
Scheme 5. Generally accepted mechanism for Pd-catalyzed aerobic alcohol oxidation.

More recently, Stahl and co-workers have also studied the role of molecular sieves in the $Pd(OAc)_2$/pyridine and $Pd(OAc)_2$/DMSO system.[60] In Uemura's early work, it was proposed that sieves were responsible for the disproportionation of the hydrogen peroxide formed during reoxidation of Pd(0).[37] Additionally, since water is formed upon disproportionation of the hydrogen peroxide, sieves could also be responsible for sequestering the water.[61] However, Stahl revealed that water did not have an inhibitory effect on the reaction rate, and by monitoring O_2 consumption, it was concluded that addition of 3 Å molecular sieves slowed the disproportionation of hydrogen peroxide. Furthermore, sieves were proposed to accelerate the oxidation by serving as a heterogeneous Brønsted base and to act as a heterogeneous surface to support Pd(0) and prevent aggregation of palladium metal.

Apart from the development of new systems for the Pd-catalyzed aerobic oxidation of alcohols and subsequent mechanistic studies, there are two recent examples of using Pd oxidation catalysts in tandem reactions. Lebel and Paquet have shown that alcohols can be converted directly to the corresponding olefin in a one-pot procedure using $Pd(IiPr)(OAc)_2(H_2O)$ to oxidize the alcohol followed by a Rh-catalyzed olefination reaction (Eq. 8).[62] In all cases, the olefins were isolated in better yields than in the corresponding two-step sequences. More recently, Robinson and Taylor have developed a one-pot procedure for preparing variety of quinoxalines from α-hydroxyketones via a tandem Pd-catalyzed aerobic alcohol oxidation/quinoxaline formation (Eq. 9).[63] Of the catalysts evaluated for the reaction, the $Pd(OAc)_2$/TEA system provided the best yields of the desired products.

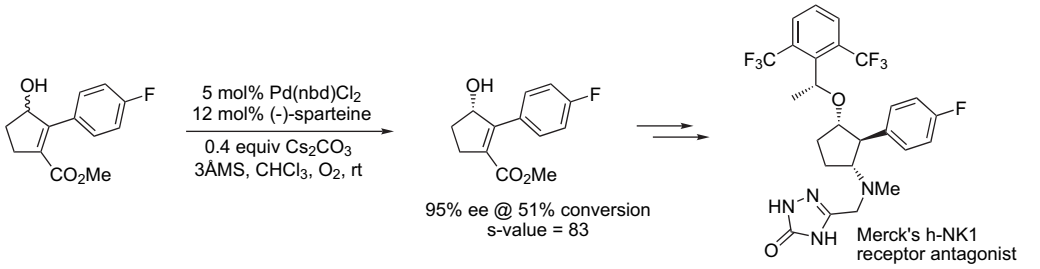


(8)



(9)

In addition to these tandem reactions, Stoltz and co-workers have demonstrated the utility of the recently developed Pd-catalyzed aerobic OKR of alcohols in the synthesis of several pharmaceutical intermediates.[64] In their best result, the 2-arylcyclopentenol precursor to Merck's human neurokinin-1 receptor antagonist[65,66] was resolved with *s*-values up to 83 (Eq. 10).



(10)

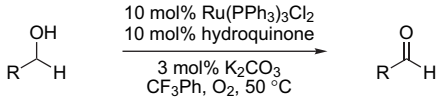
Overall, Pd(II)-catalysis represents one of the most mature fields in the aerobic oxidation of alcohols. The scope of the reaction is the most widely examined and the mechanism is best understood. Future work in this area will focus on identifying/designing new ligands that would allow the oxidation to be performed using low catalyst loadings under mild conditions.

3.7. Ruthenium

There also has been much effort placed on the development of Ru-catalyzed aerobic alcohol oxidations. In 1997, Markó and Ley simultaneously reported TPAP-catalyzed aerobic alcohol oxidations.[67,68] While the two groups used different reaction conditions, both systems worked well for a broad range of alcohols, but primary aliphatic alcohols were not oxidized well in contrast to using NMO as the stoichiometric oxidant.[69] Following these reports, several other Ru-catalysts have been developed for the aerobic oxidation of alcohols. Ishii and co-workers employed a combination of $Ru(PPh_3)_3Cl_2$ and hydroquinone to chemoselectively oxidize primary aliphatic alcohols to the corresponding aldehydes under aerobic conditions (Eq. 11).[70] A year later, Sheldon demonstrated using the same Ru-catalyst in combination with TEMPO that both primary and secondary alcohols were oxidized successfully (Eq. 12).[71,72] Unfortunately, this system requires 10 bar pressure. In 2000, Lee and Chang reported a convenient Ru-catalyzed aerobic alcohol oxidation using $[RuCl_2(p\text{-cymene})]_2$ in combination with Cs_2CO_3 for

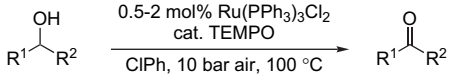
the oxidation of a broad range of alcohols. This system was limited due to the poor oxidation of primary aliphatic alcohols (Eq. 13).[73]

Ishii



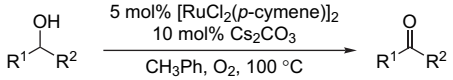
(11)

Sheldon



(12)

Chang



(13)

More recently, Katsuki and co-workers have published several papers on Ru–salen catalysts for aerobic alcohol oxidations. Catalyst **8** was employed to oxidize *o*-hydroxy benzyl alcohol derivatives to the corresponding aldehydes selectively (Eq. 14).[74] This is especially impressive since these substrates have the ability to chelate with the metal and/or undergo other coupling reactions. This catalyst also proved effective for chemoselective oxidation of primary alcohols in the presence of secondary alcohols and upon derivatization of the ligand, catalyst **9** provided better chemoselectivity (Eq. 15).[75,76] Using **9**, it was demonstrated that primary aliphatic alcohols could be selectively oxidized in the presence of secondary aliphatic, benzylic, allylic, and propargylic alcohols.

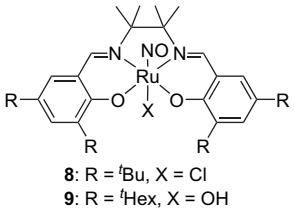
In addition to chemoselective oxidation, chiral salen ligands have been used for the Ru-catalyzed aerobic oxidative kinetic resolution of secondary alcohols and desymmetrization of *meso*-diols (Eq. 16).[77,78] After evaluating several Ru–salen complexes, **10** proved most effective for the oxidative kinetic resolution of secondary alcohols resulting in *s*-values up to 20. In contrast, Katsuki found that catalyst **11** proved most useful for the oxidative desymmetrization of a variety of *meso*-diols. Upon oxidation of the resulting lactol to the corresponding lactone, ees up to 93% were observed (Eq. 17).

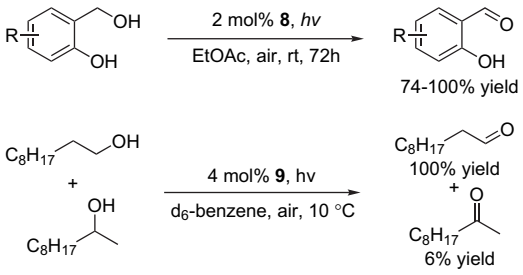
Ruthenium has proven effective for the aerobic oxidation of alcohols. This is especially true for Sheldon's system, which uses low catalyst loadings but requires elevated O_2 pressure. Additionally, Katsuki has developed an efficient chemoselective oxidation of primary aliphatic alcohols. However, much work remains in developing general alcohol oxidation catalysts that employ low catalyst loadings and perform under milder conditions.

3.8. Vanadium

Vanadium has recently been applied to the aerobic oxidation of both α-hydroxycarbonyl compounds and propargylic alcohols. In 1999, Nemoto and co-workers demonstrated that a simple procedure using 1 mol % $VOCl_3$ in acetonitrile at room temperature resulted in the oxidation of α-hydroxycarbonyls in excellent yields (Table 9).[79] This method was successful for the oxidation of a variety of substrates and could be carried out under an air atmosphere, albeit with significantly longer reaction times (entry 2). In addition, aromatic substrates oxidized much more rapidly then aliphatic substrates.

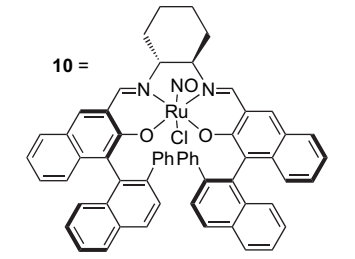
Uemura and co-workers have published a thorough study on the vanadium-catalyzed aerobic oxidation of propargyl alcohols.[80,81] Under optimized conditions (1 mol % $VO(acac)_2$, 3 Å MS, MeCN, O_2, 80 °C), a variety of propargyl alcohols including aryl, vinyl, alkynyl, and aliphatic substrates were successfully oxidized to the corresponding carbonyl products (Table 10). Of the alcohols examined, aryl substrates proved

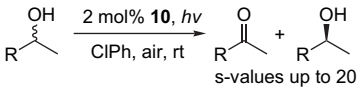




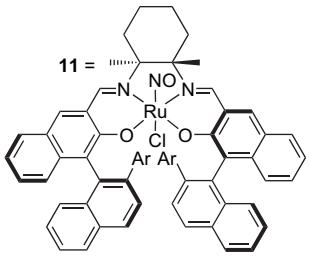
(14)

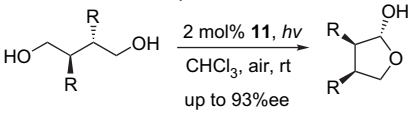
(15)





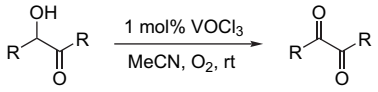
(16)





(17)

Table 9. V-catalyzed aerobic oxidation of α-hydroxycarbonyls



Entry	R	Time (h)	Yield (%)
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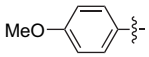
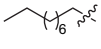
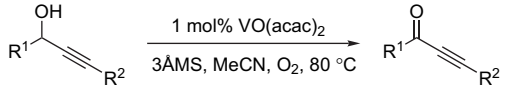
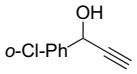
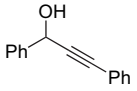
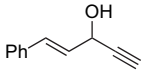
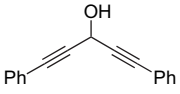
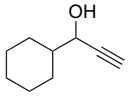
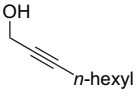
2[a]		11	89
3		1.5	>99
4		5.5	>99
5		12	>99
6		20	95

[a] Reaction carried out under an air atmosphere.

Table 10. $VO(acac)_2$-catalyzed oxidation of propargylic alcohols

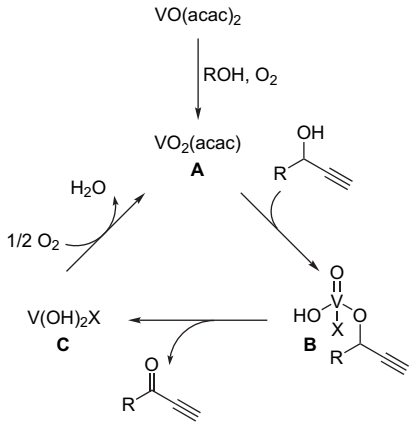


Entry	Substrate	Yield[a] (%)
1		65
2		81
3		65
4		76
5		41
6[b]		62

[a] GC yield.
[b] $VO(acac)_2$ (5 mol %).

to oxidize most effectively. Of note, a primary propargyl alcohol was successfully oxidized, but requires increased catalyst loadings (5 mol %) to obtain good yields (entry 6). On further optimization, the authors showed that the use of hexafluoroacetylacetonate as the ligand on V and as an additive led to slightly better yields for problematic substrates. Besides propargyl alcohols, an assortment of other alcohols was exposed to the reaction conditions. Unfortunately, the conditions only provided poor to moderate yields for the oxidation of simple benzylic, aliphatic, and allylic alcohols.

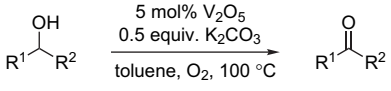
After exploring the scope of the V-catalyzed aerobic alcohol oxidation, several mechanistic experiments were performed. The stoichiometry of alcohol to oxygen was determined to be 2:1 by measuring oxygen uptake. The authors also found that radical inhibitors did not affect the rate of oxidation. Furthermore, use of electron spin resonance spectroscopy provided evidence that $VO(acac)_2$ was not involved and V(V) may be the active species in the oxidation. Combining these experiments, the authors proposed a mechanism involving initial oxidation of V(IV) to V(V) by O_2 followed by the attack of alcohol to form a V-alkoxide species **B** (Scheme 6). Elimination of the alkoxide would result in product formation and V(III) **C**, which could then be reoxidized by O_2 to reform the active V(V) catalyst **A**.



Scheme 6. Proposed mechanism for V-catalyzed aerobic oxidation of propargylic alcohols.

In addition to Uemura's work, Velusamy and Punniyamurthy have used V_2O_5 to broaden the scope of oxidation to benzylic, allylic, and aliphatic alcohols.[82] This oxidation required 5 mol % V_2O_5 and 0.5 equiv of K_2CO_3 in toluene at 100 °C under an oxygen atmosphere (Table 11). This method showed little dependence on the electronics of benzylic alcohols for the oxidation (entries 1–4). While the oxidation of secondary aliphatic alcohols proceeded well, primary aliphatic alcohols provided only moderate

Table 11. V_2O_5-catalyzed aerobic alcohol oxidation



Entry	Substrate	Time (h)	Yield (%)
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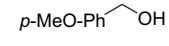
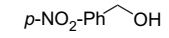
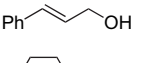
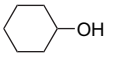
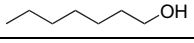
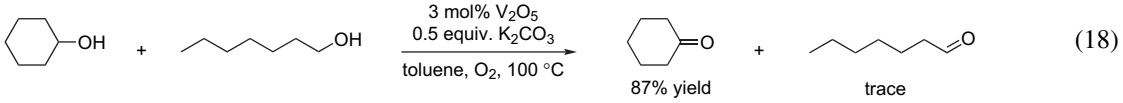
2		13	95
3		22	92
4		25	79
5		22	87
6		15	89
7		24	43

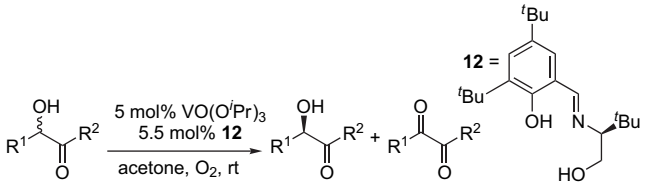
yields of the corresponding aldehydes (38–43%) (entry 7). With this in mind, the authors exposed a mixture of cyclohexanol and 1-heptanol to modified reaction conditions (3 mol % V_2O_5) to test for a chemoselective oxidation. This experiment resulted in an 87% isolated yield of cyclohexanone and only a trace amount of heptanal (Eq. 18). Therefore, this system has the potential to be used for the chemoselective oxidation of secondary aliphatic alcohols.

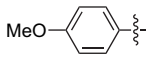
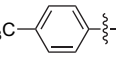
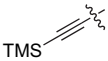


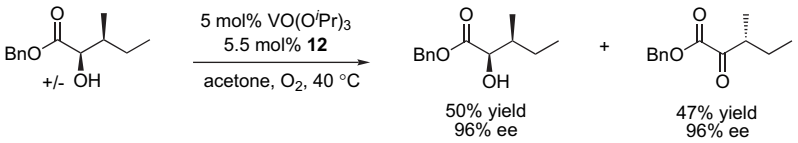
(18)

While several groups have worked on V-catalyzed general alcohol oxidations, Toste and co-workers have recently shown that chiral vanadium complexes catalyze the oxidative kinetic resolution (OKR) of α-hydroxy esters.[83] In this report, a tridentate ligand with an O,N,O-binding motif proved most effective (Table 12). A variety of racemic aryl, vinyl, and alkyl substituted α-hydroxy esters were efficiently resolved with selectivity factors ranging from 12 to >50. Unfortunately, application of this system to the OKR of a propargyl α-hydroxy ester resulted in a poor resolution (entry 6). In addition to the efficient resolution of simple α-hydroxy esters, racemic substrates with α-chiral centers were effectively resolved to yield both the enantioenriched alcohol and ketone in excellent yields and ee's (Eqs. 19 and 20).

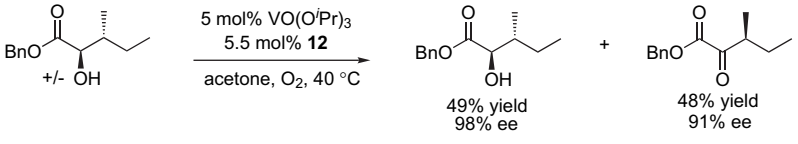
Table 12. V-catalyzed aerobic OKR of α-hydroxy esters



Entry	R^1	R^2	Time (h)	Yield (%)	ee (%)	*s*
1		OEt	10	49	99	>50
2		OMe	5.5	38	95	13
3		OMe	4	35	98	29
4		OBn	16	45	92	18
5		O^{i}Pr	90	37	98	30
6		OEt	16	53	50	6



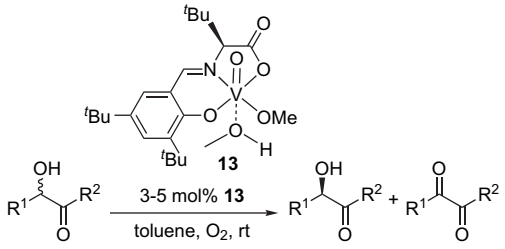
(19)

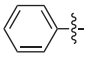
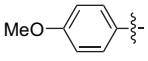
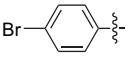


(20)

More recently, Chen and co-workers have published OKR of both α-hydroxy esters and amides using a similar O,N,O-chelating tridentate ligand (Table 13).[84] The procedure utilized 3–5 mol % catalyst **13** in toluene at room temperature under an oxygen atmosphere. The catalyst system was most efficient for the OKR of aryl α-hydroxy esters and amides with *s*-values ranging from 5 to 1057 with slightly higher *s*-values than Toste's method. The system was also successful for a vinyl α-hydroxy ester and amide but aliphatic substrates gave varying results (entries 4–6). Overall, the procedure generally resulted in higher *s*-values for the oxidation of benzyl amides relative to the benzyl esters.

Table 13. V-catalyzed aerobic OKR of α-hydroxy esters and amides



Entry	R^1	R^2	Time (h)	Yield (%)	ee (%)	*s*
1		OBn	12	45	98	458
		NBn	25	47	99	1057
2		OBn	57	49	70	10
		NBn	106	38	77	7
3		OBn	15	43	88	28
		NBn	24	45	99	>80
4		OBn	5.5	40	96	27
		NBn	9	47	>99	>211
5		OBn	63	46	71	14
		NBn	142	46	95	81
6	Me	OBn	83	36	37	2
		NBn	92	47	33	3

While the use of vanadium for the aerobic oxidation of alcohols has only been explored recently, the potential utility has been demonstrated. It has proven to be the most effective metal for the aerobic oxidation of propargylic alcohols and for the oxidative kinetic resolution of α-hydroxy esters. Continued work on the development of more effective

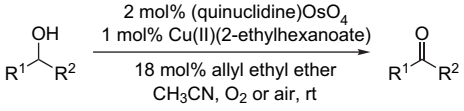
general alcohol oxidations and oxidative kinetic resolutions remains.

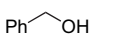
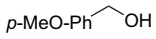
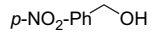
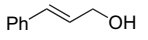
3.9. Other systems

Besides applying individual metals for aerobic alcohol oxidations, there are also a limited number of reports of bimetallic homogeneous alcohol oxidations. The use of bimetallic systems can offer the advantage of one metal activating the alcohol while the other activates molecular oxygen preventing catalyst decomposition and thus more efficient catalysis. In the late 1990's Osborn and co-workers published separate reports of Os–Cu, Ru–Cu, and Mo–Cu bifunctional aerobic alcohol oxidations.[85–87] All three of these systems employed relatively low catalyst loadings (1 mol %) and were most successful for the oxidation of primary benzylic alcohols. While very little mechanistic work was performed the authors proposed that in all the oxidations Cu was most likely responsible for the activation of O_2 and the other metal for the oxidation of alcohol. Shapley and co-workers have also reported similar Os–Cr and Ru–Cr systems for the aerobic oxidation of alcohols.[88] In these reports, primary alcohols were oxidized most effectively with the best chemoselectivity realized for aliphatic alcohols. The authors showed that the oxidation was reversible and a competitive isotope effect of 1.9 for the oxidation of PhCHDOH was observed.

A more recent report by Muldoon and Brown utilized a Os–Cu bifunctional catalyst system for the aerobic oxidation of alcohols at room temperature.[89] This oxidation occurs using 2 mol % (quinuclidine)OsO_4 and 1 mol % Cu(ethylhexanoate) (Table 14). The authors stated that the use of quinuclidine as a ligand for Os resulted in a 10-fold increase in activity. This oxidation was successful for a variety of benzylic and allylic alcohols. As with Osborn's report, aliphatic alcohols were not oxidized well.

Table 14. Os/Cu-catalyzed aerobic oxidation



Entry	Alcohol	Time (h)	Yield (%)
1		6	98
2		6	97
3		10	98
4		8	97
5		15	96

There are also several reports of other metals catalyzing the aerobic oxidation of alcohols. This includes two reports by Kim and co-workers of bifunctional systems where catalytic TEMPO or *N*-hydroxyphthalimide oxidizes a range of alcohols. Catalytic Ce is used to reoxidize the TEMPO/*N*-hydroxyphthalimide and O_2 is proposed as the terminal oxidant to reoxidize Ce.[90,91] In 1998, Ruiz and co-workers reported a unique Mn–oxamato complex that catalyzed the aerobic oxidation of secondary benzylic alcohols. A primary benzylic alcohol was oxidized to the corresponding acid and no further examples of the substrate scope were given.[92] There is also one example of a Rh-catalyzed aerobic alcohol oxidation that utilized $RhCl_3$ or $Rh(ClO_4)_3$ along with either $BiCl_3$ and/or LiCl to oxidize secondary aliphatic alcohols to the corresponding ketones.[93]

4. Conclusion

The last 10 years has seen a considerable increase of interest in the area of metal-catalyzed aerobic alcohol oxidations. Of the work presented in this review, Markó's Cu-(phen) and Sigman's $Pd(OAc)_2$/TEA and $Pd(IiPr)(OAc)_2(H_2O)$ systems are the most mature. All three of these methods have been explored for a broad scope of alcohols and work under relatively mild conditions. In addition, several of the catalyst systems discussed show high potential for synthetic utility. This is especially true for chemoselective oxidations. Unfortunately, even with the significant amount of work applied to the development of a variety of catalyst systems, application in target synthesis has yet to be realized.

Mechanistically, very little work has been performed to elucidate the fine details for many of the metal-catalyzed aerobic alcohol oxidations. Pd-catalyzed aerobic alcohol oxidations are an exception to this and the mechanistic details are well understood. While there has been relatively little mechanistic work for the majority of systems, it is interesting to note the significant differences/similarities in proposed pathways of many of the systems discussed in this review. There seems to be two general mechanistic motifs: (1) the oxidation of the alcohol occurs at the metal center, which can proceed by either one or two electron pathway and involve a β-hydride elimination or β-hydrogen abstraction as a key step and (2) a co-oxidant is responsible for the oxidation of alcohol wherein the metal activates the co-oxidant and alcohol or the metal is responsible for reoxidation of the additive with O_2.

While there has been a tremendous amount of effort applied to the development and improvement of metal-catalyzed aerobic alcohol oxidations, many improvements can be envisioned. Performing aerobic alcohol oxidation under mild conditions (room temperature, air atmosphere) while employing low catalyst loadings should be an important goal of researchers. Additionally, in order for these methods to be used in target synthesis, the scope of the individual systems must be broadened to include more complex alcohols that are synthetically relevant.

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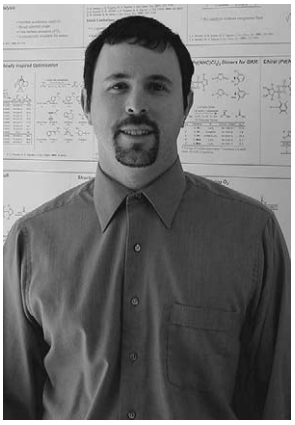
Biographical sketch

Mitchell Schultz received a B.S. in chemistry from Southwest Minnesota State University in 2001 before obtaining his Ph.D. in organic chemistry at the University of Utah under the guidance of Professor Matthew Sigman. He is currently working as a postdoctoral researcher for Professor Jeffrey Moore at the University of Illinois.



Matt Sigman was born in Los Angeles, CA. He received a B.S. in chemistry from Sonoma State University in 1992 before obtaining his Ph.D. in organometallic chemistry at Washington State University with Professor Bruce Eaton in 1996. Following an NIH postdoctoral stint with Professor Eric Jacobsen he joined the faculty of the University of Utah in 1999. His research interests are the development and applications of catalytic methods.

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The application of vinylogous iminium salt derivatives to an efficient synthesis of the pyrrole containing alkaloids Rigidin and Rigidin E

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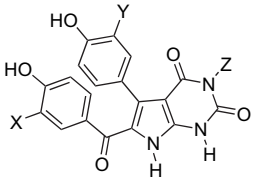
Dedicated to Professor Hiroki Yamanaka on the occasion of his retirement

Abstract—Studies directed on the synthesis of the pyrrole containing marine natural products Rigidin and Rigidin E via vinylogous iminium salts are described. The successful strategy relies on the formation of a 2,4-disubstituted pyrrole from a vinamidinium salt followed by acylation at the 5-position of pyrrole. Halogenation and aminocarbonylation at the 3-position of pyrrole followed by hydrolysis of the ester group at C-2 and subsequent Curtius rearrangement generates the pyrrolopyrimidine skeleton. A final deprotection step completes the synthesis of Rigidin and Rigidin E.

1. Introduction

Marine natural products[1] continue to attract significant attention as a result of their diverse and interesting biological properties. Consequently, synthetic organic chemists continue to develop and explore new synthetic strategies[2] for the efficient and selective preparation of such substances. The pyrrolo[2,3-*d*]pyrimidine skeleton is often encountered in important pharmacologically active substances and more recently it has been observed in a class of marine natural products known as Rigidins[3] (Fig. 1). These alkaloids have been obtained from tunicates obtained near Okinawa and New Guinea and they have been shown to exhibit very significant calmodulin antagonist activity. Edstrom and Wei[4] were the first to report a total synthesis of the parent Rigidin (**1**) and his synthetic sequence is outlined in Scheme 1.



1 X = Y = Z = H Rigidin
2 X = OCH_3 Y = Z = H Rigidin B
3 Y = OCH_3 X = Z = H Rigidin C
4 X = Y = OCH_3 Z = H Rigidin D
5 X = Y = H Z = CH_3 Rigidin E

Figure 1.

Edstrom route begins with the displacement of a 6-chloro group of a 1,3-dibenzyl protected uracil (**6**) by an *N*-benzyl protected glycine to yield the corresponding aminouracil (**7**). Heating this compound (**7**) with acetic anhydride causes cyclization to the acetoxypyrrolopyrimidine (**8**). Base mediated hydrolysis followed by reaction with triflic anhydride

Keywords: Vinamidinium salt; Pyrrole; Marine natural product; Microwave acceleration.
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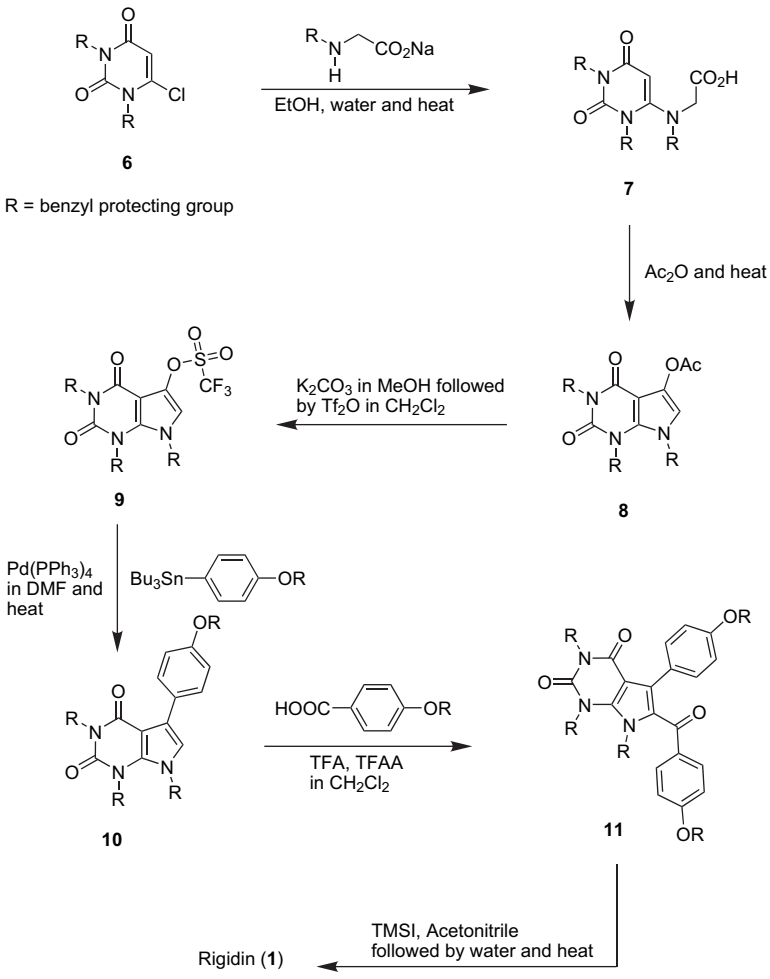


Scheme 1.

produces the triflate derivative (**9**), which then undergoes Stille cross-coupling to give the corresponding arylpyrrolopyrimidine (**10**). Acylation of compound **10** in the presence of trifluoroacetic anhydride/trifluoroacetic acid yields Rigidin analog **11**, which is then deprotected with TMSI to produce Rigidin (**1**) in an overall yield of 26%. Sakamoto and co-workers[5] have reported the only other synthesis of Rigidin (**1**) to date and it is described in Scheme 2.

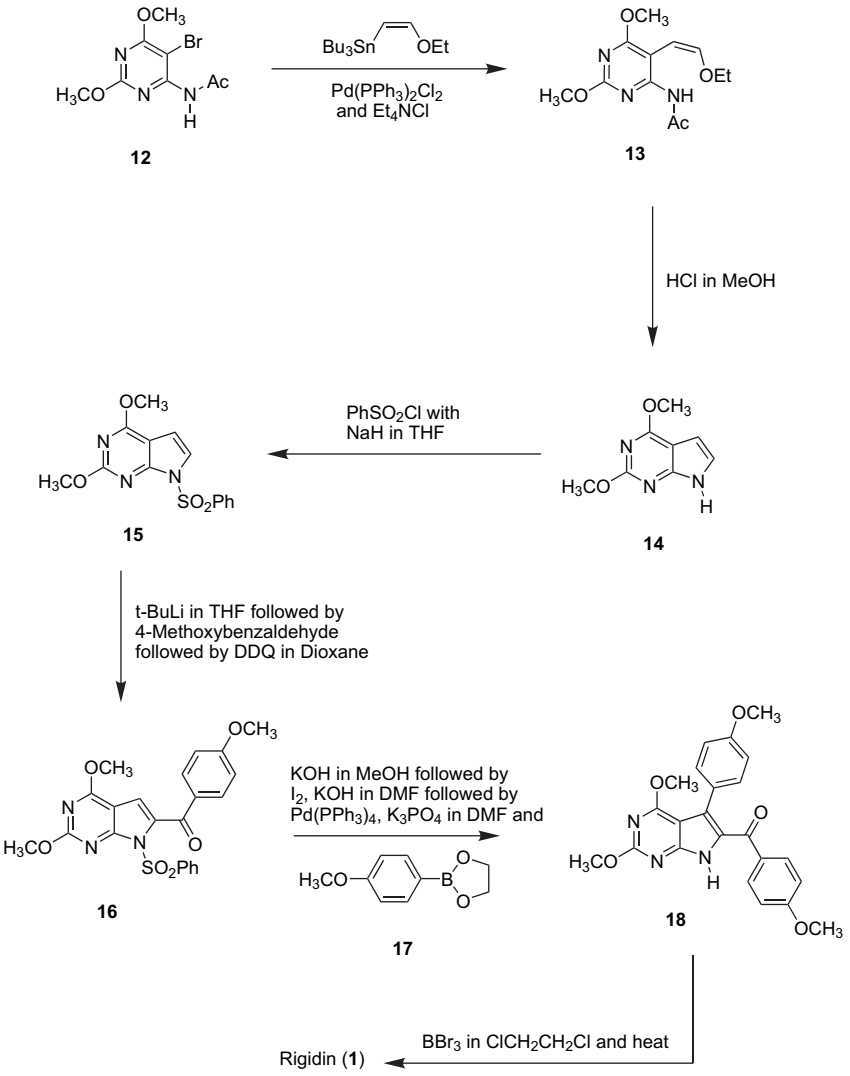
The Sakamoto synthesis begins with a Stille cross-coupling reaction of a highly functionalized bromopyrimidine (**12**) with a vinylstannane, which produces an intermediate (**13**) that is deprotected and cyclized under acidic conditions to yield pyrrolopyrimidine **14**. This pyrrolopyrimidine (**14**) is *N*-protected as the phenylsulfonyl derivative, treated with *t*-butyl lithium at −78 °C, quenched with 4-methoxybenzaldehyde, and oxidized with DDQ in dioxane to yield the corresponding acylated derivative (**16**). Removal of the phenylsulfonyl group from **16** followed by iodination, Suzuki cross-coupling, and deprotection with boron tribromide yielded Rigidin (**1**) in less than 10% overall yield.

2. Results and discussion

In the past several years we have utilized disubstituted β-chloroenals[6] (**19**) as building blocks for the preparation of 2,3,4-trisubstituted pyrroles (**20**), which served as precursors (Scheme 3) for the pyrrole containing natural products Lamellarin O (**21**), Lukianol A (**22**), and Ningalin B (**23**). A key reaction[7] involved the condensation of amino acid esters with the disubstituted β-chloroenals to produce the desired highly functionalized pyrroles.

More recently we have opted for a different strategy, which utilizes 2,4-disubstituted pyrroles[8] as the key building blocks for the preparation of pyrrole containing natural products Polycitone A and B.[9] We now describe our efforts toward the synthesis of Rigidin and Rigidin E as they relate to these two related pathways. Our initial approach was conceived a number of years ago and is depicted in Scheme 4. An aryl keto ester (**24a**) was converted in good yield (95%) to the corresponding vinylogous amide (**25a**) with DMF acetal. The initial studies were carried out with the aryl groups being phenyl. This vinylogous amide (**25a**) was then treated with either the hydrochloride salt or PTSA salt of an α-aminoketone in which case an amine exchange reaction occurred in good yield (92%) to produce the corresponding vinylogous amide (**26**).

This vinylogous amide (**26**) was then subjected to a variety of acid mediated cyclization conditions and these trials are described in Table 1. It was observed that PTSA in EtOH gave reasonable yields (56%) of the desired



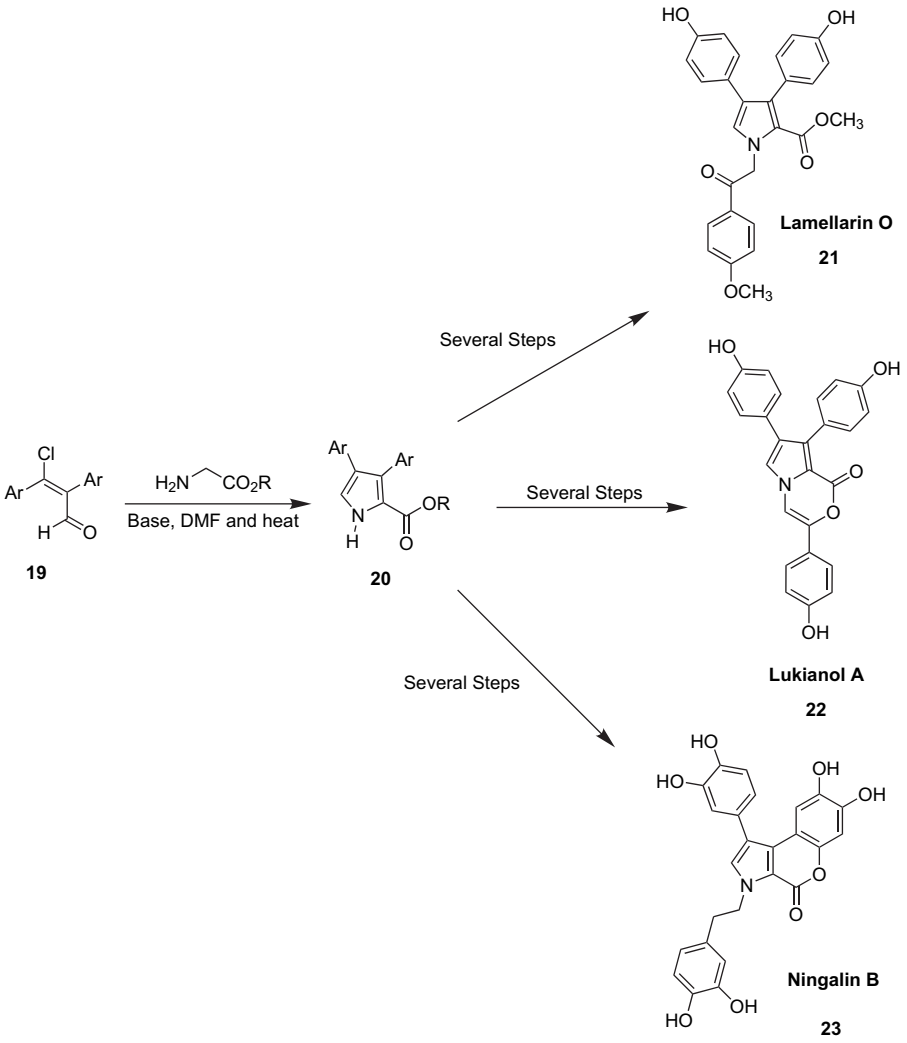
Scheme 2.

2,3,4-trisubstituted pyrrole (**27a**) and that the primary reaction byproduct (keto ester **24a**) resulted from hydrolysis of the vinylogous amide (**26**) starting material. The 2,3,4-trisubstituted pyrrole (**27a**) was then *N*-methylated under basic conditions to produce pyrrole **29**, which was subjected to NMR NOE studies that verified the indicated regiochemistry (see Section 4 for details). The 2,3,4-trisubstituted pyrrole (**27a**) was then nitrated in 35% yield at the 5-position and also brominated in 85% yield at the 5-position. It was anticipated that this tetrasubstituted pyrrole (**28a**) containing the 5-nitro group could then be converted to the pyrrolopyrimidine skeleton by reduction of the nitro group to an amino group followed by reaction with TMS isocyanate.

During the course of these studies, we developed an alternative route to the desired trisubstituted pyrrole (**27b**) and this is presented in Scheme 5.

The vinylogous amide (**25b**) was prepared in the usual manner (79% yield) with DMF acetal and this material was then treated with phosphorous oxychloride in dichloromethane followed by hydrolysis with water/THF to give a β-chloroenal (**30**) as a mixture of *E*- and *Z*-stereoisomers (87% yield) as reported for a similar case in our synthesis[6b] of Ningalin B hexamethyl ether. The crude mixture of isomers (**30**) could be used for the next step involving condensation with the PTSA salt of α-amino-4-methoxyacetophenone to yield the desired 2,3,4-trisubstituted pyrrole (**27b**) in 48% yield. Interestingly, this reaction can be carried out somewhat more efficiently under microwave accelerated conditions to produce the desired material (**27b**) in about the same yield (45%). It should be noted that this condensation reaction is carried out in the absence of external base. When the reaction was repeated using sodium hydride and DMF, a different isomeric trisubstituted pyrrole (**31**) was obtained (Scheme 6) in good yield (86%).

Both compounds **31** and **27b** were subjected to appropriate NOE NMR experiments (see Section 4 for details) to verify the regiochemical assignments. It appears that under neutral conditions an imine is formed by reaction of β-chloroenal (**30**) with glycine in the first step followed by cyclization, whereas under basic conditions the displacement of the chlorine of β-chloroenal (**30**) by the amino group of the aminoketone takes place first followed by ring closure. The ability to tune this regiochemical outcome by the presence or

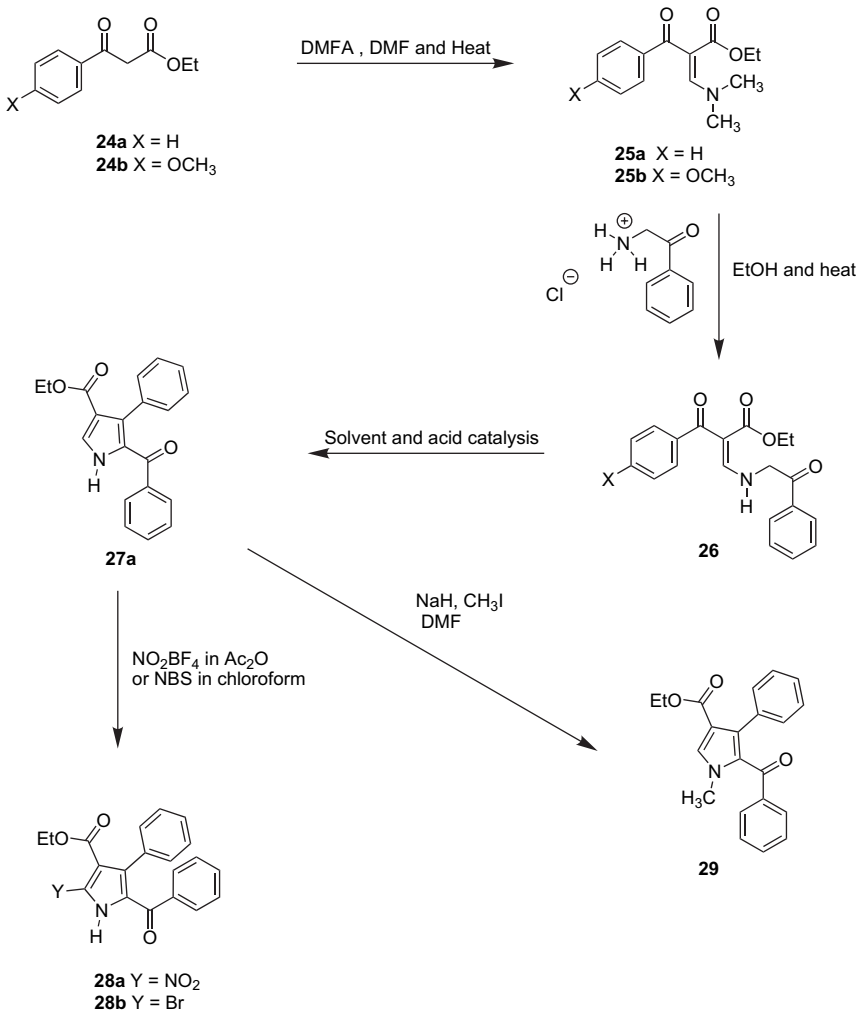


Scheme 3.

absence of strong base is quite remarkable. With the ability to generate a significant amount of the desired 2,3,4-trisubstituted pyrrole (**27b**), we turned our attention to the nitration at the 5-position of the pyrrole. After the examination of many nitration conditions including those described in Scheme 4 for the nitration of the phenyl analog, no 5-nitro analog of compound **27b** could be obtained. NMR analysis of the crude reaction products suggested that preferential nitration of the methoxyphenyl group at C-3 of the pyrrole was occurring as the primary reaction pathway.

More recently, we have successfully utilized a somewhat different strategy[9] to construct an efficient relay synthesis of the marine natural products Polycitone A and B, which is presented in Scheme 7. Pyrrole **37** was the key intermediate prepared by Steglich and co-workers[10] for the synthesis of the Polycitone natural products. Our strategy involved the use of a symmetrical vinamidinium salt (**32**) for the preparation of 2,4-disubstituted pyrrole (**33**), which could be acylated at the 5-position with 4-methoxybenzoic acid to produce 2,3,5-trisubstituted system (**34**). Bromination or iodination of this pyrrole (**34**) produced the 4-halogenated compound (**35a** or **35b**). Subsequent Suzuki cross-coupling of this halopyrrole (**35a** or **35b**) followed by ester hydrolysis and Friedel–Crafts acylation yielded the 'Steglich synthon' (**37**) for Polycitone A and B in very good overall yield (42%) with each of the individual steps being high yield reactions (>75%).

We recently recognized that pyrroles **35a** or **35b** should be well suited for the preparation of Rigidins if a carboxamide group could be introduced at the halogen bearing carbon from which a uracil ring could be constructed. This strategy is represented in Scheme 8. Upon subjecting our tetrasubstituted pyrrole (**35a**) from the Polycitone synthesis to conditions described by Larhed and Wannenberg[11] for microwave accelerated aminocarbonylation reactions, it was possible to produce either an *N*-2,4-dimethoxybenzylamide (80% yield) or an *N*-methyl amide (65% yield) depending upon which amine trapping agent was used. Both amidoesters (**38a** and **38b**) could be efficiently hydrolyzed to the desired carboxylic acids (**39a** in 89% yield and **39b** in 80% yield). The resulting acids (**39a** and **39b**) were subjected to Curtius rearrangement conditions[12] whereby the carboxylic acid groups were converted to an isocyanate followed by trapping with the neighboring amide group to generate the uracil skeleton (**40a** in 69% yield or **40b** in 53% yield). Removal of the *O*-methyl and *N*-benzyl groups from the respective pyrrolopyrimidines (**40a** or **40b**) via boron tribromide produced products, which were spectroscopically identical to Rigidin (**1** in 96% yield) and Rigidin E (**5** in 41% yield)



Scheme 4.

thereby completing a total synthesis of these pyrrole containing marine natural products.

3. Conclusions

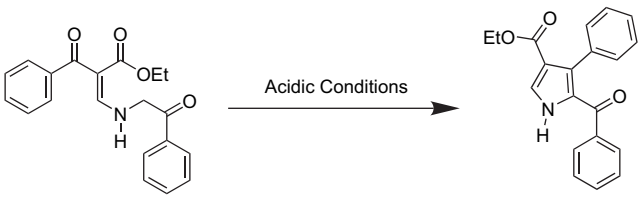
In summary, we have demonstrated a new, 'pyrrole first approach' to an important family of bioactive, pyrrole containing marine natural products. This was accomplished by constructing 2,4-disubstituted pyrroles from vinamidinium salts, electrophilically substituting the 5-position of the pyrrole followed by halogenation and a microwave accelerated, palladium mediated, aminocarbonylation reaction at the 3-position. The resulting amide group, which is proximate to the 2-carboxyl group, is transformed to a uracil via a Curtius rearrangement. A subsequent deprotection step leads to the desired natural products. It is important to note that each pyrrole substituent is introduced independently and can be easily varied so as to accommodate in depth SAR studies for Rigidin analogs. The seven-step syntheses of Rigidin and Rigidin E, from the readily available 2,4-disubstituted pyrrole (**33**) was accomplished in 40 and 10% overall yields, respectively. We are currently in the process of applying this same strategy to other important pyrrole containing marine natural products.

4. Experimental

4.1. General

All chemicals were used as received from the manufacturer (Aldrich Chemicals and Fisher Scientific) and all reactions were carried out under a nitrogen or argon atmosphere. All solvents were dried over 4 Å molecular sieves prior to their use. NMR spectra were obtained either on a GE Omega 300 MHz spectrometer, a Bruker 500 MHz spectrometer or a Varian Gemini 200 MHz spectrometer either in $CDCl_3$, DMSO-d_6 or acetone-d_6 solutions. IR spectra were recorded on a Nicolet Avatar 320 FTIR spectrometer with an HATR attachment or a Perkin–Elmer 1600 series FTIR spectrometer. High-resolution mass spectra were provided by the Midwest Center for Mass Spectrometry at the University of Nebraska at Lincoln or on a Biotof Q electrospray mass spectrometer. Low resolution GC–MS spectra were obtained on a Shimadzu QP 5050 instrument. Melting points and boiling points are uncorrected. Radial chromatographic separations were carried out on a Harrison Chromatotron using silica gel plates of 2 mm thickness with a fluorescent backing using ethyl acetate/hexane as the eluant. Flash chromatographic separations were carried out on a Biotage Horizon HFC or SP-1 instrument, which had been equipped

Table 1. Acid mediated cyclization results



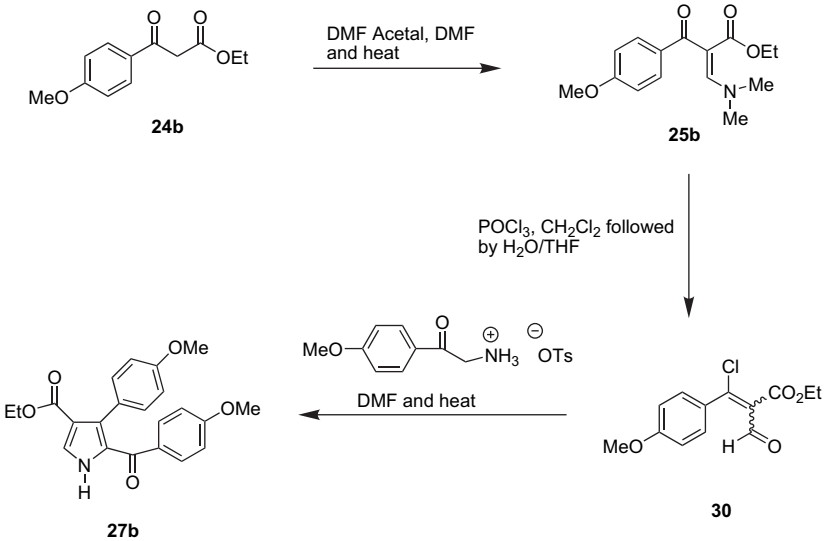
26 **27a**

Trial	Acid	Solvent	Yield (%)	Determination
1	Acetic acid	Acetic acid	48	Isolated yield
2	PTSA	$CHCl_3$	43	Isolated yield
3	PTSA	EtOH	56	Isolated yield
4	PTSA	CH_3CN	50	Isolated yield
5	PTSA	Dioxane	46	Isolated yield
6	PTSA/TFA	TFAA/$CHCl_3$	42	Isolated yield
7	$POCl_3$	$CHCl_3$	39	Isolated yield
8	Amberlyst	$CHCl_3$	Trace	NMR, TLC
9	$ZnCl_2$	$CHCl_3$	Trace	NMR, TLC
10	PPA	$CHCl_3$	Trace	NMR, TLC
11	TFA	TFAA/$CHCl_3$	Trace	NMR, TLC
12	TFA	TFAA/EtOH	Trace	NMR, TLC
13	TFA	EtOH	Trace	NMR, TLC
14	Nafion	$CHCl_3$	0	NMR, TLC
15	$AlCl_3$	$CHCl_3$	0	NMR, TLC
16	$MeO_3{}^+BF_4{}^-$	$CHCl_3$	0	NMR, TLC
17	BF_3Et_2O	$CHCl_3$	0	NMR, TLC

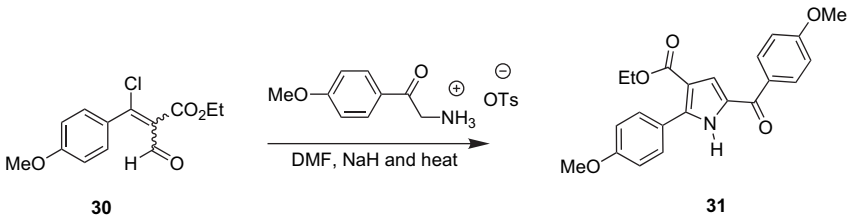
with a silica cartridge, and ethyl acetate/hexane was used as the eluant. TLC analyses were conducted on silica plates with hexane/ethyl acetate as the eluant. Vinamidinium salts utilized for pyrrole formation were prepared according to standard procedures.[7] All purified reaction products gave TLC results, GC–MS spectra, flash chromatograms, and ^{13}C NMR spectra consistent with a sample purity of >95%.

4.1.1. 2-Benzoyl-3-dimethylaminoacrylic acid ethyl ester (25a). A 250-mL, three-neck, round-bottom flask was equipped with a stir bar and a reflux condenser. Into the flask were placed 8.00 g (0.0416 mol) of ethyl benzoyl acetate, 14.70 g (0.167 mol) of *N*,*N*-dimethylformamide dimethyl acetal (DMFA), and 150 mL of DMF. The reaction mixture was heated at 75–80 °C overnight. The DMF and unreacted DMFA were removed in vacuo, yielding a yellow oil (9.76 g, 95% yield). An analytical sample was obtained by radial chromatography using an 80:20 mixture of hexane/ethyl acetate as eluent. After removal of solvent, a light yellow oil was obtained, which exhibited the following physical properties: bp 30 °C at 0.1 mm of Hg; ^1H NMR ($CDCl_3$) δ 7.60–7.80 (m, 3H), 7.30–7.50 (m, 3H), 3.95 (q, J=7.0 Hz, 2H), 2.60–3.20 (br s, 6H), and 0.87 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 15.9, 32.9, 61.7, 129.9, 130.4, 130.5, 130.8, 133.7, 157.9, 170.8, and 196.2; IR (CCl_4) 1740 and 1688 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{14}H_{17}NO_3$ 247.1208, found 247.1204.

4.1.2. 2-Benzoyl-3-(2-oxo-2-phenylethylamino)acrylic acid ethyl ester (26). A one-neck, 500-mL round-bottom flask was equipped with a stir bar and a reflux condenser. Into the flask were placed 5.00 g (20.2 mmol) of 2-benzoyl-3-dimethylaminoacrylic acid ethyl ester (**25a**), 3.82 g (22.2 mmol) of α-aminoacetophenone hydrochloride, and 200 mL of ethanol. The mixture was refluxed overnight. The solvent was removed in vacuo and the residue was taken up in 100 mL of chloroform and washed with 2×50 mL of water. The chloroform phase was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo, yielding 6.53 g (96% yield) of a yellow solid. The product was purified by dissolving it in 50 mL of ethyl acetate and passing through a short plug of silica gel, followed by recrystallization with an 80:20 mixture of hexane/ethyl acetate, yielding 6.26 g (92% yield) of a white solid. This material exhibited the following physical properties: mp 148–149 °C; ^1H NMR ($CDCl_3$) δ 10.75 (br s, 0.5H), 9.50 (br s, 0.5H), 7.86–8.20 (m, 3H), 7.31–7.70 (m, 8H), 4.88–4.91 (m, 2H), 3.96–4.1 (m, 2H), and 0.94 (t, J=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 15.7, 15.8, 56.3, 56.6, 61.6, 61.7, 103.4, 103.7, 129.2, 129.5, 129.6, 129.9, 130.2, 131.1, 131.9, 132.6, 136.0, 136.1, 136.3, 143.6, 144.4, 161.0, 162.0, 169.9, 170.7, 194.2, 194.5, 196.2, and 198.1; IR (CCl_4) 1678 and



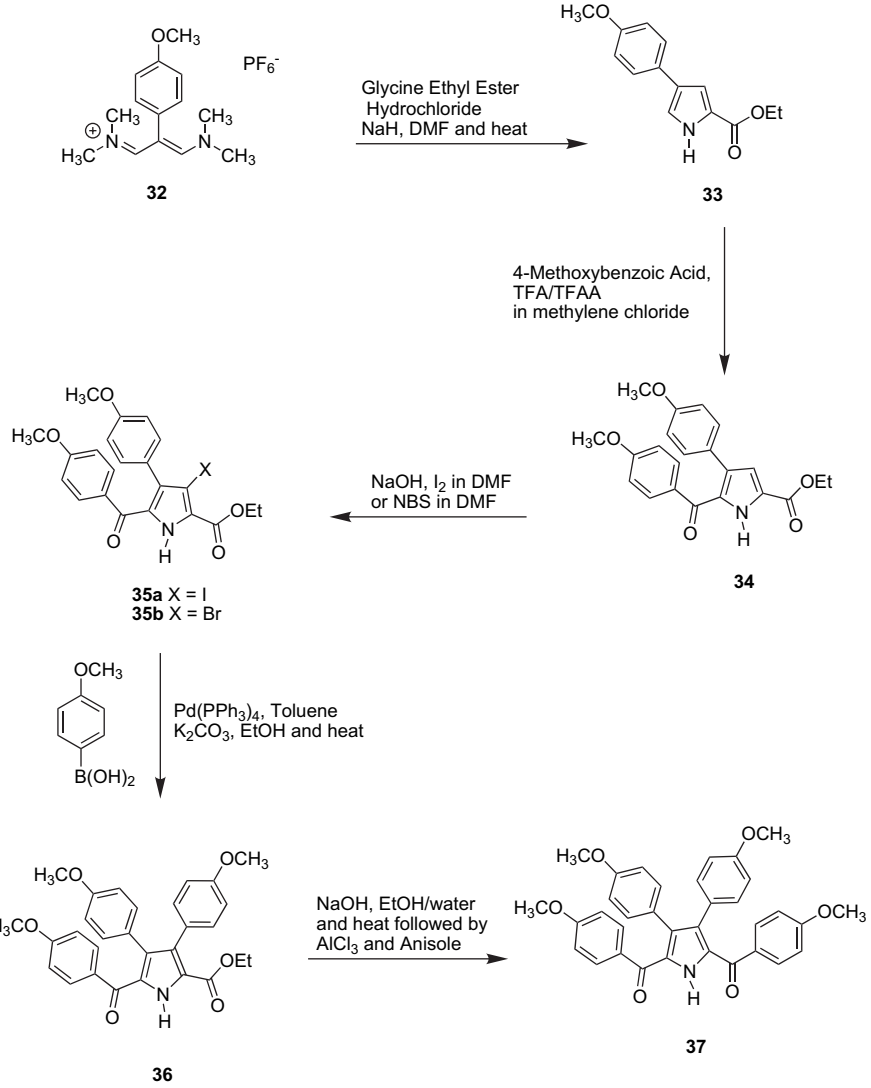
Scheme 5.



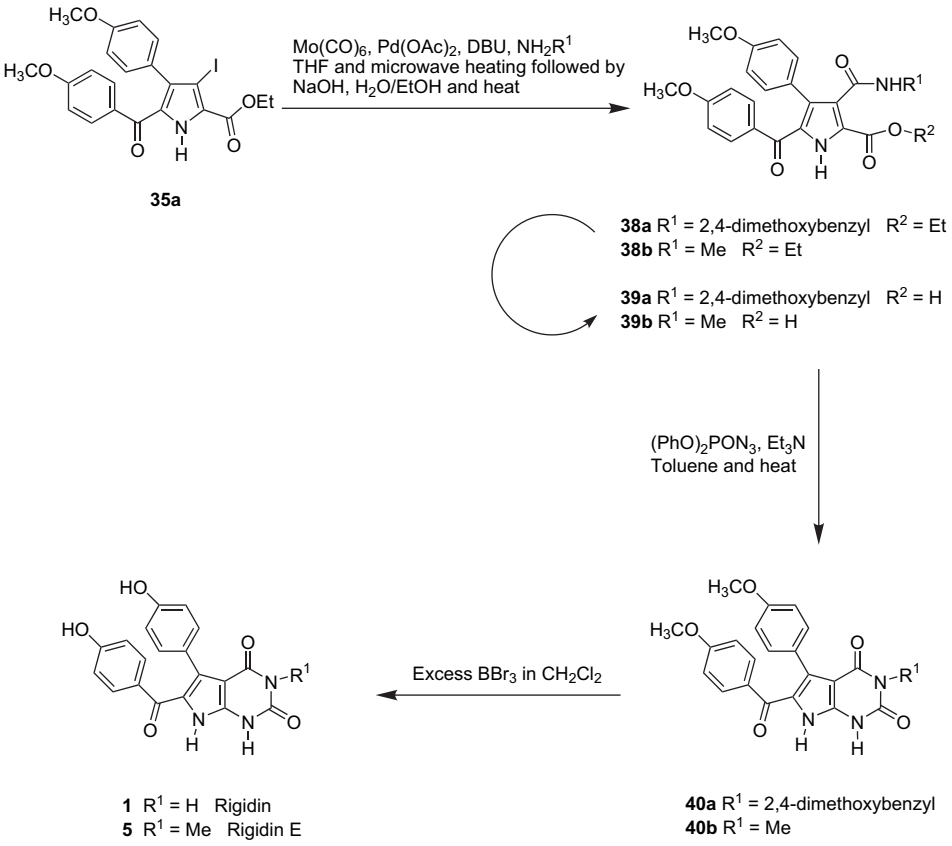
Scheme 6.

1620 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{20}H_{19}NO$ 337.1314, found 337.1292.

4.1.3. 2-Benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (27a). Into a one-neck, 200-mL round-bottom flask were placed 0.800 g (2.37 mmol) of 2-benzoyl-3-(2-oxo-2-phenylethylamino)acrylic acid ethyl ester (**26**), 0.368 g (2.37 mmol) of phosphorous oxychloride, and 80 mL of dry chloroform. The reaction mixture was refluxed for 1 h. The solvent was removed in vacuo and the residue was partitioned between chloroform (50 mL) and saturated aqueous sodium bicarbonate solution (50 mL). The chloroform phase was dried over anhydrous magnesium sulfate, filtered, and concentrated, yielding 0.730 g of a brown semi-solid. The crude material was dissolved in ethyl acetate (30 mL) and eluted through a short plug of silica gel. The product was further purified by radial chromatography using an 80:20 mixture of hexane/ethyl acetate as eluent. A 0.318 g sample (42% yield) of a light yellow solid was obtained, which exhibited the following properties: mp 141–142 °C; ^{1}H NMR (DMSO-d_6) δ 12.58 (br s, 1H), 7.73 (d, J=3.5 Hz, 1H), 7.2–7.40 (m, 3H), 7.00–7.18 (m, 7H), 4.06 (q, J=7.1 Hz, 2H), and 1.11 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 16.2, 62.0, 118.9, 129.0, 129.1, 129.4, 130.8, 130.9,



Scheme 7.



Scheme 8.

131.7, 133.1, 133.4, 135.0, 135.4, 139.1, 165.9, and 190.0; IR (CCl_4) 3246, 1718, and 1610 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{20}H_{17}NO_3$ 319.1208, found 319.1194.

4.1.4. 2-Benzoyl-1-methyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (29). A 100-mL, three-neck, round-bottom flask was equipped with a magnetic stir bar and placed under a nitrogen atmosphere. Into the flask was placed 0.291 g (7.27 mmol) of a 60% mineral oil dispersion of sodium hydride. The dispersion was washed with hexane and the hexane was removed via cannula. To the flask were added 40 mL of dry DMF, 0.400 g (1.25 mmol) of 2-benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (**27a**), and 2.890 g (20.4 mmol) of iodomethane. The mixture was stirred overnight at room temperature. The solvent was removed by Kugelrohr distillation and the residue was taken up in 50 mL of chloroform and washed with 2×30 mL of water. The chloroform phase was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was dissolved in 50 mL of ethyl acetate and passed through a short plug of silica gel. After removal of solvent, 0.409 g of a light yellow solid (98% yield) was obtained, which exhibited the following properties: mp 146–147 °C; ^{1}H NMR (DMSO-d_6) δ 7.91 (s, 1H), 7.42 (d, J=7.0 Hz, 2H), 7.30 (t, J=7.2 Hz, 1H), 6.96–7.18 (m, 7H), 4.08 (q, J=7.1 Hz, 2H), 3.85 (s, 3H), and 1.13 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 16.2, 39.3, 61.8, 115.4, 128.9, 129.5, 131.6, 132.2, 133.1, 133.9, 135.3, 135.7, 140.1, 165.8, and 191.0; IR (CCl_4) 1706 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{21}H_{19}NO_3$ 333.1365, found 333.1353. NOEDIF ($CDCl_3$): irradiating at 3.96 ppm (*N*-methyl hydrogens), an NOE was observed at 7.56 ppm (α-pyrrole hydrogen); irradiating at 7.56 ppm (α-pyrrole hydrogen), an NOE was observed at 3.96 ppm (*N*-methyl hydrogens).

4.1.5. 2-Benzoyl-5-bromo-3-phenylpyrrole-4-carboxylic acid ethyl ester (28b). A one-neck, 250-mL, round-bottom flask was equipped with a stir bar and a reflux condenser. Into the flask were placed 0.600 g (1.88 mmol) of 2-benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (**27a**), 0.336 g (1.88 mmol) of *N*-bromosuccinimide, and 120 mL of dry chloroform. The reaction mixture was refluxed for 6 h. The solvent was removed in vacuo and the residue was partitioned between chloroform (60 mL) and saturated aqueous sodium bicarbonate (30 mL) solution. The chloroform phase was dried over anhydrous magnesium sulfate and the solvent was removed in vacuo yielding 0.690 g (92% yield) of a solid. The crude material was dissolved in ethyl acetate and eluted through a short column of silica gel. The chromatographed material was further purified by recrystallization with a mixture of 80:20 hexane/ethyl acetate yielding a bright yellow solid (0.64 g, 85% yield), which exhibited the following properties: mp 155–156 °C; ^{1}H NMR (DMSO-d_6) δ 7.37 (d, J=7.9 Hz, 2H), 7.20–7.32 (m, 1H), 6.90–7.19 (m, 7H), 4.01 (q, J=7.0 Hz, 2H), and 0.96 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 15.8, 62.4, 114.5, 118.4, 129.1, 129.3, 129.4, 130.8, 131.8, 132.8, 133.5, 135.0, 136.0, 138.6, 165.0, and 188.8; IR (CCl_4) 3210, 1717, and 1612 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{20}H_{17}BrNO_3$ 397.0313, found 397.0301.

4.1.6. 2-Benzoyl-5-nitro-3-phenylpyrrole-4-carboxylic acid ethyl ester (28a). A 100 mL, three-neck, round-bottom flask was equipped with a magnetic stir bar, chilled to −78 °C in an isopropanol/dry ice slurry, and placed under a nitrogen atmosphere. Into the flask were placed 0.200 g (0.630 mmol) of 2-benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (**27a**) and 5 mL of acetic anhydride. Subsequently, 0.443 g (3.34 mmol) of nitronium tetrafluoroborate was dissolved in 15 mL of cold acetic anhydride solution and added dropwise to the reaction flask through an addition funnel. The mixture was stirred for 2 h at −78 °C, followed by room temperature stirring for 4 h. The reaction mixture was then diluted with water with cooling. The resulting reaction mixture was stirred for 2 h at room temperature and was then extracted with chloroform (3×30 mL). The combined chloroform extracts were washed with saturated aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was dissolved in ethyl acetate and eluted through a short plug of silica gel. The product was further purified by radial chromatography using a 70:30 mixture of hexane/ethyl acetate as eluent. After removal of solvent from the chromatography fractions, 0.090 g (35% yield) of a light yellow solid was obtained, which exhibited the following properties: mp 132–133 °C; ^{1}H NMR ($CDCl_3$) δ 12.58 (br s, 1H), 7.47 (dd, J=8.0, 1.1 Hz, 2H), 7.22–7.35 (m, 1H), 7.00–7.18 (m, 7H), 4.30 (q, J=7.1 Hz, 2H), and 1.21 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 15.8, 64.3, 119.6, 129.9, 130.1, 130.2, 131.2, 131.5, 132.0, 132.2, 132.8, 135.0, 137.3, 137.5, 164.5, and 189.1; IR (CCl_4) 3410, 1739, and 1638 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{20}H_{16}N_2O_5$ 364.1059, found 364.1070.

4.1.7. 2-Benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (acetic acid catalysis) (27a). Into a one-neck, 50 mL, round-bottom flask were placed 0.200 g (0.594 mmol) of 2-benzoyl-3-(2-oxo-2-phenylethylamino)acrylic acid ethyl ester (**26**) and 20 mL of acetic acid. The reaction mixture was refluxed overnight and then acetic acid was removed in vacuo. The residue was partitioned between chloroform and a saturated aqueous sodium bicarbonate solution and the chloroform phase was dried over anhydrous magnesium sulfate, filtered, and concentrated. The crude material was dissolved in ethyl acetate and purified by radial chromatography using hexane/ethyl acetate yielding 0.091 g (48% yield) of a light yellow solid (48% yield), which exhibited identical physical properties to the 2-benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester reported in Section 4.1.3.

4.1.8. 2-Benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester (PTSA catalysis) (27a). Into a one-neck, 50 mL, round-bottom flask were placed 0.200 g (0.594 mmol) of 2-benzoyl-3-(2-oxo-2-phenylethylamino)acrylic acid ethyl ester (**26**), 0.110 g (0.653 mmol) of PTSA, and 20 mL of anhydrous ethanol. The reaction mixture was refluxed overnight and the solvent was removed in vacuo. The residue was then partitioned between water and chloroform. The chloroform layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was dissolved in ethyl acetate and purified by radial chromatography using hexane/ethyl acetate yielding a light yellow solid (0.106 g, 56% yield), which exhibited identical physical properties to the 2-benzoyl-3-phenylpyrrole-4-carboxylic acid ethyl ester reported in Section 4.1.3.

4.1.9. 2-(4-Methoxybenzoyl)-3-dimethylaminoacrylic acid ethyl ester (25b). A 100 mL, one-neck, round-bottom flask was charged with 30 mL of DMF, 3-oxo-3-(4-methoxyphenyl)propionic acid ethyl ester (**24b**) (2.22 g, 0.0100 mol), and *N*,*N*-dimethylformamide dimethyl acetal (4.77 g, 0.0400 mol). The reaction mixture was heated at reflux with stirring for 24 h and subsequently cooled to room temperature. The reaction mixture was diluted with 100 mL of ethyl acetate and 50 mL of water. The aqueous layer was extracted with additional ethyl acetate (3×50 mL) and the combined ethyl acetate phases were washed with brine (3×20 mL) and dried over anhydrous magnesium sulfate. After removal of the drying agent by vacuum filtration, the filtrate was concentrated in vacuo leaving a dark oil (2.18 g, 79% yield), which could be used without further purification. An analytical sample was prepared by taking 1.00 g of the reaction product and subjecting it to chromatographic separation on a Biotage Horizon flash chromatography system with a gradient elution of ethyl acetate/hexanes. A yellow oil was obtained, which exhibited the following properties: bp 79–80 °C at 0.1 mm of Hg; ^{1}H NMR ($CDCl_3$) δ 7.60–7.85 (m, 3H), 6.88 (d, J=8.8 Hz, 2H), 3.98 (q, J=7.0 Hz, 2H), 3.84 (s, 3H), 2.91 (br s, 6H), and 0.96 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 14.1, 55.4, 59.6, 99.7, 113.2, 131.3, 133.7, 154.7, 162.7, 168.7, and 193.1; IR (neat) 1683 and 1601 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{15}H_{19}NO_4$ 278.1392, found 278.1440.

4.1.10. 3-Chloro-2-formyl-3-(4-methoxyphenyl)acrylic acid ethyl ester (30). A round-bottom flask was charged with 2-(4-methoxybenzoyl)-3-dimethylaminoacrylic acid ethyl ester (**25b**) (2.00 g, 7.22 mmol) dissolved in 50 mL of dry methylene chloride. To the stirred solution was added phosphorous oxychloride (1.68 g, 10.9 mmol) and the resulting mixture was heated at reflux for 1 h. The reaction mixture was cooled to room temperature and the solvent was removed in vacuo. The residue was then taken up in 50 mL of a 50:50 mixture of water/THF and stirred for 4 h in a round-bottom flask equipped with a stopper. The reaction mixture was subsequently diluted with 100 mL of water and extracted with 3×50 mL of ethyl acetate. The combined organic layers were then washed with brine (2×50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo yielding a dark viscous oil (1.68 g, 87% yield). An analytical sample was prepared by taking a 1.30 g sample of the crude product and subjecting it to flash chromatography using an ethyl acetate/hexane gradient elution in which case 0.75 g (50% yield) of a yellow oil was obtained. This oil exhibited the following properties: (major isomer) bp 107–109 °C at 0.3 mm of Hg; ^{1}H NMR ($CDCl_3$) δ 9.34 (s, 1H), 7.46 (d, J=9.0 Hz, 2H), 6.98 (d, J=9.0 Hz, 2H), 4.35 (q, J=7.0 Hz, 2H), 3.89 (s, 3H), and 1.38 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 14.2, 55.7, 62.2, 114.4, 126.3, 130.4, 132.2, 155.5, 162.9, 164.5, and 186.7; IR (neat) 1730, 1670, and 1600 cm^{-1}; HRMS (ES, M+H) *m/z* calcd for $C_{13}H_{14}O_4Cl$ 269.0581, found 269.0591.

4.1.11. 2-(4-Methoxybenzoyl)-3-(4-methoxyphenyl)pyrrole-4-carboxylic acid ethyl ester (27b). Method A: a round-bottom flask was equipped with a reflux condenser and magnetic stir bar and was placed under a nitrogen atmosphere. The flask was charged with 200 mL of DMF and

5.41 g (20.18 mmol) of 3-chloro-2-formyl-3-(4-methoxyphenyl)acrylic acid ethyl ester (**30**). 2′-Amino-4-methoxyacteophenone *p*-toluenesulfonic acid salt[9] (6.90 g, 20.57 mmol) was then added and the reaction mixture was stirred at room temperature for 30 min and then heated at reflux for 20 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The crude residue was chromatographed on a silica gel column using ethyl acetate/hexane gradient elution in which case 3.64 g (48% yield) of a tan solid was obtained. This solid exhibited the following physical properties: mp 151–152 °C; ^{1}H NMR ($CDCl_3$) δ 10.10 (br s, 1H), 7.72 (d, J=3.3 Hz, 1H), 7.37 (d, J=9.0 Hz, 2H), 7.03 (d, J=9.0 Hz, 2H), 6.61 (d, J=9.0 Hz, 2H), 6.52 (d, J=9.0 Hz, 2H), 4.19 (q, J=7.0 Hz, 2H), 3.72 (s, 3H), 3.70 (s, 3H), and 1.21 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 14.4, 55.3, 55.4, 56.0, 112.9, 113.0, 116.7, 125.8, 128.7, 129.7, 130.1, 131.7, 132.6, 132.7, 159.0, 162.6, 164.3, and 187.1; NOEDIF ($CDCl_3$): irradiating at 7.37 ppm (*o*-benzoyl hydrogen), NOEs were observed at 10.10 ppm (pyrrole N–H) and 7.03 ppm (*o*-methoxyphenyl hydrogen); IR (KBr) 3280 and 1680 cm^{-1}; HRMS (ES, M+H) *m*/*z* calcd for $C_{22}H_{22}NO_5$ 380.1498, found 380.1517.

Method B: a 7 mL microwave reaction vessel equipped with a stir bar was charged with 6 mL of DMF, 0.220 g (0.821 mmol) of 3-chloro-2-formyl-3-(4-methoxyphenyl)-acrylic acid ethyl ester (**30**), and 0.331 g (0.982 mmol) of 2′-amino-4-methoxyacteophenone *p*-toluenesulfonic acid salt.[9] The reaction vessel was sealed (Crymper-seal) and heated under microwaves at 150 °C for 14 min in a Liberator Microwave Reactor. After cooling to room temperature, the reaction mixture was diluted with 20 mL of water and extracted with 3×20 mL of ethyl acetate. The combined organic layers were washed with 3×20 mL of brine and dried over anhydrous magnesium sulfate. The resulting solution was filtered, concentrated in vacuo, and subjected to flash chromatography using an ethyl acetate/hexane gradient yielding 0.140 g (45% yield) of a tan solid, which was identical by NMR and TLC comparison with the material prepared by method A.

4.1.12. 5-(4-Methoxybenzoyl)-2-(4-methoxyphenyl)pyrrole-3-carboxylic acid ethyl ester (31). A three-neck round-bottom flask was placed under a nitrogen atmosphere and sodium hydride (0.47 g, 1.96 mmol) mineral oil dispersion was placed in the flask along with 80 mL of dry DMF. A mixture of 3-chloro-2-formyl-3-(4-methoxyphenyl)acrylic acid ethyl ester (**30**) (0.966 g, 3.60 mmol) and 2′-amino-4-methoxyacteophenone *p*-toluenesulfonic acid salt (1.286 g, 3.82 mmol) in 80 mL of dry DMF was prepared in a second flask and this mixture was stirred for 30 min. The 3-chloro-2-formyl-3-(4-methoxyphenyl)acrylic acid ethyl ester (0.966 g, 3.60 mmol)/2′-amino-4-methoxyacteophenone *p*-toluenesulfonic acid salt mixture in DMF was added dropwise to the reaction vessel containing sodium hydride/DMF and the resulting mixture was refluxed for 12 h. After cooling to room temperature, the reaction mixture was quenched with 5 mL of methanol and concentrated in vacuo. The residue was partitioned between water (50 mL) and methylene chloride (3×40 mL) and the combined methylene chloride extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The resulting residue was dissolved in a minimum amount of ethyl acetate and placed on a short plug of silica gel. The plug was washed with several portions of ethyl acetate and the combined washings were concentrated in vacuo to give a tan solid (1.174 g, 86% yield), which exhibited the following properties: mp 208–209 °C; ^{1}H NMR ($CDCl_3$) δ 9.66 (br s, 1H), 7.95 (d, J=8.0 Hz, 2H), 7.63 (d, J=8.0 Hz, 2H), 7.37 (d, J=2.6 Hz, 1H), 7.01 (d, J=8.0 Hz, 2H), 6.97 (d, J=8.0 Hz, 2H), 4.25 (q, J=8.0 Hz, 2H), 3.91 (s, 3H), 3.86 (s, 3H), and 1.28 (t, J=8.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 14.3, 55.3, 55.5, 60.0, 113.6, 113.7, 114.1, 121.5, 123.0, 129.7, 130.2, 130.7, 131.3, 142.2, 160.4, 163.1, 164.2, and 183.4; NOEDIF ($CDCl_3$): irradiating at 7.87 ppm (*o*-benzoyl hydrogen), NOEs were observed at 9.66 ppm (pyrrole N–H) and 7.37 ppm (pyrrole C-4 hydrogen); IR (Nujol) 3250 and 1705 cm^{-1}; HRMS (ES, M+H) *m*/*z* calcd for $C_{22}H_{22}NO_5$ 380.1498, found 380.1499.

4.1.13. 3-(2,4-Dimethoxybenzylcarbamoyl)-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid ethyl ester (38a). Into a 7 mL microwave reaction vessel, which had been equipped with a magnetic stir bar, was placed molybdenum hexacarbonyl (0.261 g, 0.99 mmol), palladium(II) acetate (0.066 g, 0.099 mmol), 3-iodo-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid ethyl ester[9] (**35a**) (0.500 g, 0.99 mmol), and tetrahydrofuran (6 mL). Then 1,8-diazabicyclo[5.4.0]undec-7-ene (0.445 mL, 2.97 mmol) and 2,4-dimethoxybenzylamine (0.446 mL, 2.97 mmol) were quickly added and the vial was capped. The reaction mixture was stirred for 5 min and subjected to microwave irradiation for 40 min at 100 °C (15–20 W). The reaction mixture was filtered through a plug of sand, silica, and Celite and then the plug was washed with tetrahydrofuran (50 mL). The filtrate was concentrated in vacuo to give a viscous red oil. The residue was adsorbed onto silica gel and purified by flash chromatography (gradient elution with ethyl acetate/hexanes) to provide 0.456 g (80% yield) of a solid, which exhibited the following properties: mp 162–164 °C; ^{1}H NMR ($CDCl_3$) δ 9.75 (br s, 1H), 7.45 (d, J=9.0 Hz, 2H), 7.04 (d, J=8.0 Hz, 1H), 6.97 (d, J=9.0 Hz, 2H), 6.56 (d, J=9.0 Hz, 2H), 6.50 (d, J=9.0 Hz, 2H), 6.35 (d of d, J=2.5, 8.0 Hz, 1H), 6.31 (d, J=2.5 Hz, 1H), 4.41 (d, J=6.0 Hz, 2H), 4.32 (q, J=7.0 Hz, 2H), 3.79 (s, 3H), 3.73 (s, 3H), 3.70 (s, 3H), 3.64 (s, 3H), and 1.32 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 14.2, 38.9, 55.0, 55.1, 55.2, 55.3, 61.5, 98.2, 103.8, 113.1, 113.3, 118.6, 122.4, 124.8, 125.9, 129.1, 129.5, 129.6, 130.2, 131.2, 131.9, 158.4, 158.8, 159.8, 160.3, 162.9, 164.1, and 186.1; IR (neat) 3346, 3276, 1695, and 1667 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{32}H_{32}N_2O_8$ 572.2158, found 572.2167.

4.1.14. 3-(2,4-Dimethoxybenzylcarbamoyl)-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid (39a). A one-necked, round-bottom flask was equipped with a magnetic stir bar and a reflux condenser. Into the flask were placed 3-(2,4-dimethoxybenzylcarbamoyl)-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid ethyl ester (**38a**) (0.521 g, 0.900 mmol), potassium hydroxide (0.173 g, 3.00 mmol), and 60 mL of a 50:50 mixture of ethanol/water. The mixture was refluxed for 24 h, cooled to room temperature, and then placed in an ice/water bath. Hydrochloric acid (6 M) was added dropwise to a pH of 2 and

a few drops of water were added to induce crystallization. The precipitate was collected by suction filtration and dried in vacuo (Kugelrohr) to yield a fine white powder (0.436 g, 89% yield), which exhibited the following properties: mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J=9.0 Hz, 2H), 7.16 (d, J=9.0 Hz, 2H), 7.04 (d, J=8.0 Hz, 1H), 6.82 (d, J=9.0 Hz, 2H), 6.75 (d, J=9.0 Hz, 2H), 6.46 (d, J=2.5 Hz, 1H), 6.45 (dd, J=2.5, 8.0 Hz, 1H), 4.30 (d, J=5.0 Hz, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H), and 3.67 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 39.2, 54.6, 54.7, 54.9, 55.0, 98.1, 104.3, 113.3, 114.1, 116.6, 123.6, 127.8, 130.0, 130.5, 131.6, 132.1, 158.5, 159.7, 159.9, 161.3, 163.3, 165.5, and 185.5; IR (neat) 3403, 3244, 1707, and 1621 cm^{-1}; HRMS (EI) m/z calcd for $C_{30}H_{28}N_2O_8$ 544.1846, found 544.1846.

4.1.15. 3-(2,4-Dimethoxybenzyl)-6-(4-methoxybenzoyl)-5-(4-methoxyphenyl)-1,7-dihydropyrrolo[2,3-*d*]pyrimidine-2,4-dione (40a). Into an argon blanketed, three-neck, round-bottom flask equipped with a thermometer, condenser, septa, and magnetic stir bar were placed 3-(2,4-dimethoxybenzylcarbamoyl)-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid (**39a**) (0.355 g, 0.65 mmol) and toluene (12 mL). Triethylamine (0.14 mL, 1.00 mmol) was added dropwise followed by diphenylphosphorylazide (0.22 mL, 1.00 mmol). The reaction mixture was then refluxed for 9 h and then stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue was subjected to flash chromatography (gradient elution with EtOAc/hexanes) to provide a light yellow solid (0.242 g, 69% yield), which exhibited the following properties: mp 196–200 °C; ^{1}H NMR ($CDCl_3$) δ 10.43 (br s, 1H), 10.10 (br s, 1H), 7.41 (d, J=9.0 Hz, 2H), 7.15 (d, J=9.0 Hz, 2H), 6.96 (d, J=8.0 Hz, 1H), 6.59 (d, J=9.0 Hz, 2H), 6.55 (d, J=9.0 Hz, 2H), 6.46 (d, J=2.4 Hz, 1H), 6.39 (d of d, J=2.4, 8.0 Hz, 1H), 5.18 (s, 2H), 3.84 (s, 3H), 3.74 (s, 6H), and 3.70 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 38.9, 55.1, 55.2, 55.3, 55.7, 98.7, 99.3, 104.3, 112.8, 112.9, 117.6, 123.8, 125.5, 127.3, 129.5, 130.9, 131.5, 132.6, 139.5, 151.4, 157.9, 159.1, 159.6, 159.9, 162.4, and 186.5; IR (neat) 1712 and 1663 cm^{-1}; HRMS (EI) m/z calcd for $C_{30}H_{27}N_3O_7$ 541.1849, found 541.1836.

4.1.16. Rigidin (1). A solution of 3-(2,4-dimethoxybenzyl)-6-(4-methoxybenzoyl)-5-(4-methoxyphenyl)-1,7-dihydropyrrolo[2,3-*d*]pyrimidine-2,4-dione (**40a**) (0.190 g, 0.350 mmol) in methylene chloride (25 mL) was prepared in a three-neck 50 mL round-bottom flask equipped with a condenser, thermometer, septa, and magnetic stir bar. The solution was placed under an argon atmosphere and cooled to −78 °C in an acetone/dry ice bath. A 1 M solution of boron tribromide in methylene chloride (7 mL, 7.00 mmol) was added to the reaction mixture slowly over 5 min and the reaction set-up was protected from the light. The reaction mixture was allowed to slowly equilibrate to room temperature over 8 h and then stirred for 60 h. After cooling the reaction mixture to −78 °C an additional amount of boron tribromide solution (2.5 mL) was added slowly over 3 min and the reaction mixture was allowed to equilibrate to room temperature and stirred for 20 h. The reaction mixture was cooled in an ice/water bath and quenched with slow addition of methanol to a total of 40 mL. This mixture was stirred in an ice bath for 5 h and 20 mL of water was added followed by the careful addition of 5% aqueous NaOH (30 mL) to a pH of 6. This solution was allowed to warm to room temperature and stirred for 16 h. The solvents were removed in vacuo and the resulting aqueous mixture was transferred to a separatory funnel and extracted with ethyl acetate (3×50 mL). The combined organic extracts were filtered through a cotton plug and concentrated in vacuo followed by drying in vacuo (Kugelrohr) to give a reddish brown solid (0.157 g). HPLC analysis of the crude product on a C-18 reverse phase column with a methanol/water gradient indicated that the material was 77.8% Rigidin (96% in situ yield). A 50 mg sample of the crude product was subjected to flash chromatography on a C-18 reverse phase column with a methanol/water gradient and this resulted in 0.030 g of a light yellow solid, which exhibited physical properties[3a,4,5] identical to those reported for Rigidin: mp>325 °C (lit.[3]>300 °C); ^{1}H NMR (DMSO-d_6) δ 11.73 (br s, 1H), 11.17 (br s, 1H), 10.62 (br s, 1H), 9.99 (br s, 1H), 9.72 (br s, 1H), 7.30 (d, J=9.0 Hz, 2H), 6.95 (d, J=9.0 Hz, 2H), 6.48 (d, J=9.0 Hz, 2H), and 6.45 (d, J=9.0 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 98.6, 114.3, 114.8, 123.2, 125.4, 128.5, 129.1, 132.0, 132.7, 141.6, 151.1, 156.9, 160.2, 161.2, and 185.7; IR (neat) 3215, 1695, 1568, 1434, 1413, and 1258 cm^{-1}; HRMS (ES, M−H) m/z calcd for $C_{19}H_{12}N_3O_5$ 362.0777, found 362.0774.

4.1.17. 3-Methylcarbamoyl-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid ethyl ester (38b). Into a 7 mL microwave reaction vessel, which had been equipped with a magnetic stir bar, were placed molybdenum hexacarbonyl (0.228 g, 0.865 mmol), palladium(II) acetate (.058 g, 0.087 mmol), 3-iodo-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid ethyl ester[9] (**35a**) (0.437 g, 0.865 mmol), and tetrahydrofuran (4 mL). Then 1,8-diazabicyclo[5.4.0]undec-7-ene (0.39 mL, 2.60 mmol) was added and the reaction vessel was capped. Through the septum was added 1.30 mL (2 M in THF, 2.60 mmol) of *N*-methyl amine. The reaction mixture was stirred for 5 min and subjected to microwave irradiation for 45 min at 100 °C (15–20 W). The reaction mixture was filtered through a plug of sand, silica, and Celite and the plug was washed with tetrahydrofuran (50 mL). The filtrate was concentrated in vacuo and the residue was adsorbed onto silica gel and purified by flash chromatography (gradient elution with ethyl acetate/hexanes) to yield 0.240 g (64.5% yield) of a yellow solid, which exhibited the following properties: mp 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 9.89 (br s, 1H), 7.48 (d, J=9.0 Hz, 2H), 7.08 (d, J=9.0 Hz, 2H), 6.64 (d, J=9.0 Hz, 2H), 6.59 (d, J=9.0 Hz, 2H), 6.29 (broad absorption, 1H), 4.43 (q, J=7.0 Hz, 2H), 3.76 (s, 3H), 3.73 (s, 3H), 2.87 (d, J=5.0 Hz, 3H), and 1.42 (t, J=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 14.3, 26.6, 55.2, 55.4, 61.7, 113.1, 113.4, 122.2, 125.0, 125.6, 129.0, 129.8, 130.1, 131.5, 131.9, 158.9, 159.9, 163.0, 164.9, and 186.2; IR (neat) 3266, 1704, and 1627 cm^{-1}; HRMS (ES, M+H) m/z calcd for $C_{24}H_{25}N_2O_6$ 437.1707, found 437.1712.

4.1.18. 3-Methylcarbamoyl-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid (39b). Into a round-bottom flask equipped with reflux condenser and magnetic stir bar were placed 0.254 g (0.582 mmol) of 3-methylcarbamoyl-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid ethyl ester (**38b**), 0.098 g (1.75 mmol) of potassium hydroxide, and 30 mL of a 1:1

mixture of ethanol/water. The mixture was heated at reflux for 24 h, cooled to room temperature, and acidified with 6 M hydrochloric acid to a pH of 2. The reaction mixture was placed in a freezer for 16 h, and the resultant precipitate was collected by vacuum filtration and dried in vacuo. The filtrate was placed back in the freezer for 24 h and a second crop of solid was obtained. Combination of the two crops yielded 0.190 g (79.8% yield) of a white solid, which exhibited the following properties; mp 176–178 °C; ^{1}H NMR (acetone-d_6) δ 7.55 (d, J=8.7 Hz, 2H), 7.24 (d, J=8.7 Hz, 2H), 6.87 (d, J=8.7 Hz, 2H), 6.82 (d, J=8.7 Hz, 2H), 3.82 (br s, 3H), 3.78 (br s, 3H), and 2.76 (s, 3H); ^{13}C NMR (acetone-d_6) δ 26.0, 54.7, 55.0, 113.3, 116.6, 114.2, 123.7, 127.8, 128.1, 130.0, 131.2, 131.6, 132.3, 159.8, 160.0, 163.3, 166.9, and 185.6; IR (neat) 3394, 1701, and 1569 cm^{-1}; HRMS (ES, M−H) m/z calcd for $C_{22}H_{19}N_2O_6$ 407.1238, found 407.1234.

4.1.19. 3-Methyl-6-(4-methoxybenzoyl)-5-(4-methoxyphenyl)-1,7-dihydropyrrolo[2,3-*d*]pyrimidine-2,4-dione (40b). Into an argon blanketed, three-neck, round-bottom flask equipped with a thermometer, condenser, septa, and magnetic stir bar were placed 0.175 g (0.429 mmol) of 3-methylcarbamoyl-5-(4-methoxybenzoyl)-4-(4-methoxyphenyl)pyrrole-2-carboxylic acid (**39b**) and 15 mL of toluene. To this mixture were added 0.10 mL (0.75 mmol) of triethylamine and 0.16 mL (0.75 mmol) of diphenylphosphorylazide. The reaction mixture was heated at 75 °C for 9 h, cooled to room temperature, and stirred for an additional 24 h at room temperature. The solvent was then removed in vacuo, and the residue was purified by automated flash chromatography using a gradient elution of hexanes/ethyl acetate to yield 0.092 g (53% yield) of a yellow solid, which exhibited the following properties: mp 300 °C; ^{1}H NMR (DMSO-d_6) δ 11.94 (br s, 1H), 7.36 (d, J=9.0 Hz, 2H), 7.05 (d, J=9.0 Hz, 2H), 6.65 (d, J=9.0 Hz, 2H), 6.62 (d, J=9.0 Hz, 2H), 3.70 (s, 3H), 3.67 (s, 3H), and 3.15 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 27.2, 55.5, 55.8, 98.3, 112.9, 113.4, 124.8, 125.7, 128.7, 130.7, 131.6, 132.7, 140.1, 151.2, 158.8, 159.5, 162.3, and 185.7; IR (neat) 3180, 1709, and 1658 cm^{-1}; HRMS (ES, M+H) m/z calcd for $C_{22}H_{19}N_2O_6$ 406.1397, found 406.1412. It should also be noted that the three-step process of aminocarbonylation, ester hydrolysis, and uracil formation has also been carried out in an overall 30% yield with only a purification (flash chromatography) being required for the last step.

4.1.20. Rigidin E (5). A three-neck, round-bottom flask was equipped with a condenser and magnetic stir bar and was placed under a nitrogen atmosphere. Into the flask were placed 0.100 g (0.247 mmol) of 3-methyl-6-(4-methoxybenzoyl)-5-(4-methoxyphenyl)-1,7-dihydropyrrolo[2,3-*d*]-pyrimidine-2,4-dione (**40b**) and 12 mL of methylene chloride. The mixture was cooled to −78 °C in an acetone/dry ice bath and 5.0 mL of a 1.0 M (5.00 mmol) boron tribromide in methylene chloride was slowly added over 5 min. The reaction mixture was protected from light, stirred, and allowed to equilibrate to room temperature for 72 h. The reaction mixture was again cooled to −78 °C in an acetone/dry ice bath and an additional 1.6 mL (1.6 mmol) of boron tribromide was added. The reaction mixture was stirred and allowed to equilibrate to room temperature over 18 h. The reaction mixture was cooled to 0 °C, slowly quenched with methanol (45 mL), and stirred at 0 °C for 6 h. Water (25 mL) was subsequently added, followed by the dropwise addition of 5% NaOH such that the pH of the solution reached 7. This solution was then stirred overnight at room temperature and the solvents were removed in vacuo. The residue was diluted with 50 mL of ethyl acetate and the aqueous phase was extracted with 3×30 mL portions of ethyl acetate. The organic layers were combined and concentrated in vacuo to give 0.182 g of a light brown solid, which was adsorbed onto silica gel and purified by flash chromatography (gradient elution with ethyl acetate/hexanes) to yield 0.170 g of a light brown solid. Trituration of this material with acetone yielded 0.038 g (40.8% yield) of a solid, which exhibited spectral properties[3c] identical to those reported for Rigidin E: mp>330 °C; ^{1}H NMR (DMSO-d_6) δ 11.90 (br s, 1H), 10.00 (br s, 1H), 9.25 (br s, 1H), 7.30 (d, J=9.0 Hz, 2H), 6.96 (d, J=9.0 Hz, 2H), 6.48 (d, J=9.0 Hz, 2H), 6.46 (d, J=9.0 Hz, 2H), and 3.16 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 27.2, 98.2, 114.2, 114.7, 123.3, 125.4, 128.6, 129.2, 132.0, 132.8, 139.9, 151.7, 156.9, 159.7, 161.1, and 185.7; IR (neat) 3200, 1689, and 1646 cm^{-1}; HRMS (ES, M+H) m/z calcd for $C_{20}H_{16}N_3O_6$ 378.1084, found 378.1100.

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The role of botrydienediol in the biodegradation of the sesquiterpenoid phytotoxin botrydial by *Botrytis cinerea*

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Abstract—The biotransformation of botrydienediol (**6**) labelled with deuterium on carbons C-10 and C-15 has been studied. This has led to modification of some previous assumptions about the biodegradative route of botrydial. The [10-^{2}H,15-^{2}H]-botry-1(9)-4-diendiol (**12**) was transformed into dehydrobotrydienediol derivatives **13**–**15** but it was not incorporated into secobotryane skeleton (**7**). In addition, three new sesquiterpenoids have been isolated, which shed further light on the secondary metabolites of *Botrytis cinerea*. From the point of view of persistence of these toxins in the food chain, the easy biotransformation and different biodegradative routes of botrydial (**1**), seem to indicate that the toxin may not persist in the plant for a long time as it will be metabolized by the fungi and the plant.

1. Introduction

The ascomycete *Botrytis cinerea* Pers.:Fr. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel] is a phytopathogenic fungus that grows as a grey mould on a variety of commercial crops causing serious economic losses.[1] A number of phytotoxins have been isolated from this fungus.[2] The best known and most active of these metabolites is botrydial (**1**), which possesses the sesquiterpenoid botryane skeleton,[3] it is responsible for the typical lesions of the fungal infection and it plays an important role in the pathogenicity of the organism in vivo.[4,5]

Botrydial (**1**) has recently been detected in ripe fruits of *Capsicum annuum* and in the leaves of *Phaseolus vulgaris* and *Arabidopsis thaliana* that have been wounded and inoculated with a conidial suspension of *B. cinerea*.[6]

The regulatory effect of botrydial (**1**) on the growth of *B. cinerea* and its biodegradation by the fungus has been reported.[7] Fungal growth ceases when the concentration of botrydial reaches a particular level. The fungus transformed botrydial (**1**) to the less active phytotoxins dihydrobotrydial (**3**) and secobotrytrienediol (**7**). It is only after its detoxification to the less harmful compounds **3**–**7** that fungal growth resumes.[7] This may have implications for our understanding of the progress of the fungal infection of a plant and, additionally, from the point of view of its persistence in the food chain, to understand the fate of this toxin in fruits and vegetables infected by this phytopathogenic fungus. Consequently, the study on biodegradation pathway of the major toxin, botrydial, it is of interest. In the light of our results two main biodegradation and detoxification pathways for botrydial (**1**) were proposed and are set out in Scheme 1 (routes a and b).[7] In this paper we report the results of investigations on the biotransformation of the putative biosynthetic intermediate botrydiendiol (**6**) by *B. cinerea* and some considerations of the secondary metabolism of the fungus.

2. Results and discussion

As indicated above, the biodegradative route leading to secobotrytrienediol (**7**) has come to our attention because this transformation could have implications for the progress of the fungal infection of a plant and for the safety of the food chain.

In addition, the biosynthesis of metabolite secobotrytrienediol (**7**) is regulated by pH.[7] The formation of **7** may involve an electrocyclic ring opening reaction from the diene **5** or its reduced derivative **6**. This is an interesting process in

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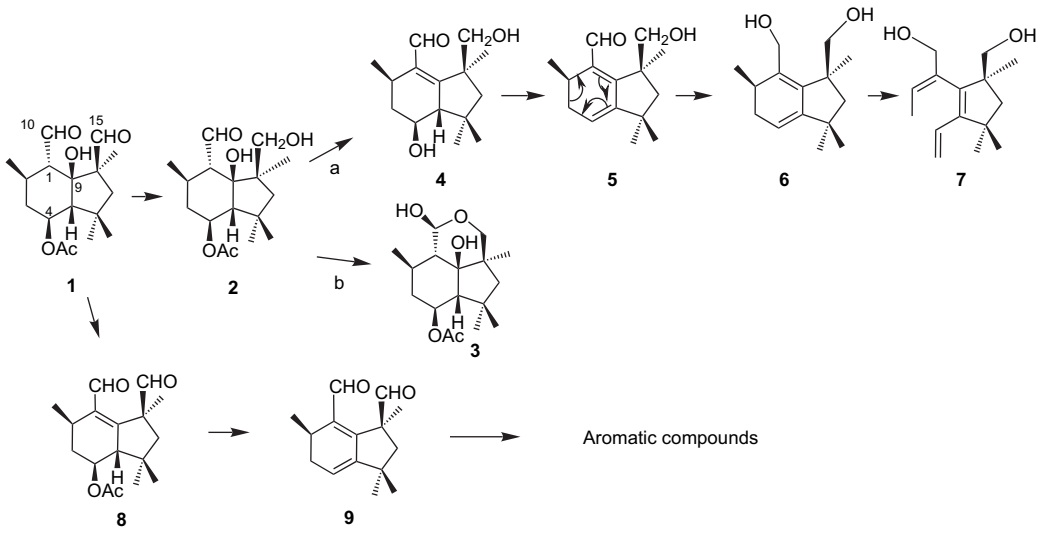


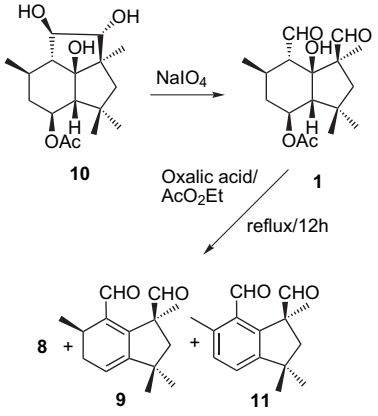
Scheme 1.

biological systems and has a parallel in the formation of precalciferol.[8]

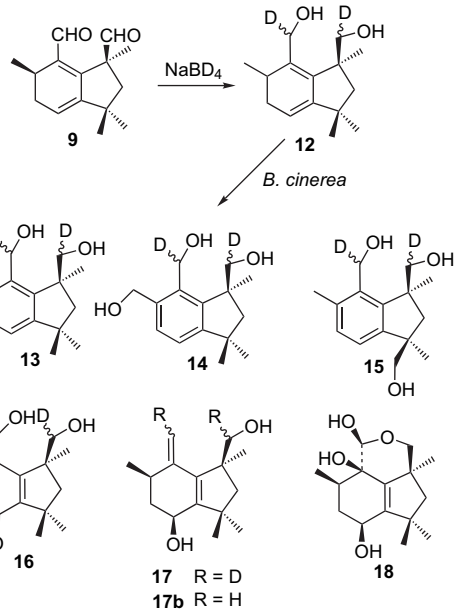
In order to gain a greater understanding of the secondary metabolism within the fungus *B. cinerea*, the biotransformation of the botrydienediol (**6**) labelled with deuterium on carbons C-10 and C-15 has been studied.

Compound (**9**) was obtained from the natural probotryane (**10**) by the bio-mimetic chemical transformation indicated in Scheme 2. Compound **10**, obtained from fermentation of *B. cinerea*, was treated with periodate to yield botrydial (**1**) (60%). Reaction of **1** with oxalic acid gave compounds **8**,[2,5] **9**[2,9] and **11**[2,9] with 15, 64 and 10% yield, respectively (Scheme 2). The reduction of **9** with deuteriated sodium borohydride yielded the deuteriated botrydiendiol (**12**) (Scheme 3).

The deuteriated compound **12** was fed to two fermentations one of which was buffered at pH 7 whilst the other was allowed to grow at the natural pH. After three days, from inoculation, (see Section 3), the mycelium of each experiment was filtered. The broths were saturated with sodium chloride and extracted four times with ethyl acetate. The solvent was evaporated and the residue was purified by chromatography



Scheme 2.



Scheme 3.

on a Si gel column. Final purification was carried out by means of semi-preparative HPLC.

The experiment using a buffered medium afforded, in addition to the deuteriated compounds **13–15**, two new deuteriated compounds 1-hydroxy-4-oxo-[10-^{2}H, 15-^{2}H]-botry-5(9)-endiol (**16**) and botry-[1(10),5(9)-^{2}H]-dien-[15-^{2}H]-ol (**17**) and an undeuteriated new metabolite 1β,4β-dihydroxy-5(9)-ene dihydrobotrydial (**18**). Compounds **13–17** were also obtained from the fermentation grown at the natural pH (Scheme 3). The structures of the metabolites were established on the basis of their one-dimensional and two-dimensional NMR spectra (^{1}H, ^{2}H, ^{13}C, HSQC and HMBC).

Compounds **13–15** showed an average deuterium incorporation of 79% and a characteristic pattern of signals, in their ^{1}H and ^{13}C NMR, corresponding to the aromatic botryane derivatives isolated previously from *B. cinerea* and previously

reported.[10] Although they could be considered as artefacts from the aromatization of diene **12** in an acidic medium, the isolation of these compounds from the biotransformation in buffered medium would indicate that compounds **13–15** arise from a biotransformation of **12** by the fungus.

Interestingly, two new compounds with a previously unknown functionality on carbon C-1 were isolated. Compound **16** was isolated as a diastereomeric mixture of deuteriated derivatives, displaying a 44% deuterium incorporation. The HREIMS of **16** showed a molecular ion corresponding to the molecular formula $C_{15}D_2H_{22}O_4$. The IR spectrum possessed absorption bands at 3375, 2930, 1660, 1355, 1059 cm^{-1}, indicating that an unsaturated ketone and hydroxyl groups were present. The ^{13}C NMR spectrum showed 15 signals arising from four methyls, four methylenes, one methines and six quaternary carbon atoms, including a ketonic carbonyl group at δ 197 and a double bond at δ 145.0 and 169.1.

The ^1H NMR spectrum of the product showed a typical pattern of botryane signals from which the stereochemistry has already been established.[2] The principal differences were the absence of the signal corresponding to a hydroxyl group at C-4 and, in this deuteriated compound, the signals (singlets) corresponding to H-10 and H-15, at δ 3.72–3.76 and 3.53, respectively. These signals could be superimposed with those signals corresponding to the compound without deuterium. The location of a carbonyl group at C-4 was confirmed by the HMBC correlation of the corresponding carbonyl signal with the methine proton H-2, at δ 2.39. Furthermore, the HMBC experiment also provided information on the location of the hydroxyl group. The long-range correlations of the carbon at δ 75.6 with the methyl doublet at C-2 clearly marked the presence of hydroxyl group at C-1.

The stereochemistry of hydroxyl group was inferred from the observed downfield shift of the methyl group on C-2, which was consistent with a β disposition for a hydroxyl group on C-1. The assignment of the signals was supported by ^1H–^1H COSY, HSQC and HMBC experiments and ^2H NMR spectrum, and was consistent with the proposed structure **16**.

Compound **17** displayed 79% deuterium incorporation and it was isolated as a diastereomeric deuteriated mixture. Its molecular formula $C_{15}D_2H_{22}O_2$ was established on the basis of HREIMS data from the *m/z* 238.1895. The ^{13}C NMR spectrum showed 15 signals arising from four methyls and four methylenes, including a methylene on a double bond; two methines, one corresponding to an oxygenated carbon; and five quaternary carbon atoms. Three quaternary carbons and one methine were assigned to two double bonds.

The ^1H NMR spectrum of the product showed a typical pattern of botryane signals. The principal difference was the presence of signals at δ 4.95 and 5.13 corresponding to an exocyclic double bond, which was easily located at C-1 by an HMBC experiment. The signal at δ 4.39 (1H, dd, H-4) was assigned to the geminal proton to a hydroxyl group on C-4. The HSQC and HMBC experiments clearly established the presence of the fully substituted C-5–C-9 double bond and exocyclic double bond between C-1–C-10. Signals at δ 2.6 and 4.39 were assigned to H-2 and H-4, respectively. The ^2H NMR spectrum was consistent with the proposed structure **17**.

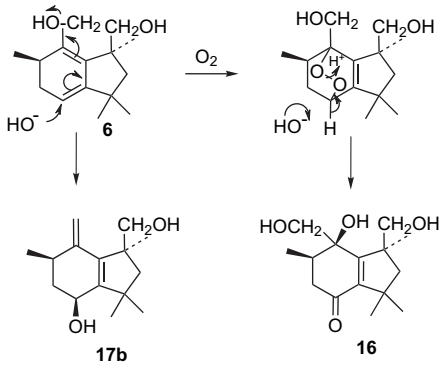
This compound had previously been isolated in very small amount from an experiment on the biotransformation of diisophorone by *B. cinerea*.[11] Its structure was not established at that time. The spectroscopic data of this compound **17b** are now included in Section 3.

The undeuteriated compound **18** was obtained from the experiment with buffered medium. The HREIMS showed a molecular ion corresponding to molecular formula $C_{15}H_{24}O_4$. The IR spectrum possessed absorption bands at 3410, 2954, 1639, 1035 and 751 cm^{-1} indicating that hydroxyl groups and an alkene were present. The ^{13}C NMR spectrum showed signals arising from four methyls, three methylenes, three methines and five quaternary carbon atoms, including a fully substituted double bond. The ^1H NMR spectrum shows a pattern of the signals comparable to those of dihydrobotrydial (**3**). However, the signal corresponding to H-10 appeared as a singlet and the signal of H-4 was shifted upfield suggesting the introduction of a new hydroxyl group in C-1 and a double bond on C-5–C-9.

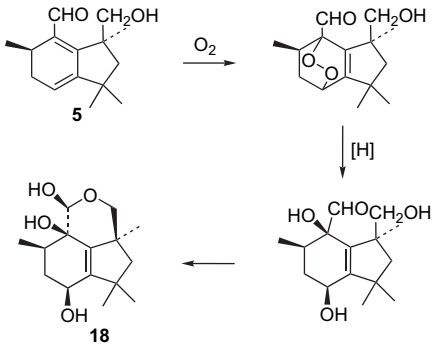
The singlet at δ 4.6, which was assigned to the proton H-10, the signals in the ^{13}C NMR at δ 72.2, C-1 and 138.1 and 146.4 corresponding to C9 and C5, respectively, were consistent with the proposed structure. The long-range experiments, which were carried out were consistent with the structure **18** proposed for this compound. The stereochemistry of the hydroxyl at C-1 was established in the same way as that of compound **16** by the downfield shift observed for the signal corresponding to methyl group on C-2. On the other hand the β configuration of hydroxyl at C-4 was assigned on the basis of the observed coupling constant and biogenetic considerations. Compound **18** has also been isolated in very small amounts in the experiment on the biotransformation of diisophorone, previously cited.[11] Its structure was not determined at that time.

The results obtained clearly show that [10-^2H,15-^2H]-botry-1(9)-4-diendiol (**12**) was not incorporated into the secobotryane derivative (**7**). However the high incorporation ratio observed in compounds **13–15** seems to indicate that, in addition to the previously proposed compound **9**,[7] the botrydiendiol (**12**) is the precursor of all the aromatic derivatives obtained from *B. cinerea*.[7,10]

Addition of oxygen to cisoid dienes to give peroxides has been reported in several fungi.[12] These epidioxides can rearrange or be reduced and several reports can be found in the literature.[12,13] The formation of compounds **16** and **17** represents two facets of the chemistry of the dienol **6**, Scheme 4. Compound **16** may be formed via the 1,4-epidioxide by a base-catalyzed cleavage whilst compounds **17/17b** are the result of a rearrangement. Compound **18** may be formed by the reductive cleavage of the 1,4-epidioxide formed from compound **5**, a putative intermediate to secobotryane **7**. If compound **18** was formed from **5** then it could explain why the former **18** had not incorporated deuterium, Schemes 5 and 6.



Scheme 4.



Scheme 5.

The new isolated compounds **16**–**18** shed further light on secondary metabolism of the fungus. Thus *B. cinerea* produce an interesting 1,4-dihydroxylation on the diene of the substrate leading to compounds **16**–**18**. From this point of view some modification on the degradative route of botrydial may be proposed, Scheme 6. Furthermore, the easy biotransformation and different biodegradative routes of botrydial seem to indicate that the toxins may not persist in the plant for a long time and can be metabolized by the fungus and the plant.

3. Experimental

3.1. General experimental procedures

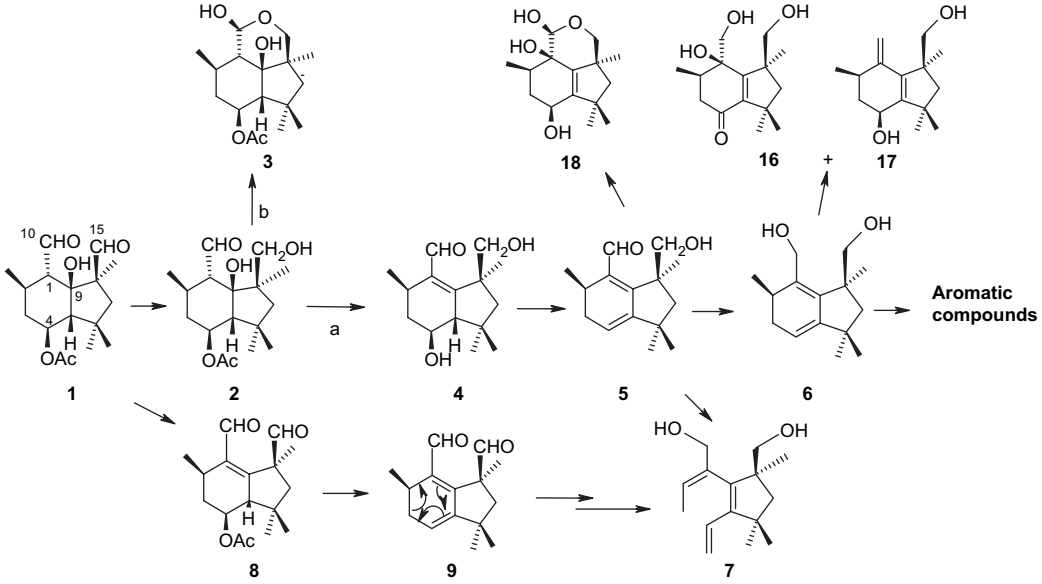
Optical rotations were determined with a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Mattson Genesis spectrophotometer, series FTIR (Fourier transform infrared). ^{1}H and ^{13}C NMR measurements were obtained on Varian Unity (^{1}H at 400 MHz, ^{13}C at 100 MHz) and Varian Unity 600 MHz (^{1}H at 600 MHz, ^{13}C at 150 MHz). Chemical shifts are quoted relative to TMS (Me_4Si) in $CDCl_3$. Mass spectra were recorded on Fisons MD800 and Finnigan MAT95 S instruments. High-performance liquid chromatography (HPLC) was performed with a Hitachi/Merck L-6270 apparatus equipped with an UV–vis detector (L4250) and a differential refractometer detector (RI-71). Thin-layer chromatography (TLC) was performed on Merck Kiesegel 60 F254, 0.2 mm thick (Catalog no. 1.05554.0001). Silica gel (Merck) was used for column chromatography. Chemicals were products of Fluka or Aldrich. Purification by means of HPLC was accomplished on a 25×1 cm Hibar 60 silica gel column. All solvents used were freshly distilled.

3.2. Organism

B. cinerea 2100 was obtained from the Colección Española de Cultivos Tipo (CECT), Facultad de Biología, Universidad de Valencia, Spain. Conidial stock suspensions of this strain were maintained viable in 80% glycerol at −40 °C.

3.3. Synthesis of [10-^{2}H,15-^{2}H]-botry-1(9)-4-diendiol 12

3.3.1. Botrydial 1. Sodium periodate (1.37 g, 6.4 mmol) was added to a solution of **10** (0.5 g, 1.6 mmol) in THF/H_2O (5:1, 10 mL). The solution was stirred for 12 h at room temperature. Sodium iodate precipitated out of the solution during the reaction and then it was filtered off. The mixture was concentrated in vacuo to remove THF. The resulting aqueous solution was extracted with EtOAc



Scheme 6.

(3×5 mL). The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo, and the crude extract was subjected to column chromatography to afford botrydial **1** (0.30 g, 60%), which was identified by its NMR spectroscopic data.

3.3.2. Botrydienal 9. A solution of **1** (100 mg, 0.32 mmol) in AcOEt (10 mL) was treated with 6% aqueous oxalic acid solution (15 mL) and refluxed for 12 h. The mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with AcOEt (×3). The organic layers were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed, and the mixture obtained was purified by means of normal-phase HPLC to afford **8**[2,5] (14 mg, 0.048 mmol, 15%), botrydienal **9**[2,9] (48.3 mg, 0.21 mmol, 65%) and aromatic derivative **11**[2,9] (7.4 mg, 0.032 mmol, 10%).

3.3.3. [10-^2H,15-^2H]-Botry-1(9)-4-diendiol 12. $NaBD_4$ (36.1 mg, 0.86 mmol) (≥99 at. % D, purchased from the Fluka Chemical Company) was added to a solution of **9** (50 mg, 0.22 mmol) in methanol (10 mL), and the resulting solution was stirred for 15 min at room temperature. The mixture was poured onto ice, acidified with 2 N HCl (15 mL), and stirred for 10 min. The solution was diluted with H_2O (45 mL) and extracted with $CHCl_3$ (30 mL, ×3). The solvent was evaporated and the crude extract chromatographed to yield **12** (50.3 mg, 0.21 mmol, 96%, HREIMS (*m/z*): 238.1908 (calcd $C_{15}H_{22}O_2D_2$, 238.1901)).

3.4. Biotransformation by *B. cinerea*

A suspension (4.2 mL) of *B. cinerea* conidia (2.48×10^6 conidia/mL) was inoculated at 25 °C and 250 rpm in shake cultures in 12 Erlenmeyer flasks (500 mL) containing 200 ml of Czapek–Dox medium: glucose (50 g), yeast extract (1 g), KH_2PO_4 (5 g), $NaNO_3$ (2 g), $MgSO_4$ (0.5 g) and $FeSO_4$ (10 mg) per litre of distilled water. Before the inoculation, the pH medium was carefully adjusted to 7.0 with aqueous NaOH. After 72 h, the mycelium was transferred, into 10 flasks (500 mL) containing 200 mL of Czapek–Dox medium (without glucose) and the substrate **12** (90 ppm). The additional two flasks were used as control. After three days, the mycelium was filtered. The pH was measured (control: 6.09 pH; inoculated mediums broth: 6.03 pH). The broth (2 L) was saturated with sodium chloride and extracted four times with ethyl acetate. Extracts were dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. The residue (203 mg) was purified by chromatography on a Si gel column and then by HPLC affording dehydrobotrydienol (**13**; 1.8 mg), 11-hydroxydehydrobotrydienol (**14**; 120 mg) and 12-hydroxydehydrobotrydienol (**15**; 3.5 mg), novel deuteriated compounds 1-hydroxy-4-oxo-[10-^{2}H, 15-^{2}H]-botry-5(9)-endiol (**16**; 0.3 mg) and botry-[1(10),5(9)-^2H]-dien-[15-^2H]-ol (**17**; 4 mg). Their structures were established on the basis of their NMR spectroscopy: ^{1}H, ^{2}H and ^{13}C HSQC, HMBC.

3.5. Biotransformation by *B. cinerea* on buffered medium

Twelve Erlenmeyer flasks (500 mL) were filled with 200 mL of Czapek–Dox medium as cited above. The pH was adjusted to 7.0 with aqueous NaOH, and the flasks were inoculated with a suspension (2.3 mL) of *B. cinerea* conidia (8×10^6 conidia/mL). The flasks were incubated at 25 °C for three days and stirred at 250 rpm; the mycelium was then filtered and transferred into 10 flasks (500 mL) containing 200 mL of medium, which consisted of equal volumes of sterile phosphate buffer (pH 7 with phosphate buffer 0.4 M) and Czapek–Dox (as above but without glucose) and 90 ppm of the substrate botry-4,9-dien-[15,10-^2H]-ol (**12**) per flask. The remaining two flasks were used as control. The broth and the mycelia were separated further by filtration after three days. The pH was measured (control: 6.58 pH units; inoculated medium broth: 6.51 pH units) and the broth was saturated with NaCl, and extracted four times with ethyl acetate. The extract was dried over anhydrous sodium sulfate, and the solvent was then evaporated under vacuum. Fractionation of the extract (210 mg) was carried out by means of column chromatography on silica gel (SiCC), eluting with hexane/ethyl acetate (80:20). Final purification was carried out by means of semi-preparative HPLC to afford deuteriated known compounds dehydrobotrydienol (**13**; 3 mg), 11-hydroxydehydrobotrydienol (**14**; 130 mg) and 12-hydroxydehydrobotrydienol (**15**; 1.1 mg), unknown deuteriated compounds, 1-hydroxy-4-oxo-[10-^{2}H, 15-^{2}H]-botry-5(9)-endiol (**16**; 0.6 mg) and botry-[1(10),5(9)-^2H]-dien-[15-^2H]-ol (**17**; 1.5 mg) and a new, undeuteriated, metabolite compound 1β,4β-dihydroxy-5(9)-ene dihydrobotrydial (**18**; 0.7 mg). Their structures were established on the basis of their one-dimensional and two-dimensional NMR analyses (^{1}H, ^{2}H, ^{13}C, HSQC and HMBC).

3.5.1. 1-Hydroxy-4-oxo-[10-^2H,15-^2H]-botry-5(9)-endiol 16. Colorless oil, IR (film): ν_{max} 3375, 2930, 1660, 1355, 1059, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm): 1.09 (3H, d, J_{11-2}=6.2 Hz, H-11), 1.27* (3H, s, H-13), 1.28* (3H, s, H-12), 1.38 (3H, s, H-14), 1.51 (1H, d, $J_{7\alpha-7\beta}$=12.9 Hz, H-7α), 1.76 (1H, d, $J_{7\beta-7a}$=12.9 Hz, H-7β), 2.39 (3H, m, H-2, H-3β, H-3α), 3.72–3.74 (2H, m, H10, H10′), 3.53 (1H, s, H-15), 3.76 (1H, s, H-15′). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm): 14.6 (q, C-11), 26.1 (q, C-14), 28.2* (q, C-13), 29.8* (q, C-12), 42.0 (d, C-2), 42.4 (t, C-6), 44.3 (s, C-3), 52.0 (s, C-7), 52.4 (t, C-8), 64.4 (dt$^{\#}$, C-10), 68.4 (dt$^{\#}$, C-15), 75.6 (s, C-1), 145.0 (s, C-5), 169.1 (s, C-9), 197 (s, C-4). *: Interchangeable; $^{\#}$: coupling ^{13}C–^{1}H (d) and ^{13}C–^{2}H (t). EIMS (*m/z*): 270 [M$^+$] (3%), 252 [M$^+$–H_2O] (0.2%), 238 [M$^+$–CDHOH] (55%), 220 [M$^+$–H_2O–CDHOH] (100%). HREIMS (*m/z*): 270.1772 (calcd $C_{15}D_2H_{22}O_4$, 270.1800). Deuterium percentage: 44%.

3.5.2. Botry-[1(10),5(9)-^2H]-dien-[15-^2H]-ol 17. Colorless oil, $[\alpha]_D^{25}$ +12.20 (*c* 1.5, ethyl acetate); IR (film): ν_{max} 3386, 2950, 2933, 1459, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.12 (3H, d, J_{11-2}=6.7 Hz, H-11), 1.25 (3H, s, H-12), 1.17 (3H, s, H-13), 1.29 (3H, s, H-14), 1.54 (1H, d, $J_{7\alpha-7\beta}$=13.0 Hz, H-7α), 2.09 (1H, d, $J_{7\beta-7a}$=13.0 Hz, H-7β), 1.65 (1H, ddd, $J_{3\alpha-3\beta}$=13.2 Hz, $J_{3\alpha-2}$=10 Hz, $J_{3\alpha-4}$=4.8 Hz, H-3α), 1.8 (1H, ddd, $J_{3\beta-3\alpha}$=13.2 Hz, $J_{3\beta-4}$=4.8 Hz, $J_{3\beta-4}$=3.9 Hz, H-3β), 2.6 (1H, m, H-2), 3.41 (1H, br s, H-15), 3.8 (1H, br s, H-15′), 4.39 (1H, dd, $J_{4-3\alpha}$=4.8 Hz, $J_{4-3\beta}$=4.8 Hz, H-4), 4.9 (1H, s, H-10), 5.11 (1H, s, 10). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.2 (q, C-11), 19.3 (q, C-14), 31.15 (q, C-12), 28.39 (q, C-13), 33.12 (d, C-2), 41.62 (t, C-3), 50.67

(s, C-8), 43.43 (s, C-6), 53.25 (t, C-7), 63.59 (d, C-4), 137.6 (s, C-1), 69.30 (C-15), 145.9 (s, C-9), 150.7 (s, C-5), 107.3 (C-10). EIMS (*m/z*): 238 [M^+] (79%), 223 [M^+–Me] (7%), 205 [M^+–H_2O–Me] (79%), 188 [M^+–H_2O–CDHOH] (100%). HREIMS (*m/z*): 238.1895 (calcd $C_{15}D_2H_{22}O_2$, 238.1902). Deuterium percentage: 79%.

3.5.3. Botry-1(10),5(9)-dien-15-ol 17b. Colorless oil, $[\alpha]_D^{25}$ +12.14 (*c* 1.4, ethyl acetate); IR (film): ν_{max} 3386, 2958, 2930, 1459, 1375 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.12 (3H, d, J_{11-2}=6.7 Hz, H-11), 1.25 (3H, s, H-12), 1.16 (3H, s, H-13), 1.29 (3H, s, H-14), 1,53 (1H, d, $J_{7\alpha-7\beta}$=13.0 Hz, H-7α), 2.08 (1H, d, $J_{7\beta-7a}$=13.0 Hz, H-7β), 1.65 (1H, ddd, $J_{3\alpha-3\beta}$=13.2 Hz, $J_{3\alpha-2}$=10 Hz, $J_{3\alpha-4}$= 4.8 Hz, H-3α), 1.8 (1H, ddd, $J_{3\beta-3\alpha}$=13.2 Hz, $J_{3\beta-4}$= 4.8 Hz, $J_{3\beta-4}$=3.9 Hz, H-3β), 2.6 (1H, m, H-2), 3.43 (1H, d, $J_{15-15'}$=11.1 Hz, H-15), 3.8 (1H, d, $J_{15'-15}$=11.1 Hz, H-15′), 4.39 (1H, dd, $J_{4-3\alpha}$=4.8 Hz, $J_{4-3\beta}$=4.8 Hz, H-4), 4.95 (1H, s, H-10), 5.13 (1H, s, 10). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 24.2 (q, C-11), 19.13 (q, C-14), 31.15 (q, C-12), 28.39 (q, C-13), 33.12 (d, C-2), 41.62 (t, C-3), 50.67 (s, C-8), 43.43 (s, C-6), 53.25 (t, C-7), 63.59 (d, C-4), 137.6 (s, C-1), 69.77 (t, C-15), 145.9 (s, C-9), 150.7 (s, C-5), 107.61 (t, C-10). EIMS (*m/z*): 237 [M^++1] (79%), 236 [M^+] (7%), 218 [M^+–H_2O]. HREIMS (*m/z*): 236.1735 (calcd $C_{15}H_{24}O_2$, 236.1731).

3.5.4. 1β,4β-Dihydroxy-5(9)-ene dihydrobotrydial 18. Colorless oil, $[\alpha]_D^{25}$ −55.2 (*c* 0.096, $CHCl_3$); IR (film): ν_{max} 3410, 2954, 2924, 1639, 1455, 1035, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.1 (3H, d, J_{11-2}= 7.5 Hz, H-11), 1.2 (3H, s, H-12), 1.24 (3H, s, H-13), 1.4 (3H, s, H-14), 1,6 (2H, d, $J_{7\alpha-7\beta}$=1.2 Hz, H-7), 1.7 (1H, ddd, $J_{3\alpha-3\beta}$=14.5 Hz, $J_{3\alpha-2}$=2.8 Hz, $J_{3\alpha-4}$=1.8 Hz, H-3α), 2.01 (1H, m, H-2), 2.15 (1H, dt, $J_{3\beta-3\alpha}$=14.5 Hz, $J_{3\beta-2}$= $J_{3\beta-4}$=4.7 Hz, H-3β), 3.2 (1H, d, $J_{15\alpha-15\beta}$=10.7 Hz, H-15α), 3.8 (1H, d, $J_{15\beta-15\alpha}$=10.7 Hz, H-15β), 4.3 (1H, dd, $J_{4-3\alpha}$=1.6 Hz, $J_{4-3\beta}$=4.8 Hz, H-4), 4.6 (1H, s, H-10). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.3 (q, C-11), 27.0 (q, C-14), 28.07 (q, C-12), 30.7 (q, C-13), 33.9 (d, C-2), 35.9 (t, C-3), 43.7 (s, C-8), 46.2 (s, C-6), 51.7 (t, C-7), 62.8 (d, C-4), 72.2 (s, C-1), 77.2 (t, C-15), 97.3 (s, C-10), 138.1 (s, C-9), 146.4 (s, C-5). EIMS (*m/z*): 268 [M^+] (0.2%), 250 [M^+–H_2O] (11%), 239 (27%), 204 (49%), 191 (100%), 189 (52%), 165 (37%), 161 (32%), 148 (44%), 133 (51%), 91 (42%). HREIMS (*m/z*): 250.1579 (–H_2O) (calcd $C_{15}H_{22}O_3$, 250.1569).

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Comprehensive synthesis of ER related high-mannose-type sugar chains by convergent strategy

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Abstract—Systematic synthesis of high-mannose-type sugar chains of asparagine-linked glycoproteins is described. To construct the target sugar chains, we employed the convergent route, using three oligosaccharide components, the common hexasaccharide, branched tri-, tetra- and pentasaccharides, and mono-, di-, and triglucosyl fragments. Construction of the β-mannoside linkage was performed using *p*-methoxybenzyl-assisted intramolecular aglycon delivery. The hexasaccharide fragment was coupled with the branched mannooligosaccharide donors such as M5, M4B, M4C, and M3 to give undecasaccharide (M9), decasaccharide (M8B and M8C), and nonasaccharide (M7), respectively. Incorporation of mono-, di-, and triglucosyl fragments toward them gave tetradecasaccharide (G3M9), tridecasaccharide (G2M9), dodecasaccharide (G1M9), undecasaccharide (G1M8B and G1M8C), and decasaccharide (G1M7), respectively.

1. Introduction

N-Glycosylation of secretory and membrane-bound proteins is an essential and highly conserved protein modification of eukaryotes[1] (Fig. 1). The key step of protein N-glycosylation pathway is the en bloc transfer of a triglucosylated high-mannose-type tetradecasaccharide ($Glc_3Man_9GlcNAc_2$; G3M9) from lipid carrier dolichyl pyrophosphate (Dol-PP) to asparagine (Asn) residue of Asn-X-Ser/Thr sequences of nascent polypeptide chains. It occurs during their translocation across the endoplasmic reticulum (ER) membrane.[2] This transformation is catalyzed by a large multisubunit enzyme, oligosaccharyltransferase (OST) complex. Subsequently, glucosidase I, an integral membrane protein with a luminally oriented catalytic domain, removes the terminal α1-2 linked glucose residue of the triglucosyl sequence. Trimming of penultimate glucose residues takes place by the action of glucosidase II to give $Glc_1Man_9GlcNAc_2$ (G1M9) and then $Man_9GlcNAc_2$ (M9) glycoforms.[3,4]

Partial trimming of mannose residues also occurs in the ER, which is initiated by two distinct enzymes, ER α1-2 mannosidases I and II. The proposed function of the former enzyme is to remove the terminal sugar of the middle branch to generate the M8B isomer, which is the proposed ligand of mannosidase-like protein (MLP).[5,6] On the other hand, mannosidase II preferentially releases the terminal mannose linked to the α1-6 branch to yield M8C isomer. An additional mannosidase that may be involved in early processing is Man9 mannosidase, which has an ability to trim M9 to $Man_7GlcNAc_2$ (M7) and eventually $Man_5GlcNAc_2$ (M5).[7]

Recent investigations have revealed the fundamental roles of these N-linked high-mannose-type glycans in protein quality control (folding, transport, and degradation).[8–11] A number of proteins, such as calreticulin (CRT), calnexin (CNX), ER-Golgi intermediate compartment (ERGIC)-53, vesicular integral membrane protein (VIP) 36, and mannosidase-like proteins (MLP, EDEM/Htm1p/Mnl1p) have been revealed or suggested to recognize these N-linked glycans. CNX and CRT are ER-resident lectin-chaperones that specifically recognize monoglucosylated glycans, most typically G1M9.[12,13] They constitute CNX/CRT cycle, together with UDP-Glc: glycoprotein glucosyltransferase (UGGT). Intriguingly, the latter enzyme functions as the 'folding sensor', glucosylating prematurely deglucosylated glycoproteins having M9, M8 or M7 glycans and regenerate monoglucosylated structures G1M9∼7, in order that they can engage in multiple rounds of interactions with CNX/CRT.[14] On the other hand, ERGIC-53 and VIP36 are cargo receptors that mediate glycoprotein transport between ER and Golgi,[15] while MLPs are proposed to capture malfolded glycoproteins destined for degradation. Although MLPs are considered to recognize M8B, their lectin activity is yet to be revealed.

These functions of high-mannose-type glycans in protein quality control are attracting recent attention. Most of the

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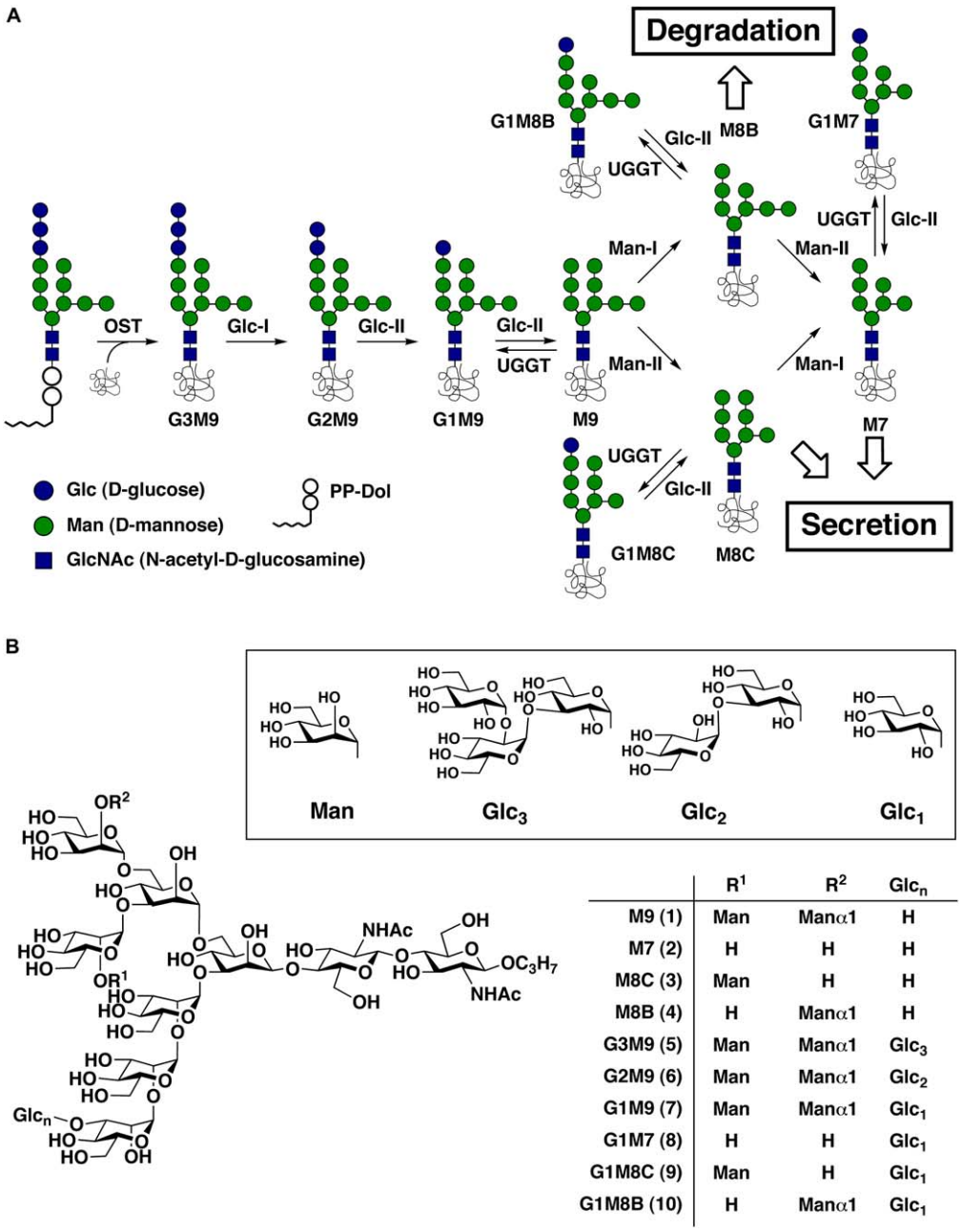


	R^1	R^2	Glc_n
M9 (1)	Man	Manα1	H
M7 (2)	H	H	H
M8C (3)	Man	H	H
M8B (4)	H	Manα1	H
G3M9 (5)	Man	Manα1	Glc_3
G2M9 (6)	Man	Manα1	Glc_2
G1M9 (7)	Man	Manα1	Glc_1
G1M7 (8)	H	H	Glc_1
G1M8C (9)	Man	H	Glc_1
G1M8B (10)	H	Manα1	Glc_1

Figure 1. (A) Glycoprotein glycan processing in ER. (B) Structure of ER-type *N*-glycans synthesized in this study.

previous studies were conducted with glycoproteins or glycan chains derived from biological sources. In order to gain precise understanding of carbohydrate–protein interplays in protein quality control, access to homogeneous and structurally defined oligosaccharides is highly important. With this respect, approaches based on chemical synthesis are more advantageous, in terms of their flexibility and ability to provide homogeneous glycans in larger amount. We wish to describe here the results of our program toward comprehensive synthesis of ER-related *N*-glycans.[11] Parts of this study have been reported in preliminary forms (M9 and G1M9,[16] M8B and G1M8B,[17] G2M9 and G3M9.[18]

Considering the diversity of the targets, we adopted a convergent strategy as shown in Figure 2. Thus, fragments corresponding to an invariant hexasaccharide **11**, branched penta-, tri-, and tetramannoside (**M5**, **M3**, **M4B**, and **M4C**) fragments, and mono-, di-, and triglucoside donors (**G1**, **G2**, and **G3**) were chosen. Combination of these fragments should allow for the construction of all types of high-mannose-type sugar chains.

2. Results and discussion

2.1. Synthesis of an invariant hexasaccharide

Synthesis of hexasaccharide **11** was carried out as depicted in Scheme 1. It started with the preparation of trisaccharide **25**. Its β-mannoside linkage was constructed using the 2-*O*-*p*-methoxybenzyl (PMB) equipped thioglycoside **20** as a donor. Thus, coupling with the 4-*O*-unprotected fluoride

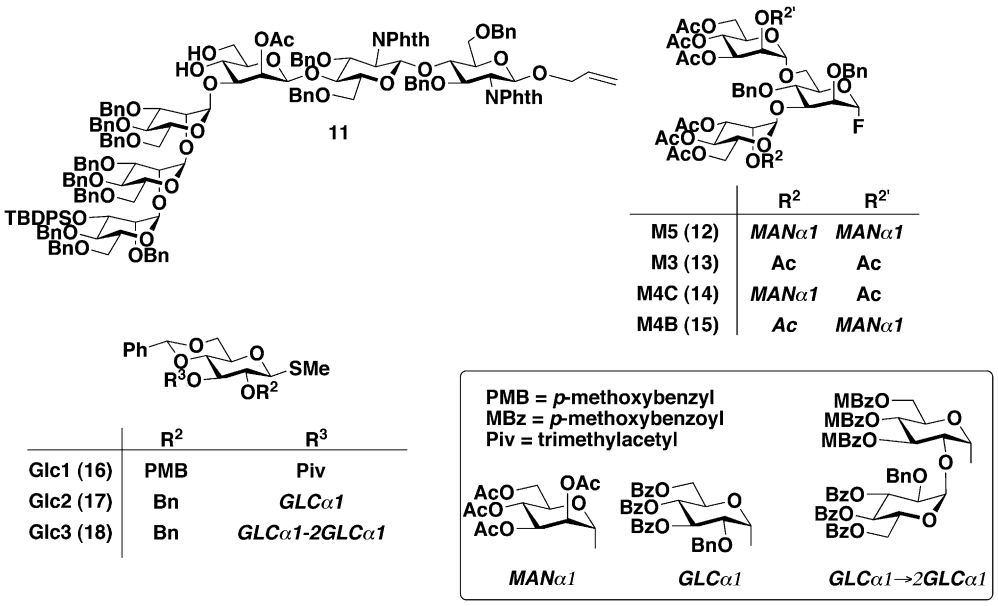


Figure 2. Designed oligosaccharide fragments.

19 was conducted according to our standard protocol; DDQ-mediated formation of the mixed acetal and subsequent intramolecular aglycon delivery (IAD) that was promoted by MeOTf to afford disaccharide **21** as a single stereoisomer in 84% yield (two steps).[19,20] NMR of **21** revealed the presence of newly formed β-mannoside linkage [δ_H 4.37 ($J_{1,2}$=~0 Hz), δ_C 99.7 ($^1J_{C-H}$=159 Hz)].[21] After acetylation, it was condensed with a reducing-end GlcN component **23** through the activation with Cp_2HfCl_2/AgOTf[22,23] to give **24**. Subsequent desilylation proceeded most cleanly under high-pressure conditions (1 GPa) with HF/pyridine[24] to give **25**.

The linear mannotriose **31** was synthesized in a stepwise manner. Coupling of a thiomannoside **26** with chloride **27** was performed under standard conditions (AgOTf/CH_2Cl_2) to give mannobiose **28**,[25,26] which was deacetylated to **29**. As the third mannose residue, the 3-*O*-TBDPS protected fluoride **30** was used and trisaccharide **31** was obtained in a quantitative yield. Three anomeric signals appeared in the region of α-Man linkage [δ_H 5.75, 5.54 and 5.21 (s)], while that of β-Man linkage is typically less than 5 ppm,[27] confirming its stereochemistry. Coupling with **25** was achieved using MeOTf to afford hexasaccharide **32**. Although rigorous assignment of αMan1-3Man linkage was not made at this stage, homogeneity of isolated **32** was obvious and we assumed its stereochemical integrity based on well-established intrinsic α-selectivity of mannosyl donor as well as on our own results. Subsequent removal of the cyclohexylidene group completed the synthesis of the common hexasaccharide fragment **11**.

2.2. Synthesis of oligomannose branch

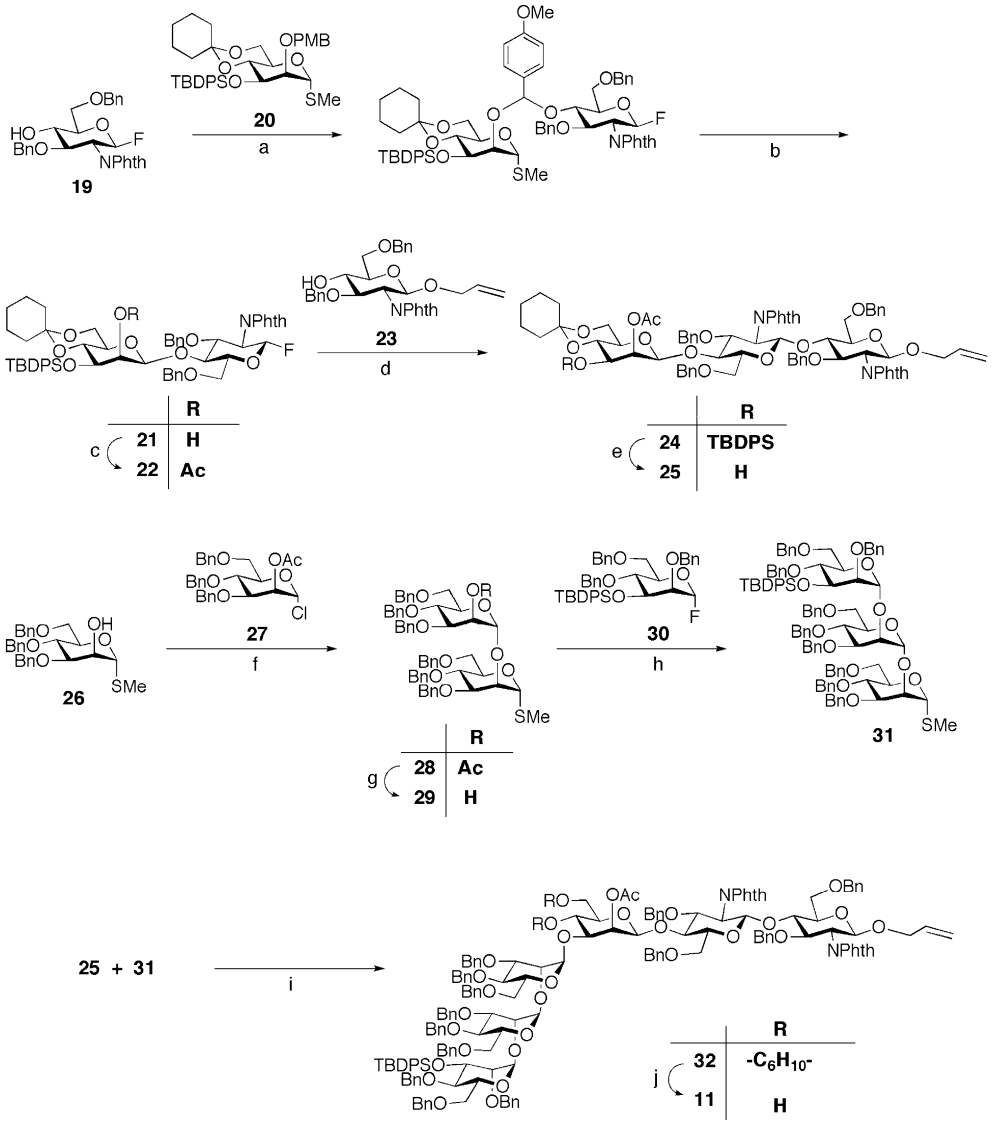
Syntheses of branched mannooligosaccharide fragments, M5 (**12**), M3 (**13**), M4C (**14**), and M4B (**15**) were conducted as shown in Scheme 2. Preparation of **12** and **13** was straightforward, being achieved by double glycosylation of diol **35** with **33**/**34** to give **36**/**37**, spectral data of which were identical with previous reported ones.[17,28] They were treated with *N*-bromosuccinimide (NBS)/diethylaminosulfur trifluoride (DAST)[29] to give corresponding fluorides **12** [δ_H 5.66 (d, $^2J_{H-F}$=51.5 Hz)] and **13** [δ_H 5.59 (d, $^2J_{H-F}$=52.0 Hz)] in 89% and 76% yield, respectively. These were used as M5 (**12**) and M3 (**13**) donors for subsequent transformations.

Construction of the heterogeneously branched M4C fragment necessitated the discrimination of *O*-3 and *O*-6 positions. To that end, compound **35** was regioselectively protected by treatment with chloroacetic anhydride (CAc_2O) in toluene/dichloromethane (1:1) at 60 °C to give 6-*O*-CAc derivative **39**.[30] The latter was glycosylated with a disaccharide donor **38**[28] using TfOH as a promoter to give trisaccharide **40** [δ_C 100.57, 99.17, and 84.27 (*C*-1)]. Subsequent removal of the chloroacetyl group provided **41**, which was further glycosylated with **34**, giving branched tetramannoside **42** in 50% yield [δ_C 100.52, 99.16, 97.79, and 84.58 (*C*-1)]. M4B fragment **43** was synthesized from diol **35** as previously reported.[30] These branched oligosaccharides were converted to fluoride **14** [δ_H 5.52 ($^2J_{H-F}$=50.7 Hz)] and **15** [δ_H 5.66 ($^2J_{H-F}$=50.8 Hz)] using NBS–DAST.

2.3. Synthesis of oligoglucose fragments

Mono-, di-, and triglucosyl donors were designed as **16**, **17**, and **18**, respectively (Fig. 2, Scheme 3). Our previous studies revealed that 4,6-*O*-benzylidene-protected thioglucoside was highly suitable for α-selective glucosylation at C-3 position of mannose. The G1 donor **16** was synthesized from **44**. For the synthesis of disaccharide (**17**) and trisaccharide (**18**) fragments, **48** and **52** were chosen as donors, respectively. We anticipated that the presence of electron-withdrawing acyloxy groups[31] at 3-, 4-, and 6-positions would be favorable for the stereoselective formation of α-glucosidic linkages (vide infra).

For the preparation of these donors, the 2-OH derivative **49** and hemiacetal **47** were chosen as key intermediates. These compounds were prepared from glucosyl bromides via cyclic acetals, as described by Suzuki et al.[32,33] Hemiacetal



Scheme 1. Reagents and conditions: (a) DDQ, CH_2Cl_2, rt, 3 h; (b) MeOTf, $ClCH_2CH_2Cl$, 45 °C, 23 h (84% two steps); (c) Ac_2O, DMAP, pyr., 40 °C, 8 h (97%); (d) Cp_2HfCl_2, AgOTf, CH_2Cl_2, −10 °C, 4 h (78%); (e) 10% HF–pyr., DMF, 1 GPa; (f) AgOTf, $ClCH_2CH_2Cl$, −30 °C to rt, 12 h (83%); (g) NaOMe, MeOH/THF, rt, 12 h (95%); (h) Cp_2HfCl_2, AgOTf, CH_2Cl_2, −45 to −20 °C, 1 h (99%); (i) MeOTf, $ClCH_2CH_2Cl$, rt, 12 h (82%); (j) $TsOH\cdot H_2O$, CH_3CN, rt, 6 h (74%).

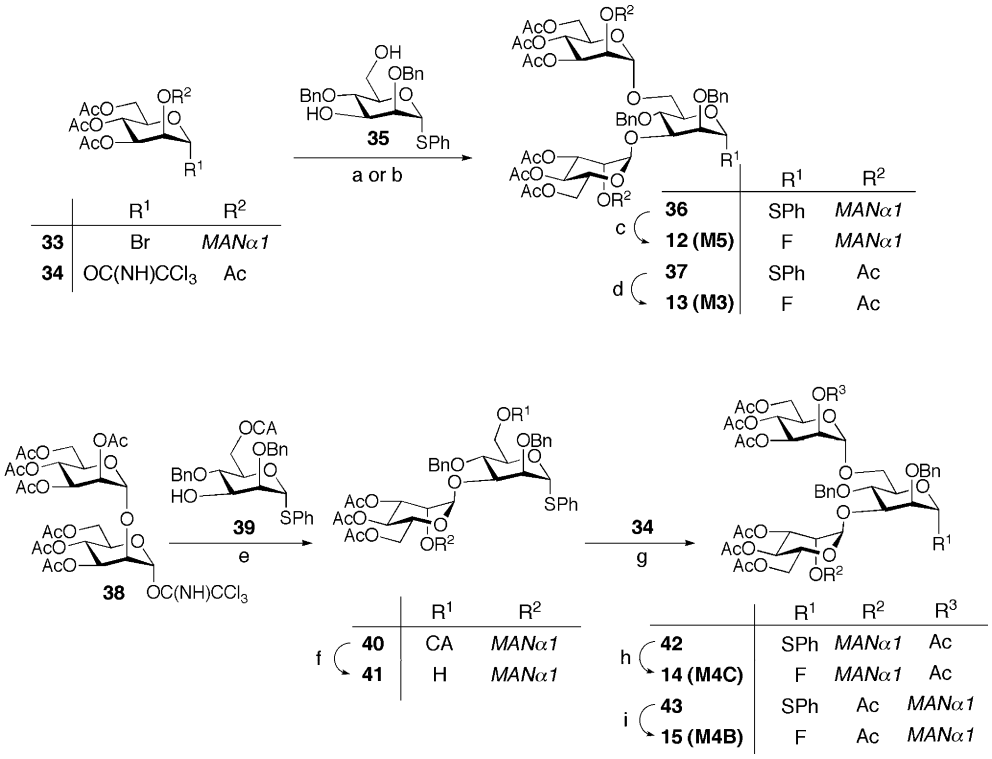
47 was converted to fluoride **48** using DAST and used for the glycosylation with **46** through activation with Cp_2HfCl_2/AgOTf. It afforded disaccharide **17** as a single stereoisomer [δ_H 5.73 (d, J=3.6 Hz, H-1$^{\alpha\text{-Glc}}$)] in 63% yield. The G3 fragment **18** was synthesized by the stepwise elongation of glucose residues from the non-reducing end. To begin with, glycosylation of **49** with **48** using Cp_2HfCl_2/AgOTf afforded disaccharide **50** as a single stereoisomer [δ_H 5.14 (d, J= 3.6 Hz, H-1$^{\alpha\text{-Glc}}$)] in 92% yield. Removal of the *p*-methoxybenzyl group using DDQ afforded hemiacetal **51** that was converted to α-imidate **52**. Coupling of **52** and **46** through activation with TMSOTf in toluene provided trisaccharide **18** in 45% yield, whose ^{1}H NMR reveled the presence of three anomeric protons with α-configuration [δ_H 5.82 (J=3.6 Hz), 5.40 (s), 4.93 (J=3.2 Hz)].

2.4. Systematic synthesis of high-mannose-type glycans

With all fragments in hand, the construction of high-mannose-type skeletons (undeca-, deca-, and nonasaccharide) and the introduction of glucose fragments were undertaken as shown in Scheme 4.

Pentamannoside **12** was used as the glycosyl donor to react with diol **11** using Cp_2HfCl_2/AgOTf in toluene to provide undecasaccharide **53** in 87% yield as a single stereoisomer, which is currently obtainable in multigram quantity (see Section 4). Similar conditions were applied to **13**, **14**, and **15**, and nonasaccharide **55**, as well decasaccharides **57** and **59** were obtained in 77%, 75%, and 84% yield, respectively. Although we were not able to confirm rigorously their stereochemistry at this stage, contamination of stereoisomer was not detectable by 400 MHz NMR in each case. Since selective formation of β-isomer is highly unlikely, we assumed their structure as depicted. Full confirmation was made after complete deprotection (vide infra).

In order to incorporate the pendant glucose residue(s), removal of the TBDPS group from **53**, **55**, **57**, and **59** was required. Mindful of the difficulty we previously encountered



Scheme 2. Reagents and conditions: (a) **33**, AgOTf, $ClCH_2CH_2Cl$, −30 °C to rt (72%); (b) **34**, TfOH, $ClCH_2CH_2Cl$, −20 °C (39%); (c) NBS, DAST, CH_2Cl_2, −20 to −10 °C (89%); (d) NBS, DAST, CH_2Cl_2, −30 °C to rt (76%); (e) TfOH, $ClCH_2CH_2Cl$, −20 to −10 °C (84%); (f) DABCO, EtOH, 50 °C (95%); (g) TfOH, $ClCH_2CH_2Cl$, −20 to −10 °C (50%); (h) NBS, DAST, CH_2Cl_2, −40 to −30 °C (87%); (i) NBS, DAST, CH_2Cl_2, −30 °C to rt (90%).

in the deprotection of TBDPS groups installed at the *O*-3-position of mannose, all of these reactions were conducted with HF/pyridine under high-pressure reaction conditions.[24] For instance, treatment of undecasaccharide **53** in DMF with 10% HF/pyridine at 30 °C under 1 GPa for 24 h cleanly gave the desired product **54** in 95% yield. In a similar manner, **56**, **58,** and **60** were obtained from **55**, **57**, and **59** in 86%, 70% and 97% yield, respectively.

As we hoped, incorporation of mono-, di-, and triglucosyl fragment proceeded selectively to provide desired α-linked products. Namely, MeOTf-promoted coupling of the undecasaccharide **54** with triglucoside **18** gave tetradecasaccharide **61** in 57% yield, and tridecasaccharide **62** was obtained in 85% yield under similar conditions, when **17** was used as the donor. Introduction of a glucose residue to M9, M7, M8C, and M8B type of sugar chain was achieved using the donor **16** to give **63**, **64**, **65**, and **66** in 93%, 86%, 91%, and 79% yield, respectively.

Finally, deprotection of these compounds afforded the ER-type sugar chains in good yield. Figure 3 showed the ^1H NMR spectra of these compounds, which were in good agreement with those reported for closely related compounds.[34]

3. Conclusion

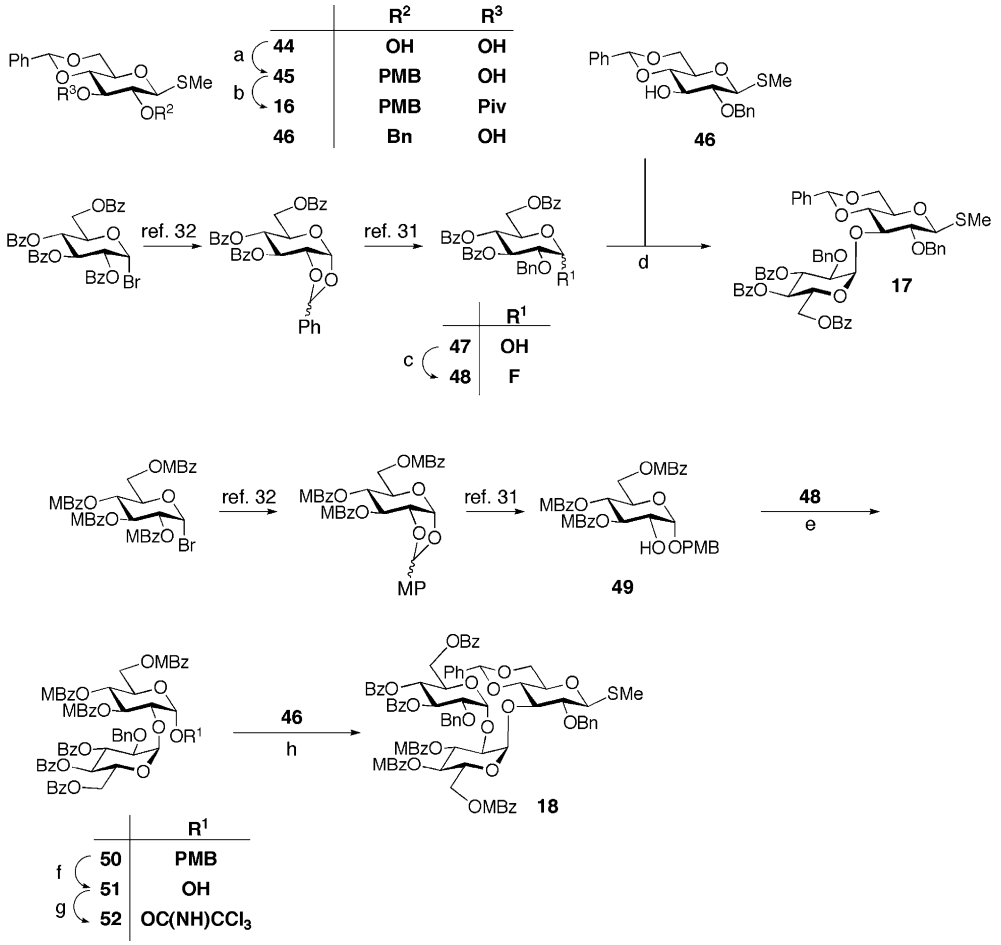
In summary, convergent and stereoselective synthetic route to ER-related *N*-glycan chains was established. Although oligosaccharides were prepared as chemically inert *n*-propyl glycosides in this study, tactics for the incorporation of probes and proteins have been established.[11,35–38] Currently, these glycan chains and derivatives are in extensive use as molecular probes to clarify various issues related to glycoprotein quality control system, including specificities of protein–oligosaccharide interactions and glycan processing enzymes. These results will be reported in due course.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were measured on JEOL EX-400 spectrometer in $CDCl_3$ and were referenced to Me_4Si unless otherwise mentioned. Silica gel column chromatography was performed using Silica gel-60 (E Merck). Preparative thin layer chromatography (PTLC) was developed on E Merck Silica Gel 60 F_{254} plates (0.5 mm thickness). MALDI-TOF MS spectra were recorded in the positive ion mode on an AXIMA CFR (Shimadzu/KRATOS) equipped with nitrogen laser with an emission wavelength of 337 nm. High-resolution fast atom bombardment mass spectrometry was performed on a JEOL IMS-HX-100 mass spectrometer.

4.1.1. Compound 22. Under ice-water cooling, a mixture of DDQ (3.16 g, 15.9 mmol) and molecular sieves 4 Å (15 g) in CH_2Cl_2 (40 mL) was stirred under Ar. A solution of **20** (9.90 g, 15.3 mmol) and **19** (6.25 g, 12.7 mmol) in CH_2Cl_2 (60 mL) was added and the mixture was stirred at room temperature for 3 h. It was then quenched with aq solution of ascorbic acid (0.7%)/citric acid (1.3%)/NaOH (0.9%), stirred for 5 min, diluted with CH_2Cl_2/brine, and filtered

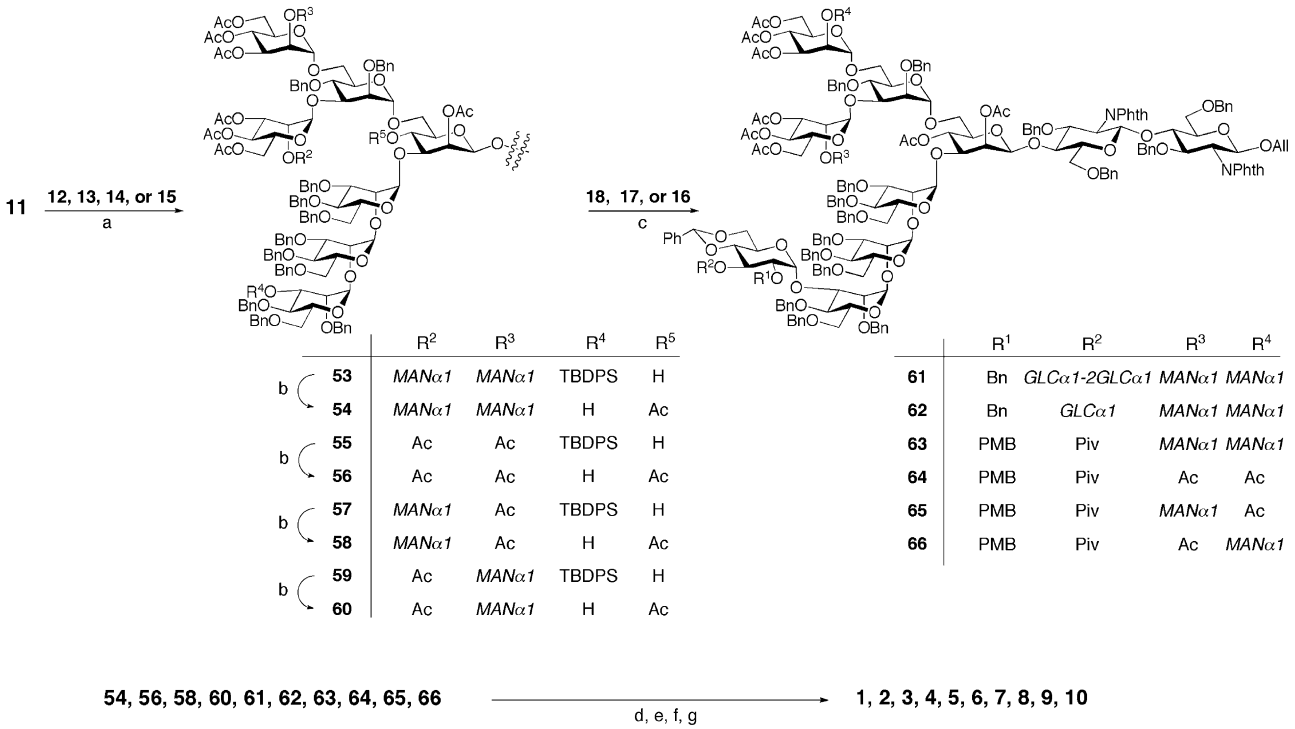


Scheme 3. Reagents and conditions: (a) PMBCl, Bu_4NHSO_4, aq NaOH, 45 °C (52%); (b) PivCl, pyr., 0 °C to rt (61%); (c) DAST (92%); (d) Cp_2HfCl_2, AgOTf, toluene/ether, −40 °C (63%); (e) Cp_2HfCl_2, AgOTf, CH_2Cl_2, −10 °C (90%); (f) DDQ, CH_2Cl_2, H_2O, rt (73%); (g) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1 h (quant.); (h) TMSOTf, toluene, −40 °C (45%).

through Celite. The filtrate was washed with aq $NaHCO_3$ and the organic layers were dried over Na_2SO_4 and evaporated in vacuo. The residue was mixed with 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) (10.66 g, 51.91 mmol) and co-evaporated with toluene three times. The residue was stirred at room temperature with molecular sieves 4 Å (30 g) in $ClCH_2CH_2Cl$ (200 mL). Then, a solution of 1 M MeOTf in $ClCH_2CH_2Cl$ (36.1 mL, 36.1 mmol) was added at 0 °C and stirred at 45 °C for 23 h. The reaction was quenched with Et_3N, diluted with EtOAc/aq $NaHCO_3$, and filtered through Celite. The filtrate was washed with aq $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 5:1) to afford **21** (10.14 g, 82%). Disaccharide **21** and DMAP (122 mg) were dissolved in-pyridine (20 mL) and Ac_2O (10 mL). The mixture was stirred at 40 °C for 8 h and evaporated in vacuo. The residue was diluted with EtOAc, and washed with aq HCl, brine, aq $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, and evaporated in vacuo to give **22** (10.27 g, 97%). Physical data were consistent with those reported previously (Ref. 16).

4.1.2. Compound 24. To a stirred mixture of Cp_2HfCl_2 (3.73 g, 9.62 mmol), AgOTf (5.19 g, 20.2 mmol), and molecular sieves 4 Å (14.5 g) in dry CH_2Cl_2 (500 mL) was added a solution of **22** (19.52 g, 19.25 mmol) and **23** (12.23 g, 23.10 mmol) in dry CH_2Cl_2 (800 mL) at −10 °C. The mixture was stirred for 4 h. Insoluble materials were removed by passage through Celite, and the filtrate was then diluted with CH_2Cl_2, and washed with brine, aq $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and evaporated in vacuo. The residue was subjected to a silica gel column chromatography (toluene/EtOAc, 20:1 to 10:1) to afford compound **24** (22.90 g, 78%). Physical data were consistent with those reported previously (Ref. 16).

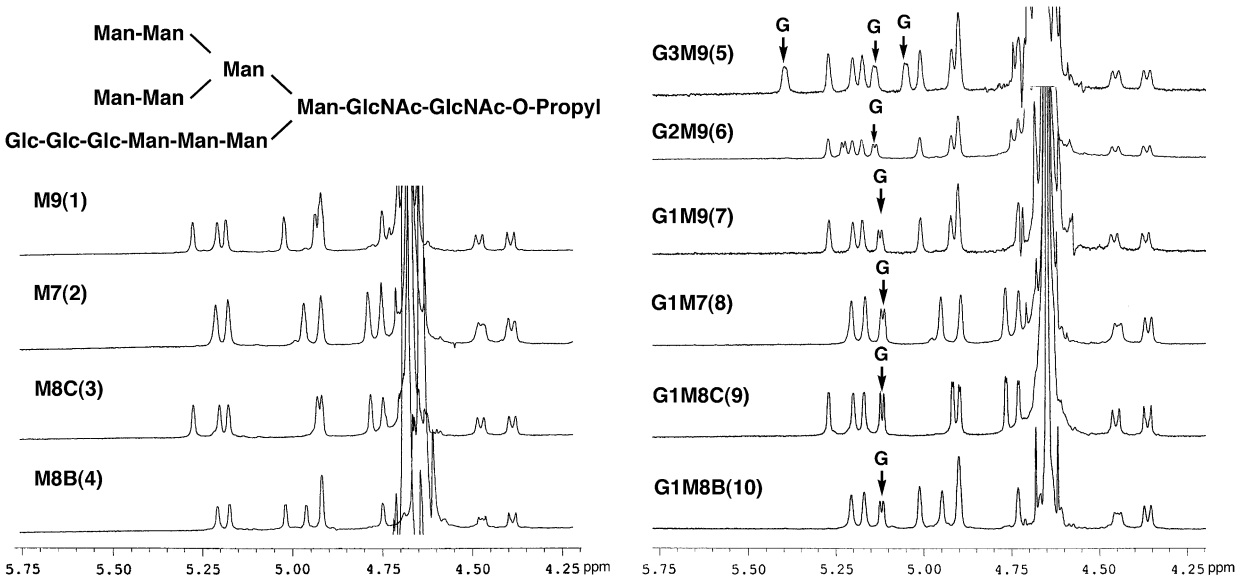
4.1.3. Compound 25. Compound **24** (16.0 g, 10.5 mmol) was dissolved in DMF (24 mL) containing 10% HF/pyridine. The mixture was divided to eight portions and transferred to 3 mL Teflon reaction vessels. It was compressed to 1.0 GPa and left at 30 °C for 5 h. Combined mixture was diluted with EtOAc and washed with aq $NaHCO_3$ and brine, successively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The combined mixtures were subjected to a silica gel column chromatography (hexane/EtOAc=2:1–3:2) to give compound **25** (11.1 g, 86% yield) as a colorless solid: ^1H NMR (400 MHz, $CDCl_3$) δ 7.64–7.00 (m, 28H), 5.50 (m, 1H), 5.27 (d, 1H, J=2.4 Hz), 5.23 (d, 1H, J=8.0 Hz), 5.01–4.91 (m, 3H), 4.84 (d, 1H, J=12.8 Hz), 4.83 (d, 1H, J=12.8 Hz), 4.68 (br s, 1H), 4.58 (d, 1H, J=12.0 Hz),



	R^2	R^3	R^4	R^5
53	*MANα1*	*MANα1*	TBDPS	H
54	*MANα1*	*MANα1*	H	Ac
55	Ac	Ac	TBDPS	H
56	Ac	Ac	H	Ac
57	*MANα1*	Ac	TBDPS	H
58	*MANα1*	Ac	H	Ac
59	Ac	*MANα1*	TBDPS	H
60	Ac	*MANα1*	H	Ac

	R^1	R^2	R^3	R^4
61	Bn	*GLCα1-2GLCα1*	*MANα1*	*MANα1*
62	Bn	*GLCα1*	*MANα1*	*MANα1*
63	PMB	Piv	*MANα1*	*MANα1*
64	PMB	Piv	Ac	Ac
65	PMB	Piv	*MANα1*	Ac
66	PMB	Piv	Ac	*MANα1*

Scheme 4. Reagents and conditions: (a) Cp_2HfCl_2, AgOTf, toluene, −30 to −10 °C; **12** (87%), **13** (77%), **14** (75%), **15** (84%); (b) (1) Ac_2O, pyr., 50 °C; (2) 10% HF–pyr., DMF, 1 Gpa; **54** (95%), **56** (86%), **58** (70%), **60** (97%); (c) MeOTf, CH_2CH_2, cyclohexane, (1) **61** (**54**+**18**; 57%), **62** (**54**+**17**; 85%), **63** (**54**+**16**; 93%), **64** (**56**+**16**; 86%), **65** (**58**+**16**; 91%), **66** (**60**+**16**; 79%); (d) ethylenediamine, *n*-BuOH; (e) Ac_2O, pyr.; (f) NaOMe, MeOH; (g) $Pd(OH)_2$/C, aq AcOH, H_2, **1** (54%), **2** (84%), **3** (47%), **4** (87%), **5** (56%), **6** (65%), **7** (54%), **8** (84%), **9** (66%), **10** (58%).



	GlcNAc (Hz)		Man								Glc (Hz)		
M9 (1)	4.36 (*J*=7.5)	4.45 (*J*=7.6)	4.73	4.90	4.90	4.92	5.01	5.17	5.20	5.27	***	***	***
M7 (2)	4.36 (*J*=6.8)	4.45 (*J*=6.6)	4.73	4.77	4.91	4.96	***	5.17	5.21	***	***	***	***
M8C (3)	4.36 (*J*=7.6)	4.45 (*J*=7.6)	4.73	4.77	4.91	4.92	***	5.17	5.20	5.27	***	***	***
M8B (4)	4.36 (*J*=8.0)	4.45 (*J*=7.5)	4.73	4.90	4.90	4.95	5.01	5.17	5.20	***	***	***	***
G3M9 (5)	4.36 (*J*=6.8)	4.45 (*J*=7.2)	4.76	4.90	4.90	4.92	5.01	5.17	5.20	5.27	5.04(*J*=3.2)	5.13(*J*=2.4)	5.39(*J*=2.4)
G2M9 (6)	4.35 (*J*=6.8)	4.57 (*J*=7.0)	4.71	4.89	4.89	4.90	5.00	5.16	5.19	5.26	***	5.12(*J*=3.9)	5.21(*J*=3.6)
G1M9 (7)	4.37 (*J*=7.5)	4.46 (*J*=7.3)	4.73	4.90	4.90	4.92	5.01	5.17	5.20	5.27	***	5.12(*J*=3.7)	***
G1M7 (8)	4.36 (*J*=7.3)	4.44 (*J*=6.6)	4.73	4.77	4.90	4.95	***	5.17	5.21	***	***	5.11(*J*=3.6)	***
G1M8C (9)	4.36 (*J*=8.0)	4.45 (*J*=7.5)	4.73	4.77	4.90	4.92	***	5.17	5.20	5.27	***	5.11(*J*=4.0)	***
G1M8B (10)	4.36 (*J*=7.2)	4.45 (*J*=6.8)	4.68	4.90	4.90	4.95	5.01	5.17	5.21	***	***	5.11(*J*=3.9)	***

Figure 3. ^{1}H NMR spectra of high-mannose-type oligosaccharides.

4.51–4.36 (m, 5H), 4.23–4.09 (m, 7H), 3.87 (m, 1H), 3.73–3.69 (m, 2H), 3.61–3.38 (m, 6H), 3.29 (m, 1H), 3.18 (m, 1H), 2.98 (m, 1H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.16, 167.22, 138.52, 138.42, 138.08, 137.69, 137.61, 133.73, 133.55, 133.28, 131.52, 131.25, 128.82, 128.34, 128.06, 128.02, 127.92, 127.84, 127.70, 127.61, 127.58, 127.38, 127.29, 127.13, 126.86, 126.64, 125.10, 123.41, 122.19, 116.93, 99.87, 99.09, 97.02, 98.87, 78.92, 76.82, 75.71, 74.47, 74.44, 73.16, 72.93, 72.71, 71.35, 71.24, 70.38, 70.15, 69.43, 68.15, 67.82, 67.60, 61.02, 60.35, 56.54, 55.66, 41.99, 37.92, 27.99, 27.07, 25.62, 25.07, 22.82, 22.58, 21.53, 21.16, 21.11, 14.30; HRMS (FAB) *m/z* calcd for $C_{73}H_{76}N_2O_{19}Na$ 1307.4940 $(M+Na)^+$, found 1307.4940.

4.1.4. Compound 28. A mixture of AgOTf (12.7 g, 49.6 mmol) and molecular sieves 4 Å (30 g) in dry toluene (105 mL) was stirred at −40 °C for 30 min. A solution of compounds **27** (10.8 g, 21.3 mmol) and **26** (8.5 g, 18 mmol) in dry $ClCH_2CH_2Cl$ (159 mL) was added dropwise over 40 min and the mixture was stirred at −30 °C for 1 h and at ambient temperature for 12 h. The reaction was quenched with TEA (20 mL). The reaction mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with aq $NaHCO_3$ and brine, successively. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 10:1 to 1:1) to afford **28** (14.1 g, 83%). Physical data were consistent with those reported previously (Ref. 16).

4.1.5. Compound 29. To a stirred solution of **28** (14.1 g, 14.8 mmol) in THF/MeOH (1:1, 148 mL) was added 28% NaOMe/MeOH (1.0 mL) at room temperature. The mixture was stirred for 12 h, neutralized with Amberlyst 15 (H^+) resin, and evaporated in vacuo. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 5:1 to 1:1) to give **29** (12.9 g, 95%). Physical data were consistent with those reported previously (Ref. 16).

4.1.6. Compound 31. To a stirred mixture of Cp_2HfCl_2 (5.71 g, 15.0 mmol), AgOTf (7.74 g, 30.1 mmol), and molecular sieves 4 Å (5 g) in dry CH_2Cl_2 (200 mL) was added a solution of **30** (8.04 g, 11.6 mmol) and **29** (9.17 g, 10.0 mmol) in dry CH_2Cl_2 (50 mL) at −45 °C. The mixture was gradually warmed up to −20 °C and stirred for 1 h. The reaction was quenched with TEA (20 mL) and processed as described for **28**. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 10:1 to 5:1) to afford the compound **31** (15.9 g, 99%). Physical data were consistent with those reported previously (Ref. 16).

4.1.7. Compound 32. A mixture of **25** (2.73 g, 2.12 mmol), **31** (5.05 g, 3.19 mmol), and molecular sieves 4 Å (15 g) in dry CH_2Cl_2 (200 mL) was stirred at −40 °C for 30 min, to which was added 1 M MeOTf (31.8 mL, 31.8 mmol) in $ClCH_2CH_2Cl$. The mixture was stirred at −40 °C for 30 min and at ambient temperature (12 h). The reaction was quenched with TEA (10 mL) and processed as described for **28**. The residue was subjected to a silica gel column chromatography (toluene/EtOAc, 30:1) to afford **32** (4.92 g, 82%). Physical data were consistent with those reported previously (Ref. 16).

4.1.8. Compound 11. To a stirred solution of compound **32** (4.29 g, 1.74 mmol) in dry CH_3CN was added *p*-toluenesulfonic acid monohydrate (0.83 g, 4.4 mmol) and stirred for 6 h at room temperature. The reaction was quenched with TEA (0.1 mL) and concentrated in vacuo. The residue was subjected to a silica gel column chromatography (toluene/EtOAc, 10:1) to afford the compound **11** (3.54 g, 74%). Physical data were consistent with those reported previously (Ref. 16).

4.1.9. Compound 36. A mixture of AgOTf (14.8 g, 57.6 mmol), molecular sieves 4 Å (100 g), and compound **35** (5.64 g, 12.8 mmol) in dry $ClCH_2CH_2Cl$ (100 mL) was stirred at 0 °C for 30 min, then cooled at −30 °C. The mixture was added a solution of **33** (20.2 g, 28.9 mmol) in dry $ClCH_2CH_2Cl$ (100 mL) dropwise over 15 min. The reaction mixture was stirred at −30 °C for 1 h and at ambient temperature (12 h). It was then quenched with TEA (1 mL) and processed as described for **28**. The residue was subjected to silica gel column chromatography (hexane/EtOAc, 1:1 to 1:3) to afford **36** (21.0 g, 72%). Physical data were consistent with those reported previously (Ref. 28).

4.1.10. Compound 12. A mixture of compound **36** (6.00 g, 3.56 mmol) and NBS (948 mg, 5.33 mmol) in CH_2Cl_2 (100 mL) was added DAST (1.41 mL, 10.6 mmol) at −30 °C. The mixture was stirred at −30 °C for 1 h and at ambient temperature (3 h). The reaction mixture was processed as described for **28**. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 3:2) to afford **12** (5.05 g, 89%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.29 (m, 10H), 5.66 (d, 1H, J=51.5 Hz), 5.40–5.21 (m, 10H), 5.07 (br s, 1H), 4.89 (d, 1H, J=12 Hz), 4.81–4.64 (m, 4H), 4.20–3.71 (m, 19H), 2.15 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.087 (s, 3H), 2.07 (s, 3H), 2.05 (s, 9H), 2.04 (s, 3H), 2.03 (s, 6H), 2.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.73, 170.55, 170.39, 170.31, 170.13, 169.61, 169.57, 169.54, 169.34, 169.30, 169.20, 169.10, 137.48, 137.08, 128.51, 128.40, 127.84, 127.73, 127.55, 127.15, 105.51, 100.08, 99.25, 99.14, 99.08, 78.38, 77.78, 77.49, 75.79, 75.45, 74.91, 74.25, 73.44, 72.41, 70.10, 69.91, 69.83, 69.67, 69.53, 69.27, 69.10, 68.55, 68.40, 66.25, 66.19, 66.11, 65.90, 62.45, 62.28, 62.13, 61.89, 20.86, 20.81, 20.68, 20.52; MALDI-TOF mass *m/z* calcd for $C_{72}H_{91}FO_{39}Na$ 1621.5 $(M+Na)^+$, found 1622.2.

4.1.11. Compound 37. A mixture of **35** (1.11 g, 2.45 mmol), TfOH (20 μL), and molecular sieves AW 300 (6 g) in dry $ClCH_2CH_2Cl$ (20 mL) was stirred at −20 °C for 30 min. The donor **33** (2.90 g, 5.89 mmol) in dry $ClCH_2CH_2Cl$ (5 mL) was added dropwise over 10 min. The reaction was quenched with aq $NaHCO_3$ and processed as described for **28**. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 5:1 to 1:1) to afford **37** (1.01 g, 39%). Physical data were consistent with those reported previously (Ref. 28).

4.1.12. Compound 13. A mixture of compound **37** (229 mg, 0.206 mmol) and NBS (73.4 mg, 0.412 mmol) in CH_2Cl_2 (2 mL) was added DAST (82 μL, 0.62 mmol) at −40 °C. The reaction mixture was stirred at −30 °C for 1 h and then at ambient temperature. After 12 h, MeOH (0.1 mL)

was added and the mixture was diluted with EtOAc, washed successively with aq $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to a silica gel column chromatography (toluene/EtOAc, 5:1 to 2:1) to afford compound **13** (162 mg, 76%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.24 (m, 10H), 5.59 (d, 1H, J=52.0 Hz), 5.40–5.17 (m, 9H), 5.15 (br s, 1H), 4.90 (d, 1H, J=2.0 Hz), 4.87 (d, 1H, J=11.6 Hz), 4.80 (d, 1H, J=12.4 Hz), 4.71 (d, 1H, J=12.4 Hz), 4.64 (d, 1H, J=11.6 Hz), 4.21 (dd, 1H, J=4.8, 12.0 Hz), 4.14–3.72 (m, 16H), 2.14 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.36, 170.26, 170.16, 169.47, 169.43, 169.37, 169.32, 169.27, 169.24, 137.18, 137.09, 128.48, 128.41, 128.34, 128.32, 128.22, 127.85, 127.72, 127.66, 127.57, 127.56, 105.26, 99.05, 97.94, 75.05, 73.90, 73.85, 72.60, 69.37, 69.31, 68.92, 68.84, 68.71, 68.47, 66.38, 66.13, 66.06, 65.98, 62.46, 62.23, 20.92, 20.75, 20.69, 20.59; MALDI-TOF mass m/z calcd for $C_{48}H_{59}FO_{23}Na$ 1045.3 $(M+Na)^+$, found 1045.4.

4.1.13. Compound 40. A mixture of compound **39** (0.497 g, 0.939 mmol), TfOH (10 μL), and molecular sieves AW 300 (4 g) in dry $ClCH_2CH_2Cl$ (10 mL) was stirred at −20 °C for 30 min. A solution of the glycosyl donor **38** (0.90 g, 1.2 mmol) in dry $ClCH_2CH_2Cl$ (10 mL) was added dropwise over 10 min and stirred at −10 °C for 8 h. The reaction was quenched with aq $NaHCO_3$ and processed as described for **28**. The residue was subjected to a silica gel column chromatography (toluene/EtOAc, 5:1 to 1.5:1) to afford compound **40** (0.89 g, 84%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.27 (m, 15H), 5.66 (d, 1H, H-1), 5.38–5.23 (m, 6H), 4.85 (d, 1H, J=11.6 Hz), 4.77 (d, 1H, J=11.6 Hz), 4.71 (d, 1H, J=1.6 Hz), 4.60 (d, 1H, J=11.6 Hz), 4.50 (d, 1H, J=11.6 Hz), 4.37–3.93 (m, 16H), 2.15 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.42, 170.24, 170.05, 169.48, 169.39, 169.08, 168.89, 166.56, 166.56, 137.31, 136.95, 133.23, 131.30, 128.99, 128.39, 128.34, 127.99, 127.71, 127.65, 127.61, 127.34, 100.57, 99.17, 84.27, 81.01, 78.72, 77.75, 75.24, 74.16, 71.05, 70.63, 69.98, 69.60, 69.29, 69.05, 68.30, 66.19, 65.86, 64.53, 62.60, 62.31, 40.63, 20.93, 20.82, 20.75, 20.73, 20.71; MALDI-TOF mass m/z calcd for $C_{54}H_{63}ClO_{23}SNa$ 1169.3 $(M+Na)^+$, found 1168.5.

4.1.14. Compound 41. Trisaccharide **40** (453 mg, 0.423 mmol) was dissolved in EtOH (20 mL) and treated with DABCO (300 mg) at 50 °C for 2 h. The mixture was neutralized with Amberlist 15 E [H^+]. Insoluble materials were removed by filtration and the filtrate was concentrated in vacuo. The residue was subjected to a silica gel column chromatography (toluene/EtOAc, 3:1 to 1:1) to give **41** (430 mg, 95%). Physical data were consistent with those reported previously (Ref. 28).

4.1.15. Compound 42. A mixture of compound **41** (377 mg, 0.352 mmol), TfOH (15 μL), and molecular sieves AW 300 (4 g) in dry $ClCH_2CH_2Cl$ (10 mL) was stirred at −20 °C for 10 min. A solution of glycosyl donor **34** (472 mg, 0.958 mmol) in dry $ClCH_2CH_2Cl$ (10 mL) was added dropwise over 10 min and stirred at −10 °C for 1 h. Resulting mixture was processed as described for **28**. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 5:1 to 1:2) to afford **42** (249 mg 50%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.27 (m, 15H), 5.62 (d, 1H, J=1.2 Hz), 5.36–5.21 (m, 9H), 4.89 (d, 1H, J=10.8 Hz), 4.81 (d, 1H, J=12.0 Hz), 4.70 (d, 1H, J=2.0 Hz), 4.64 (d, 1H, J=11.6 Hz), 4.49 (d, 1H, J=12.4 Hz), 4.23–3.80 (m, 17H), 2.15 (s, 3H), 2.14 (s, 3H), 2.12 (s, 6H), 2.10 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.49, 170.31, 169.98, 169.51, 169.45, 169.35, 169.33, 169.10, 168.95, 137.66, 137.14, 133.64, 131.35, 129.08, 128.43, 128.33, 127.60, 127.55, 127.42, 127.39, 100.52, 99.16, 97.79, 84.58, 78.74, 77.82, 75.23, 74.62, 72.22, 71.00, 70.04, 69.63, 69.38, 69.29, 68.99, 68.95, 68.37, 66.70, 66.22, 66.08, 65.92, 62.58, 62.29, 62.15, 20.99, 20.85, 20.83, 20.79, 20.77, 20.75; MALDI-TOF mass m/z calcd for $C_{66}H_{80}O_{31}SNa$ 1423.4 $[M+Na]^+$, found 1422.7.

4.1.16. Compound 14. Compound **42** (180 mg, 0.128 mmol) was treated with NBS (68 mg, 0.39 mmol) and DAST (51 μL, 0.39 mmol) as described for **13** and purified by silica gel column chromatography (toluene/EtOAc, 5:1 to 2:3) to afford compound **14** (146 mg, 87%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.05 (m, 10H), 5.52 (d, 1H, J=50.7 Hz), 5.27–5.10 (m, 7H), 4.80–4.53 (m, 5H), 4.17–3.57 (m, 14H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 6H), 1.93 (s, 6H), 1.92 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.52, 170.40, 170.29, 170.05, 169.58, 169.51, 169.49, 169.22, 169.06, 137.49, 137.11, 128.84, 128.49, 128.41, 128.36, 128.03, 127.86, 127.79, 127.68, 127.63, 127.60, 127.51, 127.40, 105.17, 100.26, 99.14, 98.02, 78.71, 77.81, 75.01, 73.97, 73.44, 72.44, 69.84, 69.53, 69.34, 69.24, 69.03, 68.82, 68.43, 68.34, 66.23, 66.12, 66.04, 65.90, 62.46, 62.16, 20.83, 20.71, 20.66, 20.63, 20.50; MALDI-TOF mass m/z calcd for $C_{60}H_{75}FO_{31}Na$ 1333.4 $(M+Na)^+$, found 1333.0.

4.1.17. Compound 15. Compound **43** (537 mg, 0.382 mmol) was treated with NBS (135 mg, 0.759 mmol) and DAST (0.10 mL, 0.75 mmol) as described for **13** and purified by silica gel column chromatography (toluene/EtOAc, 5:1 to 2:1) to afford compound **15** (452 mg, 90%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.29 (m, 10H), 5.66 (d, 1H, J=50.8 Hz), 5.41–5.10 (m, 23H), 4.89–4.64 (m, 5H), 4.21–3.75 (m, 30H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 6H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.81, 170.47, 170.43, 170.09, 169.64, 169.60, 169.49, 169.30, 169.28, 137.32, 137.18, 136.92, 128.89, 128.61, 128.53, 128.34, 128.08, 127.86, 127.78, 127.67, 127.45, 125.16, 105.51, 100.08, 99.25, 99.14, 99.08, 77.41, 75.94, 75.42, 75.10, 74.24, 73.86, 72.61, 71.89, 69.95, 69.71, 69.31, 69.10, 69.02, 68.73, 68.61, 68.43, 66.29, 66.16, 65.98, 62.50, 62.34, 61.99, 20.85, 20.71, 20.66, 20.56; MALDI-TOF mass m/z calcd for $C_{60}H_{75}FO_{31}Na$ 1333.4 $(M+Na)^+$, found 1332.7.

4.1.18. Compound 16. To a stirred solution of compound **44** (73.0 mg, 0.245 mmol), p-methoxybenzyl chloride (41 μL), Bu_4NHSO_4 (40 mg) in CH_2Cl_2 (4 mL) was added 5% aq

NaOH (0.5 mL). The mixture was stirred at 45 °C for 12 h. The reaction mixture was diluted with $CHCl_3$ and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was subjected to a PTLC (toluene/EtOAc, 8:1) to afford compound **45** (53.2 mg, 52%) and its regioisomer (33.0 mg, 32%). Compound **45** (327 mg, 0.782 mmol) was dissolved in pyridine (3 mL) and added PivCl (0.57 mL) at 0 °C. The mixture was stirred at room temperature for 12 h. The reaction was added MeOH (1 mL) and evaporated in vacuo, The residue was diluted with EtOAc and washed with aq $CuSO_4$, brine, satd aq $NaHCO_3$, and brine. The organic layer was dried over $NaSO_4$ and evaporated in vacuo. The residue was crystallized from 2-propanol to give **16** (238 mg, 61%) as a colorless needles: mp 124–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–6.85 (m, 9H), 5.58 (s, 1H), 5.41 (t, 1H, J=9.2 Hz), 4.52 (d, 1H, J=9.2 Hz), 4.58–4.53 (m, 2H), 4.38 (dd, 1H, J=4.8, 10.4 Hz), 3.97 (s, 3H), 3.63 (t, 1H, J=9.6 Hz), 3.55–3.51 (m, 2H), 2.26 (s, 3H), 1.22 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.01, 159.26, 136.86, 129.58, 129.38, 128.79, 128.07, 125.80, 113.75, 100.97, 86.31, 79.15, 79.14, 74.75, 74.33, 70.19, 68.63, 55.31, 27.23, 13.46; Anal. Calcd for $C_{27}H_{34}O_7S$: C, 64.52; H, 6.92; S, 6.38. Found: C, 64.26; H, 6.77; S, 6.20.

4.1.19. Compound 48. To a solution of compound **47** (378 mg, 0.649 mmol) in CH_2Cl_2 (10 mL) was added DAST (171 μL, 1.30 mmol) at −40 °C for 30 min. MeOH (0.5 mL) was added to the reaction mixture, which was diluted with EtOAc and washed successively with aq $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, concentrated and subjected to a silica gel column chromatography (toluene/EtOAc, 5:1) to afford **48** (348 mg, 92%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.06 (m, 20H), 5.64–5.53 (m, 2H), 5.44 (dd, 1H, J=6.0, 46.0 Hz), 4.74 (d, 1H, J=12.0 Hz), 4.62–4.53 (m, 3H), 4.38 (dd, 1H, J=5.2, 12.4 Hz), 4.16 (m, 1H), 3.73 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.12 (d, J=220.1 Hz), 77.72 (d, J=25.1 Hz), 73.62, 72.81, 72.02, 68.67, 62.89; MALDI-TOF mass m/z calcd for $C_{34}H_{29}FO_8Na$ 607.6 $(M+Na)^+$, found 607.7.

4.1.20. Compound 17. A mixture of Cp_2HfCl_2 (187 mg, 0.493 mmol), AgOTf (273 mg, 1.06 mmol), and molecular sieves 4 Å (1.4 g) was added a solution of compounds **48** (160 mg, 0.274 mmol) and **46** (107 mg, 0.274 mmol) in ether/toluene (2:1, 30 mL) at −40 °C. The mixture was stirred at −40 °C for 9 h. The reaction was quenched with TEA and processed as described for **28**. The residue was subjected to a column of Bio-Beads SX-4 (toluene) to afford **17** (165 mg, 63%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02–6.69 (m, 30H), 6.01 (t, 1H, J=9.6 Hz), 5.73 (d, 1H, J=3.6 Hz), 5.47 (s, 1H), 5.41 (t, 1H, J=9.9 Hz), 5.20 (d, 1H, J=10.4 Hz), 4.93 (d, 1H, J=10.4 Hz), 4.51 (d, 1H, J=9.6 Hz), 4.36 (dd, 1H, J=4.8, 10.4 Hz), 4.25–4.11 (m, 3H), 3.89 (t, 1H, J=9.6 Hz), 3.83–3.75 (m, 2H), 3.69 (t, 1H, J=9.2 Hz), 3.60 (dd, 1H, J=3.6, 10.0 Hz), 3.54 (m, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.83, 165.60, 165.06, 137.11, 136.77, 136.73, 133.08, 132.86, 132.69, 129.76, 129.71, 129.62, 129.58, 129.42, 129.38, 128.91, 128.85, 128.35, 128.19, 128.10, 128.05, 128.02, 128.01, 127.97, 127.68, 127.52, 127.41, 127.19, 126.33, 125.13, 102.21, 95.56, 86.09, 81.97, 78.62, 75.54, 75.17, 71.33, 70.57, 69.87, 69.02, 68.72, 67.56, 62.05, 21.42, 12.85; MALDI-TOF mass m/z calcd for $C_{55}H_{52}O_{13}SNa$ 975.3 $(M+Na)^+$, found 975.7.

4.1.21. Compound 50. A mixture of Cp_2HfCl_2 (80.9 mg, 0.213 mmol), AgOTf (110 mg, 0.426 mmol), and molecular sieves 4 Å (1.4 g) in dry CH_2Cl_2 (2 mL) was stirred at −10 °C. A solution of glycosyl donor **48** (83.5 mg, 0.142 mmol) and glycosyl acceptor **49** (52 mg 0.070 mmol) in dry CH_2Cl_2 (2 mL) was added. The mixture was stirred at −10 °C for 7 h and processed as described for **28**. The residue was subjected to a column of Bio-Beads SX-4 (toluene) to afford compound **50** (82 mg, 90%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01–7.12 (m, 36H), 6.03 (t, 1H, J=9.6 Hz), 5.82 (t, 1H, J=9.6 Hz), 5.41 (t, 1H, J=9.6 Hz), 5.29 (t, 1H, J=9.6 Hz), 5.14 (d, 1H, J=3.6 Hz), 4.93 (d, 1H, J=3.2 Hz), 4.66 (d, 1H, J=11.6 Hz), 4.51 (d, 1H, J=11.6 Hz), 4.48–4.40 (m, 2H), 4.35–4.28 (m, 2H), 4.22 (m, 1H), 4.05–3.98 (m, 3H), 3.82 (s, 3H), 3.77 (s, 3H), 3.73 (s, 3H), 3.55 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.85, 165.76, 165.18, 165.07, 165.02, 164.97, 163.48, 163.29, 163.18, 159.31, 137.31, 133.01, 132.92, 132.82, 131.93, 131.66, 130.01, 129.69, 129.58, 129.57, 128.73, 128.59, 128.30, 128.16, 128.13, 127.78, 122.12, 121.68, 121.25, 113.83, 113.55, 96.91, 94.98, 72.56, 71.78, 71.23, 69.83, 69.72, 69.36, 68.11, 67.99, 63.12, 62.64, 55.40, 55.21, 55.15; MALDI-TOF mass m/z calcd for $C_{72}H_{66}O_{21}Na$ 1289.4, found 1290.3.

4.1.22. Compound 52. To a solution of **50** (264 mg, 0.209 mmol) in CH_2Cl_2 (5 mL) was added DDQ (273 mg, 1.20 mmol), followed by H_2O (1 mL). The mixture was stirred at room temperature for 12 h, diluted with EtOAc, quenched with ascorbate buffer. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 5:1 to 2:3) to give hemiacetal **51** (175 mg, 73%): MALDI-TOF mass m/z calcd for $C_{64}H_{58}O_{20}Na$ 1169.3 $(M+Na)^+$, found 1169.8. Hemiacetal **51** (157 mg, 0.137 mmol) was dissolved in CCl_3CN (1 mL) and CH_2Cl_2 (1 mL) and added DBU (10 μL) at 0 °C. After stirring for 1 h, the mixture was subjected to a silica gel column chromatography (toluene/EtOAc, 10:1 to 3:1 in 0.1% TEA) to give **52** (177 mg, quant.) as a slightly yellow amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.02–6.63 (m, 33H), 6.13 (t, 1H, J=10.0 Hz), 5.77 (t, 1H, J=9.8 Hz), 5.57 (t, 1H, J=10.1 Hz), 5.34 (t, 1H, J=10.0 Hz), 5.08 (d, 1H, J=3.4 Hz), 4.35 (dd, 1H, J=5.3, 12.4 Hz), 4.25 (m, 2H), 4.11 (m, 1H), 3.94 (m, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.69 (dd, 1H, J=3.4, 10.0 Hz), 3.57 (s, 3H).

4.1.23. Compound 18. A mixture of **52** (65 mg, 0.057 mmol), **46** (16 mg, 0.042 mmol), and molecular sieves AW 300 (700 mg) in dry toluene (3 mL) was stirred at −40 °C for 30 min, to which was added TMSOTf (5 μL). The mixture was stirred at −40 °C for 2 h, then quenched with TEA (20 μL) and processed as described for **28**. The residue was subjected to a column of Bio-Beads SX-4 (toluene/EtOAc, 1:1), then PTLC (toluene/EtOAc, 5:1) to afford **18** (28 mg, 45%) as a white solid: R_f 0.48 (toluene/EtOAc, 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00–6.63 (m, 42H), 6.14 (t, 1H, J=10.0 Hz), 5.86 (t, 1H, J=10.0 Hz), 5.82

(d, 1H, J=3.6 Hz), 5.45 (t, 1H, J=10.0 Hz), 5.40 (s, 1H), 5.27 (t, 1H, J=10.0 Hz), 5.19–5.02 (m, 2H), 4.93 (d, 1H, J=3.2 Hz), 4.65–4.16 (m, 9H), 3.94 (dd, 1H, J=3.7, 10.5 Hz), 3.83, 3.81, 3.65 (dd, 1H, J=3.2, 10.0 Hz), 3.58 (t, 1H, J=9.3 Hz), 3.50 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.94, 165.66, 165.20, 165.10, 164.85, 163.40, 163.13, 138.15, 137.38, 136.87, 133.01, 132.86, 131.83, 131.68, 129.64, 129.59, 129.52, 129.15, 128.58, 128.43, 128.26, 128.15, 128.11, 127.74, 127.45, 126.03, 122.36, 121.64, 121.33, 113.53, 113.37, 95.95, 95.03, 86.05, 81.41, 78.74, 76.60, 75.54, 74.57, 70.98, 70.83, 69.98, 69.52, 69.17, 68.47, 67.84, 67.77, 62.93, 62.21, 55.40, 55.07, 13.01; MALDI-TOF mass m/z calcd for $C_{85}H_{80}O_{24}SNa$ 1539.5 $(M+Na)^+$, found 1540.2.

4.1.24. Compound 53. A mixture of AgOTf (465 mg, 1.81 mmol), Cp_2HfCl_2 (344 mg, 0.906 mmol), and molecular sieves 4 Å (6 g) in dry toluene (50 mL) was stirred at room temperature for 30 min, then cooled at −30 °C. A solution of donor **12** (1.45 g, 0.906 mmol) and acceptor **11** (1.66 g, 0.604 mmol) in dry toluene (10 mL) was added dropwise over 5 min. The mixture was stirred at −10 °C for 4 h. The reaction was quenched with TEA (1 mL) and processed as described for **28**. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 1:1 to 2:3) to afford **53** (2.27 g, 87%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75–6.70 (m, 93H), 5.56 (m, 1H), 5.39–5.20 (m, 15H), 4.98–3.20 (m, 96H), 3.10 (m, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 1.98 (s, 12H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.91 (s, 3H), 1.89 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.71, 170.45, 170.14, 169.96, 169.91, 169.58, 169.48, 169.38, 169.25, 169.17, 168.94, 167.90, 167.29, 138.63, 138.43, 138.29, 138.23, 138.18, 138.10, 138.06, 137.90, 137.76, 137.66, 133.61, 133.35, 131.60, 131.26, 128.42, 128.23, 128.11, 128.06, 127.86, 127.77, 127.70, 127.65, 127.59, 127.56, 127.50, 127.36, 127.27, 127.07, 126.72, 123.29, 123.00, 117.00, 101.11, 99.90, 99.31, 99.08, 98.62, 96.91, 79.79, 79.45, 79.17, 78.48, 78.25, 75.88, 75.26, 75.03, 74.86, 74.58, 74.42, 74.34, 74.21, 73.37, 73.19, 73.07, 72.76, 72.58, 72.47, 72.25, 72.18, 71.60, 71.15, 70.04, 69.83, 69.58, 69.51, 69.31, 69.11, 69.00, 68.80, 68.72, 68.55, 68.26, 68.05, 67.93, 67.43, 67.27, 67.09, 66.22, 66.10, 65.87, 65.45, 62.02, 61.83, 61.53, 56.49, 55.56, 38.68, 30.33, 28.88, 27.15, 23.73, 22.94, 21.42, 20.87, 20.81, 20.76, 20.63, 20.60, 20.57, 19.34, 14.03, 10.95; MALDI-TOF mass m/z calcd for $C_{236}H_{260}O_{73}N_2Na$ 4343.6 $(M+Na)^+$, found 4343.5; Anal. Calcd for $C_{236}H_{260}N_2O_{73}Si$: C, 65.60; H, 6.07; N, 0.65. Found: C, 65.56; H, 6.12; N, 0.56.

4.1.25. Compound 54. To a stirred solution of compound **53** (2.27 g, 0.525 mmol) and DMAP (6.4 mg) in pyridine (40 mL) was added acetic anhydride (20 mL). The mixture was stirred at 50 °C for 12 h. To the mixture was added methanol (20 mL) and volatiles were removed by evaporation in vacuo. The residue was diluted with EtOAc and washed successively with aq $CuSO_4$, brine, aq $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and evaporated in vacuo. The residue was subjected to a silica gel column chromatography (hexane/EtOAc 5:1 to 1:3) to give the acetylated undecasaccharide (2.22 g, 97%). The acetylated compound (2.22 g, 0.509 mmol) was dissolved in a 3 mL Teflon reaction vessel in DMF (2 mL) containing 10% HF/pyridine. It was compressed to 1.0 GPa and left at 30 °C for 24 h and the resultant mixture was diluted with EtOAc and washed successively with aq $NaHCO_3$ and brine, successively. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was subjected to a silica gel column chromatography (hexane/EtOAc 10:1 to 1:2) to give **54** (1.99 g, 95%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–6.71 (m, 83H), 5.59 (m, 1H), 5.41–5.11 (m, 16H), 5.01–4.91 (m, 5H), 4.86–4.77 (m, 7H), 4.69–4.14 (m, 23H), 4.33–4.23 (m, 57H), 3.48 (m, 1H), 3.26 (m, 1H), 2.07–3.14 (m, 2H), 2.11 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.04 (s, 6H), 2.02 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.71, 170.45, 170.14, 169.96, 169.91, 169.58, 169.48, 169.38, 169.25, 169.17, 168.94, 167.90, 167.29, 138.63, 138.43, 138.29, 138.23, 138.10, 138.06, 137.90, 137.76, 137.66, 133.61, 133.35, 131.60, 131.26, 128.42, 128.23, 128.11, 128.06, 127.88, 127.77, 127.70, 127.65, 127.59, 127.50, 127.36, 127.27, 127.07, 126.72, 123.29, 123.00, 117.00, 101.14, 100.77, 99.29, 99.18, 98.68, 98.56, 98.33, 96.96, 96.86, 79.90, 79.71, 78.91, 78.00, 77.53, 77.20, 76.23, 75.74, 75.05, 74.81, 74.70, 74.60, 74.43, 74.29, 74.03, 73.34, 73.21, 73.05, 72.86, 72.76, 72.67, 72.59, 72.50, 72.02, 71.87, 71.56, 71.47, 70.83, 70.07, 69.87, 69.63, 69.57, 69.39, 69.18, 69.05, 68.90, 68.71, 68.54, 68.67, 67.99, 67.21, 66.28, 66.11, 65.88, 65.48, 62.00, 61.89, 61.43, 56.56, 55.61, 20.95, 20.85, 20.71, 20.68, 20.64, 20.60, 20.54; MALDI-TOF mass m/z calcd for $C_{222}H_{244}N_2NaO_{74}$ 4144.5 $(M+Na)^+$, found 4144.2; Anal. Calcd for $C_{222}H_{244}N_2O_{74}$: C, 64.65; H, 5.96; N, 0.68. Found: C, 64.44; H, 5.97; N, 0.65.

4.1.26. Compound 55. A mixture of AgOTf (80 mg, 0.31 mmol), Cp_2HfCl_2 (52 mg, 0.14 mmol), and molecular sieves 4 Å (2 g) in dry toluene (4 mL) was stirred at room temperature for 30 min, then cooled at −30 °C. To a solution of **13** (128 mg, 0.125 mmol) and **11** (208 mg, 0.0759 mmol) in dry toluene (15 mL) the mixture was added dropwise over 5 min. The mixture was stirred at −10 °C for 12 h. The mixture was processed as described for **53** and purified by PTLC (toluene/EtOAc, 3:1) to afford **55** (219 mg, 77%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76–6.68 (m, 93H), 5.56 (m, 1H), 5.35–5.14 (m, 10H), 4.99–3.23 (m, 98H), 3.13 (m, 1H), 2.12 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.23, 169.41, 169.34, 138.53, 138.44, 138.41, 138.19, 138.16, 138.05, 138.00, 137.97, 137.92, 137.86, 137.82, 137.77, 137.60, 135.95, 135.87, 133.48, 133.20, 129.33, 128.28, 128.19, 128.09, 128.06, 128.00, 127.95, 127.86, 127.82, 127.70, 127.64, 127.50, 127.32, 127.30, 127.22, 127.19, 127.16, 127.07, 126.83, 126.73, 126.58, 116.84, 100.91, 99.80, 99.49, 99.26, 97.51, 96.87, 79.74, 79.38, 78.20, 75.28, 75.03, 74.89, 74.40, 74.32, 73.91, 73.42, 73.10, 72.80, 72.55, 72.36, 72.17, 71.43, 71.08, 70.98, 69.35, 69.32, 69.21, 69.01, 68.52, 68.32, 67.98, 67.38, 67.06, 66.20, 65.93, 65.80, 62.20, 62.14, 56.49, 55.58, 20.92, 20.85, 20.76, 20.71, 20.68, 19.39; MALDI-TOF mass m/z calcd for $C_{212}H_{228}O_{74}N_2SiNa$ 3764.5 $(M+Na)^+$, found 3764.4;

Anal. Calcd for $C_{212}H_{228}N_2O_{57}Si$: C, 68.01; H, 6.14; N, 0.75. Found: C, 67.77; H, 6.19; N, 0.69.

4.1.27. Compound 56. Compound **55** (205 mg, 0.0548 mmol) was acetylated and desilylated as described for **54**. The residue was subjected to a PTLC (toluene/EtOAc 2:1) to give **56** (168 mg, 86%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–6.72 (m, 83H), 5.58 (m, 1H), 5.38 (d, 1H, J=2.9 Hz), 5.34–3.49 (m, 93H), 3.46–3.31 (m, 2H), 3.34–3.18 (m, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.99 (s, 6H), 1.97 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.31, 170.03, 169.48, 169.44, 163.31, 169.28, 169.23, 167.77, 167.15, 138.54, 138.35, 138.32, 138.21, 138.12, 138.05, 137.98, 137.94, 137.84, 137.81, 137.71, 137.65, 133.61, 133.50, 131.31, 131.16, 128.34, 128.24, 128.14, 128.11, 128.05, 128.03, 127.98, 127.87, 127.80, 127.77, 127.69, 127.65, 127.61, 127.56, 127.52, 127.46, 127.41, 127.31, 127.27, 127.22, 127.19, 127.15, 127.01, 126.78, 126.65, 123.32, 122.89, 116.95, 101.08, 100.68, 99.18, 98.32, 97.45, 96.92, 96.77, 79.85, 79.53, 78.78, 77.95, 77.82, 77.20, 76.20, 75.69, 75.04, 74.85, 74.78, 74.69, 74.55, 74.38, 74.29, 74.01, 73.86, 73.33, 73.17, 73.04, 72.85, 72.72, 72.64, 72.49, 72.00, 71.83, 71.63, 71.50, 71.43, 71.18, 70.85, 69.39, 69.26, 69.17, 69.03, 68.95, 68.69, 68.60, 68.37, 67.98, 67.86, 67.22, 66.71, 66.23, 65.93, 65.68, 62.24, 62.00, 56.54, 55.61, 21.04, 20.94, 20.90, 20.83, 20.75, 20.66; MALDI-TOF mass m/z calcd for $C_{198}H_{212}O_{58}N_2Na$ 3568.4 $(M+Na)^+$, found 3568.1; Anal. Calcd for $C_{198}H_{212}N_2O_{58}$: C, 67.03; H, 6.02; N, 0.79. Found: C, 66.86; H, 6.00; N, 0.75.

4.1.28. Compound 57. A mixture of AgOTf (84.9 mg, 0.330 mmol), Cp_2HfCl_2 (62.8 mg, 0.165 mmol), and molecular sieves 4 Å (5 g) in dry toluene (8 mL) was stirred at room temperature for 30 min, then cooled at −30 °C. To a solution of **14** (146 mg, 0.111 mmol) and **11** (268 mg, 0.0978 mmol) in dry toluene (15 mL) the mixture was added dropwise over 5 min and stirred at −10 °C for 3 h. The reaction mixture was processed as described for **53** and purified by silica gel column chromatography (toluene/EtOAc, 5:1 to 1:1) to afford **57** (295 mg, 75%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75–6.72 (m, 93H), 5.64 (m, 1H), 5.34–3.22 (m, 104H), 3.14 (m, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 6H), 2.03 (s, 6H), 2.02 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.12 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.36–169.30, 138.45–126.76, 116.91, 101.08, 99.68, 99.58, 99.25, 98.62, 97.79, 97.51, 96.94, 96.85, 56.53, 55.58, 27.22, 20.93, 20.84, 20.76, 20.70, 20.66, 19.42, 14.29; MALDI-TOF mass m/z calcd for $C_{224}H_{244}O_{65}N_2SiNa$ 4052.6 $(M+Na)^+$, found 4053.7; Anal. Calcd for $C_{224}H_{244}N_2O_{65}Si$: C, 66.72; H, 6.10; N, 0.69. Found: C, 66.50; H, 6.13; N, 0.61.

4.1.29. Compound 58. Compound **57** (275 mg, 0.0682 mmol) was acetylated and desilylated as described for **54**. Purification by silica gel column chromatography (hexane/EtOAc, 5:1 to 1:1) gave **58** (182 mg, 70%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–6.73 (m, 83H), 5.58 (m, 1H), 5.54 (d, 1H, J=4.8 Hz), 5.40–3.12 (m, 107H), 2.10 (s, 3H), 2.03 (s, 9H), 2.02 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.89 (s, 6H), 1.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.36–169.30, 138.35–127.00, 116.95, 101.15, 100.59, 99.30, 98.69, 98.29, 97.49, 96.91, 96.80, 79.89, 79.65, 78.97, 77.95, 77.64, 77.14, 77.02, 76.67, 76.18, 75.71, 75.05, 74.86, 74.79, 74.62, 74.40, 74.26, 74.01, 73.05, 72.86, 72.74, 72.65, 72.56, 71.86, 71.52, 71.45, 71.14, 70.85, 70.03, 69.58, 69.15, 69.02, 68.96, 68.76, 68.65, 68.49, 68.39, 67.97, 67.86, 67.28, 66.18, 66.10, 65.87, 65.51, 62.21, 62.05, 61.43, 56.58, 55.62, 21.54, 20.90, 20.84, 20.76, 20.68; MALDI-TOF mass m/z calcd for $C_{210}H_{228}O_{66}N_2Na$ 3856.5 $(M+Na)^+$, found 3857.6; Anal. Calcd for $C_{210}H_{228}N_2O_{66}$: C, 65.75; H, 5.99; N, 0.73. Found: C, 65.76; H, 6.19; N, 0.68.

4.1.30. Compound 59. A mixture of AgOTf (103 mg, 0.401 mmol), Cp_2HfCl_2 (77.7 mg, 0.201 mmol), and molecular sieves 4 Å (4 g) in dry toluene (5 mL) was stirred at room temperature for 30 min, then cooled at −30 °C. To a solution of **15** (381 mg, 0.291 mmol) and **11** (550 mg, 0.201 mmol) in dry toluene (45 mL) the mixture was added dropwise over 5 min. The reaction mixture was stirred at −10 °C for 3 h and processed as described for **53**. The residue was subjected to a silica gel column chromatography (hexane/EtOAc, 1:1 to 2:3) to afford **59** (682 mg, 84%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68–6.70 (m, 93H), 5.57 (m, 1H), 5.37–5.12 (m, 12H), 5.01–3.43 (m, 89H), 3.30 (m, 2H), 3.22 (m, 2H), 3.11 (m, 1H), 2.00 (s, 6H), 1.92 (s, 9H), 1.92 (s, 3H), 1.90 (s, 6H), 1.88 (s, 3H), 1.87 (s, 3H), 1.84 (s, 3H), 1.83 (s, 3H), 1.00 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.60, 170.13, 170.01, 169.35, 169.30, 169.29, 169.07, 138.49, 138.45, 138.42, 138.23, 138.21, 138.09, 138.03, 137.84, 137.79, 136.00, 135.92, 133.54, 128.31, 128.22, 128.13, 128.05, 128.00, 127.90, 127.83, 127.75, 127.73, 127.61, 127.56, 127.37, 127.35, 127.31, 127.21, 127.13, 127.10, 126.91, 126.80, 126.60, 126.45, 116.91, 101.01, 99.97, 99.41, 99.09, 98.41, 96.93, 96.87, 79.78, 79.50, 79.10, 78.28, 77.71, 77.56, 77.20, 75.86, 75.32, 75.05, 74.89, 74.48, 74.34, 73.89, 73.41, 73.22, 73.16, 72.81, 72.61, 72.47, 72.21, 71.77, 71.53, 71.29, 71.14, 69.94, 69.77, 69.64, 69.38, 69.21, 69.05, 68.91, 68.63, 68.56, 68.37, 68.02, 67.48, 67.32, 67.01, 66.29, 66.15, 65.94, 65.81, 62.11, 61.90, 56.53, 55.63, 27.26, 20.99, 20.91, 20.84, 20.73, 19.45; MALDI-TOF mass m/z calcd for $C_{224}H_{244}O_{65}N_2SiNa$ 4053.5 $(M+Na)^+$, found 4053.8; Anal. Calcd for $C_{224}H_{244}N_2O_{65}Si$: C, 66.72; H, 6.10; N, 0.69. Found: C, 66.51; H, 6.13; N, 0.66.

4.1.31. Compound 60. Compound **59** (303 mg, 0.0751 mmol) was acetylated and desilylated as described for **54**. Purification by silica gel column chromatography (toluene/EtOAc, 5:1 to 1:1) afforded **60** (285 mg, 97%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–6.73 (m, 83H), 5.58 (m, 1H), 5.38–4.40 (m, 46H), 4.32–3.45 (m, 48H), 3.67 (m, 1H), 3.31 (m, 1H), 3.23 (m, 1H), 3.17 (m, 1H), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.64, 170.08, 169.73, 169.50, 169.43, 169.34, 169.15, 138.53, 138.36, 138.31, 138.18, 138.13, 138.05, 138.02, 137.98, 137.82, 137.76, 137.66, 133.54, 128.37, 128.28, 128.19, 128.14, 128.09, 128.07, 128.02, 127.95, 127.87, 127.82, 127.13,

127.66, 127.60, 127.56, 127.42, 127.32, 127.30, 127.23, 127.12, 127.03, 126.68, 126.42, 117.00, 101.07, 100.78, 99.17, 98.33, 97.11, 96.94, 96.82, 79.90, 79.62, 78.76, 77.98, 77.74, 77.55, 77.20, 76.23, 75.73, 75.08, 74.86, 74.64, 74.42, 74.34, 74.06, 73.68, 73.38, 73.21, 73.07, 72.86, 72.79, 72.66, 72.55, 72.47, 72.03, 71.88, 71.68, 71.54, 71.47, 71.27, 70.85, 69.96, 69.63, 69.44, 69.22, 69.05, 68.91, 68.73, 68.65, 68.55, 68.37, 68.21, 68.00, 67.18, 66.28, 66.17, 65.90, 65.77, 62.10, 62.00, 61.94, 56.53, 55.64, 21.06, 20.96, 20.87, 20.80, 20.76, 20.73; MALDI-TOF mass *m/z* calcd for $C_{210}H_{228}O_{66}N_2Na$ 3856.5 $(M+Na)^+$, found 3856.6; Anal. Calcd for $C_{210}H_{228}N_2O_{66}$: C, 65.75; H, 5.99; N, 0.73. Found: C, 65.76; H, 5.89; N, 0.75.

4.1.32. Compound 61. A mixture of **18** (28.1 mg, 0.0185 mmol), **54** (49.8 mg, 0.0120 mmol), DTBMP (38 mg, 0.012 mmol), and molecular sieves 4 Å (500 mg) in dry $ClCH_2CH_2Cl$ (1 mL) and cyclohexane (4 mL) was stirred at room temperature for 2 h, then added 1 M MeOTf (0.15 mL, 0.15 mmol) in $ClCH_2CH_2Cl$. The reaction mixture was stirred at 50 °C for 12 h. The reaction was quenched with TEA (0.1 mL). The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with aq $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to a column of Bio-Beads SX-4 (toluene/EtOAc, 1:1), then PTLC (toluene/EtOAc, 1:2) to afford **61** (38.1 mg, 57%) as a colorless amorphous: R_f 0.75 (toluene/EtOAc, 2:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97–6.56 (m, 125H), 6.10 (t, 1H, J=10.0 Hz), 5.76 (t, 1H, J=10.0 Hz), 5.66 (d, 1H, J=3.2 Hz), 5.55–5.47 (m, 1H), 5.43 (t, 1H, J=10.0 Hz), 2.03, 2.00, 1.99, 1.96, 1.95, 1.93, 1.92, 1.91, 1.91, 1.90, 1.89, 1.88; MALDI-TOF mass *m/z* calcd for $C_{306}H_{320}N_2O_{98}Na$ 5612.9 $(M+Na)^+$, found 5611.3.

4.1.33. Compound 62. A mixture of **17** (30.6 mg, 0.0321 mmol), **54** (77.4 mg, 0.0188 mmol), DTBMP (52 mg, 0.26 mmol), and molecular sieves 4 Å (500 mg) in dry $ClCH_2CH_2Cl$ (3 mL) and cyclohexane (9 mL) was stirred at room temperature for 30 min then added 1 M MeOTf (0.2 mL, 0.2 mmol) in $ClCH_2CH_2Cl$. The reaction mixture was stirred at 50 °C for 12 h and processed as described for **61**. Purification by PTLC (toluene/EtOAc, 5:4) afforded **62** (80.4 mg, 85%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00–6.61 (m, 113H), 5.94 (t, 1H, J=9.0 Hz), 5.65 (d, 1H, J=4.0 Hz), 5.63–5.54 (m, 1H), 5.42–3.45 (m, 124H), 3.30 (m, 1H), 3.25–3.18 (m, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.98 (s, 6H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H), 1.89 (s, 3H); MALDI-TOF mass *m/z* calcd for $C_{276}H_{292}N_2O_{87}Na$ 5052.2 $(M+Na)^+$, found 5052.4; Anal. Calcd for $C_{276}H_{292}N_2O_{87}$: C, 65.91; H, 5.85; N, 0.56. Found: C, 65.51; H, 5.80; N, 0.49.

4.1.34. Compound 63. A mixture of **54** (500 mg, 0.121 mmol), **16** (244 mg, 0.485 mmol), DTBMP (117 mg, 0.558 mmol), and molecular sieves 4 Å (5 g) in dry $ClCH_2CH_2Cl$ (11 mL) and cyclohexane (55 mL) was stirred at room temperature for 30 min, then added 1 M MeOTf (0.68 mL, 0.68 mmol) in $ClCH_2CH_2Cl$. The reaction mixture was stirred at 50 °C for 12 h and processed as described for **61**. Purification by PTLC (toluene/EtOAc, 1:2) afforded **63** (555 mg, 93%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–6.64 (m, 92H), 5.71 (t, 1H, J=9.8 Hz), 5.58 (m, 1H), 5.41 (s, 1H), 5.38 (br d, 1H, J=2.8 Hz), 5.36–3.42 (m, 117H), 3.33 (m, 1H), 3.23 (m, 1H), 3.17 (m, 2H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 9H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H), 1.71 (s, 9H); MALDI-TOF mass *m/z* calcd for $C_{248}H_{274}N_2O_{81}Na$ 4601.8 $(M+Na)^+$, found 4601.7; Anal. Calcd for $C_{248}H_{274}N_2O_{81}$: C, 65.05; H, 6.03; N, 0.61. Found: C, 64.51; H, 5.97; N, 0.58.

4.1.35. Compound 64. A mixture of **56** (36.7 mg, 0.0103 mmol), **16** (36.6 mg, 0.0728 mmol), DTBMP (30 mg, 0.14 mmol), and molecular sieves 4 Å (1.0 g) in dry $ClCH_2CH_2Cl$ (2 mL) and cyclohexane (4 mL) was stirred at room temperature for 30 min, then added 1 M MeOTf (0.100 mL, 0.100 mmol) in $ClCH_2CH_2Cl$. The reaction mixture was stirred at 45 °C for 19 h and processed as described for **61**. The mixture was subjected to a PTLC (toluene/EtOAc, 3:4) to afford **64** (35.6 mg, 86%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75–6.42 (m, 92H), 5.72 (t, 1H, J=9.6 Hz), 5.58 (m, 1H), 5.42 (s, 1H), 5.38 (d, 1H, J=3.2 Hz), 5.34–3.45 (m, 102H), 3.33 (m, 2H), 3.23 (m, 1H), 3.18 (m, 2H), 2.05 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.88 (s, 3H), 1.17 (s, 9H); MALDI-TOF mass *m/z* calcd for $C_{224}H_{242}N_2O_{65}Na$ $(M+Na)^+$ 4022.6, found 4022.5; Anal. Calcd for $C_{224}H_{242}N_2O_{65}$: C, 67.22; H, 6.09; N, 0.70. Found: C, 67.43; H, 6.35; N, 0.56.

4.1.36. Compound 65. A mixture of **58** (71.2 mg, 0.0186 mmol), **16** (37.9 mg, 0.0754 mmol), DTBMP (70.3 mg, 0.570 mmol), and molecular sieves 4 Å (2.0 g) in dry $ClCH_2CH_2Cl$ (4 mL) and cyclohexane (10 mL) was stirred at room temperature for 30 min, then added 1 M MeOTf (0.400 mL, 0.400 mmol) in $ClCH_2CH_2Cl$. The reaction mixture was stirred at 45 °C for 12 h. The mixture was processed as described for **61** and purified by PTLC (toluene/EtOAc, 3:4) to afford **65** (72.5 mg, 91%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57–6.63 (m, 92H), 5.71 (t, 1H, J=10.0 Hz), 5.90 (m, 1H), 5.41 (br s, 2H), 5.33–3.14 (m, 114H), 2.10 (s, 3H), 2.03 (s, 9H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 6H), 1.98 (s, 3H), 1.96 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H), 1.16 (s, 9H); MALDI-TOF mass *m/z* calcd for $C_{236}H_{258}N_2O_{73}Na$ 4310.6 $(M+Na)^+$, found 4311.7; Anal. Calcd for $C_{236}H_{258}N_2O_{73}$: C, 66.06; H, 6.06; N, 0.65. Found: C, 65.81; H, 5.92; N, 0.59.

4.1.37. Compound 66. A mixture of **60** (276 mg, 0.0719 mmol), **16** (111 mg, 0.221 mmol), DTBMP (83.5 mg, 0.407 mmol), and molecular sieves 4 Å (3.0 g) in dry $ClCH_2CH_2Cl$ (10 mL) and cyclohexane (20 mL) was stirred at room temperature for 30 min, then added 1 M MeOTf (0.288 mL, 0.288 mmol) in $ClCH_2CH_2Cl$. The reaction mixture was stirred at 45 °C for 60 h and processed as described for **61**. Purification by PTLC (toluene/EtOAc, 1:2) afforded **66** (245 mg, 79%) as a colorless amorphous: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–6.64 (m, 92H), 5.72 (t, 1H, J=9.3 Hz), 5.58 (m, 1H), 5.42 (s, 1H), 5.38–3.30 (m, 112H), 3.20 (m, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H),

1.96 (s, 3H), 1.95 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.91 (s, 3H), 1.71 (s, 9H); MALDI-TOF mass *m/z* calcd for $C_{236}H_{258}N_2O_{73}Na$ 4310.6 $(M+Na)^+$, found 4311.2; Anal. Calcd for $C_{236}H_{258}N_2O_{73}$: C, 66.06; H, 6.06; N, 0.65. Found: C, 65.94; H, 6.02; N, 0.59.

4.1.38. Compound 1 (M9). A solution of undecasaccharide **51** (28.7 mg, 0.00696 mmol) in *n*-butanol (2 mL) containing 0.5 mL ethylenediamine was stirred at 90 °C for 15 h. Volatiles were removed by evaporation in vacuo and the residue was dissolved in pyridine (0.3 mL). The solution was treated with Ac_2O (0.2 mL) at 0 °C for 24 h and evaporated in vacuo. The residue was dissolved in MeOH (5 mL) and 1 N NaOMe/MeOH (0.1 mL) was added at 0 °C. The mixture was stirred at 60 °C for 12 h, neutralized with Amberlyst 15 (H^+) resin, and evaporated in vacuo. The residue was applied to a gel filtration chromatography (Sephadex LH20, $CHCl_3$/MeOH, 1:1) to collect the deacetylated compound and evaporated in vacuo. The residue was hydrogenated over $Pd(OH)_2$/C (20 wt %, 5 mg) in 60% aq AcOH (5 mL) at room temperature for 24 h. The mixture was filtered through Celite. The filtrate was concentrated in vacuo. The residue was subjected to a gel filtration (Sephadex LH 20, H_2O) to afford **1** (7.3 mg, 54%) as a white powder: ^1H NMR (400 MHz, D_2O) δ 5.27 (br s, 1H), 5.20 (br s, 1H), 5.17 (br s, 1H), 5.01 (br s, 1H), 4.92 (br s, 1H), 4.90 (br s, 2H), 4.73 (br s, 1H), 4.45 (d, 1H, J=7.6 Hz), 4.36 (d, 1H, J=7.8 Hz), 4.09 (br s, 1H), 4.02–3.37 (m, 67H), 1.93 (s, 3H), 1.88 (s, 3H), 1.40 (m, 2H), 0.72 (t, 3H, J=7.3 Hz); MALDI-TOF mass *m/z* calcd for $C_{73}H_{124}N_2O_{56}Na$ 1947.7 $(M+Na)^+$, found 1947.7.

4.1.39. Compound 2 (M7). A solution of nonasaccharide **56** (100 mg, 0.0282 mmol) in *n*-butanol (3 mL) containing 2 mL of ethylenediamine was stirred at 90 °C for 15 h. Volatiles were removed by evaporation in vacuo and the residue was dissolved in pyridine (3 mL). The solution was treated with Ac_2O (1.5 mL) at 0 °C for 24 h and evaporated in vacuo to give acetylated compound (quantitative yield). The acetylated compound (22.6 mg, 0.0067 mmol) was hydrogenated over $Pd(OH)_2$/C (20 wt %, 20 mg) in a mixture of MeOH (10 mL) and 60% aq AcOH (5 mL) at room temperature for 24 h. The mixture was filtered through Celite. The filtrate was concentrated in vacuo. The residue was dissolved in MeOH (5 mL) and 1 N NaOMe/MeOH (0.1 mL). The mixture was stirred at 60 °C for 12 h, neutralized with Amberlyst 15 (H^+) resin, and evaporated in vacuo. The residue was subjected to a Sep-Pak C18 cartridge (500 mg, Waters) (H_2O only to H_2O/MeOH=20:1) to give **2** (9.1 mg, 84%) as a white powder: ^1H NMR (400 MHz, D_2O) δ 5.21 (br s, 1H), 5.17 (br s, 1H), 4.96 (br s, 1H), 4.91 (br s, 1H), 4.77 (br s, 1H), 4.73 (br s, 1H), 4.45 (br d, 1H, J=6.6 Hz), 4.36 (d, 1H, J=6.8 Hz), 4.10 (br s, 1H), 4.01 (br s, 1H), 3.97–3.37 (m, 54H), 1.93 (s, 3H), 1.89 (s, 3H), 1.41 (m, 2H), 0.73 (t, 3H, J=7.3 Hz); MALDI-TOF mass *m/z* calcd for $C_{61}H_{104}N_2O_{46}Na$ 1623.6 $(M+Na)^+$, found 1624.7.

4.1.40. Compound 3 (M8C). In a manner as described for **1**, decasaccharide **58** (14.5 mg, 0.00378 mmol) was subjected to a series of reactions. Purification by gel filtration (Sephadex LH20, H_2O) gave compound **3** (6.0 mg, 87%) as a white powder: ^1H NMR (400 MHz, D_2O) δ 5.27 (br s, 1H), 5.20 (br s, 1H), 5.17 (br s, 1H), 4.92 (br s, 1H), 4.91 (br s, 1H), 4.77 (br s, 1H), 4.73 (br s, 1H), 4.45 (d, 1H, J=7.6 Hz), 4.36 (d, 1H, J=7.6 Hz), 4.10 (br s, 1H), 4.02–3.38 (m, 54H), 1.94 (s, 3H), 1.89 (s, 3H), 1.41 (m, 2H), 0.73 (t, 3H, J=7.3 Hz); MALDI-TOF mass *m/z* calcd for $C_{67}H_{114}N_2O_{51}Na$ 1785.6 $(M+Na)^+$, found 1785.2.

4.1.41. Compound 4 (M8B). Decasaccharide **60** (28.7 mg, 0.00748 mmol) was subjected to a series of reactions in a manner as described for **1**. The mixture was subjected to a Sep-Pak C18 cartridge (500 mg, Waters) (H_2O only to H_2O/MeOH, 20:1) to give **4** (6.2 mg, 47%) as a white powder. ^1H NMR (400 MHz, D_2O) δ 5.20 (br s, 1H), 5.17 (br s, 1H), 5.01 (br s, 1H), 4.95 (br s, 1H), 4.90 (br s, 2H), 4.73 (br s, 1H), 4.45 (br d, 1H, J=7.5 Hz), 4.36 (d, 1H, J=8.0 Hz), 4.09 (br d, 1H, J=2.4 Hz), 4.01–3.36 (m, 54H), 1.93 (s, 3H), 1.89 (s, 3H), 1.41 (m, 2H), 0.72 (t, 3H, J=7.3 Hz); MALDI-TOF mass *m/z* calcd for $C_{67}H_{114}N_2O_{51}Na$ 1785.6 $(M+Na)^+$, found 1786.3.

4.1.42. Compound 5 (G3M9). Tetradecasaccharide **61** (26.7 mg, 0.00477 mmol) was subjected to a series of reactions in a manner as described for **1**. The mixture was purified by Sep-Pak C18 cartridge (500 mg, Waters, H_2O only to H_2O/MeOH, 20:1), then with HPLC (column Fluofix, H_2O) to give **5** (6.5 mg, 56%) as a white powder: ^1H NMR (400 MHz, D_2O) δ 5.39 (d, 1H, J=2.4 Hz), 5.27 (br s, 1H), 5.20 (br s, 1H), 5.17 (br s, 1H), 5.13 (br d, 1H, J=2.4 Hz), 5.04 (br d, 1H, J=3.2 Hz), 5.01 (br s, 1H), 4.12 (br s, 1H), 4.90 (br s, 2H), 4.76 (br s, 1H), 4.45 (d, 1H, J=7.2 Hz), 4.36 (d, 1H, J=6.8 Hz), 4.10 (br s, 2H), 4.02–3.30 (m, 84H), 1.93 (s, 3H), 1.89 (s, 3H), 1.40 (m, 2H), 0.72 (t, 3H, J=7.6 Hz); MALDI-TOF mass *m/z* calcd for $C_{91}H_{154}N_2O_{71}Na$ 2433.8 $(M+Na)^+$, found 2432.8.

4.1.43. Compound 6 (G2M9). Tridecasaccharide **62** (80.4 mg, 0.0160 mmol) in *n*-butanol (2 mL) containing ethylenediamine (1 mL) was stirred at 80 °C for 12 h. Volatiles were removed by evaporation in vacuo and the residue was dissolved in pyridine (4 mL). The solution was treated with Ac_2O (2 mL) and stirred at 40 °C for 12 h and evaporated in vacuo. The residue was diluted with EtOAc and washed with 1 N HCl, brine, aq $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was dissolved in MeOH (10 mL) and 1 N NaOMe/MeOH (0.5 mL) was added at 0 °C. The mixture was stirred at 40 °C for 5 h, neutralized with Amberlyst 15 (H^+) resin, and evaporated in vacuo. The residue was subjected to a PTLC ($CHCl_3$/MeOH, 5:1) to give the deacetylated compound (44 mg, 72%). The deacetylated compound (28.8 mg, 0.00745 mmol) was hydrogenated over $Pd(OH)_2$–C (20 wt %, 20 mg) in 50% aq AcOH (5 mL) at room temperature for 12 h. The mixture was filtered through Celite. The filtrate was concentrated in vacuo. The residue was subjected to a Sep-Pak C18 cartridge (500 mg, Waters) (H_2O only to H_2O/MeOH, 20:1) to give **6** (11.0 mg, 65%) as a white powder: ^1H NMR (400 MHz, D_2O) δ 5.26 (br s, 1H), 5.21 (d, 1H, J=3.6 Hz), 5.19 (br s, 1H), 5.16 (br s, 1H), 5.12 (d, 1H, J=3.9 Hz), 5.00 (br s, 1H), 4.90 (br s, 1H), 4.89 (br s, 2H), 4.71 (br s, 1H), 4.57 (br d, 1H, J=7.0 Hz), 4.35 (br d, 1H, J=6.8 Hz), 4.09 (br s, 2H), 4.01–3.30 (m, 80H), 1.92 (s, 3H), 1.88 (s, 3H), 1.40 (m, 2H), 0.71 (t, 3H, J=7.3 Hz); MALDI-TOF mass *m/z* calcd for $C_{85}H_{144}N_2O_{66}Na$ 2271.8 $(M+Na)^+$, found 2271.4.

4.1.44. Compound 7 (G1M9). Dodecasaccharide **63** (34.7 mg, 0.00757 mmol) was subjected to a series of reactions in a manner as described for **1**. Subsequent purification by gel filtration (Sephadex LH 20, H_2O) afforded compound **7** (8.5 mg, 54%) as a white powder: ^{1}H NMR (400 MHz, D_2O) δ 5.27 (br s, 1H), 5.20 (br s, 1H), 5.17 (br s, 1H), 5.12 (d, 1H, J=3.7 Hz), 5.01 (br s, 1H), 4.92 (br s, 1H), 4.90 (br s, 2H), 4.73 (br s, 1H), 4.46 (d, 1H, J=7.3 Hz), 4.37 (d, 1H, J=7.5 Hz), 4.10 (br s, 2H), 4.02–3.24 (m, 71H), 1.94 (s, 3H), 1.89 (s, 3H), 1.41 (m, 2H), 0.73 (t, 3H, J=7.3 Hz); MALDI-TOF mass m/z calcd for $C_{79}H_{134}N_2O_{61}Na$ 2109.7(M+Na)$^+$, found 2109.4.

4.1.45. Compound 8 (G1M7). Decasaccharide **64** (35.6 mg, 0.00898 mmol) was subjected to a series of reactions in a manner as described for **1**. The mixture was subjected to a Sep-Pak C18 cartridge (2 g, Waters) (H_2O only to H_2O/MeOH, 10:1) to give **8** (15.1 mg, 84%) as a white powder: ^{1}H NMR (400 MHz, D_2O) δ 5.21 (br s, 1H), 5.17 (br s, 1H), 5.11 (d, 1H, J=3.6 Hz), 4.95 (br s, 1H), 4.90 (br s, 1H), 4.77 (br s, 1H), 4.73 (br s, 1H), 4.44 (br d, 1H, J=6.6 Hz), 4.36 (d, 1H, J=7.3 Hz), 4.10 (br s, 2H), 4.01–3.24 (m, 61H), 1.93 (s, 3H), 1.89 (s, 3H), 1.41 (m, 2H), 0.72 (t, 3H, J=7.3 Hz); MALDI-TOF mass m/z calcd for $C_{67}H_{114}N_2O_{51}Na$ 1785.6 (M+Na)$^+$, found 1785.6.

4.1.46. Compound 9 (G1M8C). Undecasaccharide **65** (68.8 mg, 0.0160 mmol) was subjected to a series of reactions in a manner as described for **1**. The mixture was subjected to a Sep-Pak C18 cartridge (2 g, Waters) (H_2O only to H_2O/MeOH, 20:1) to give **9** (20.7 mg, 66%) as a white powder: ^{1}H NMR (400 MHz, D_2O) δ 5.27 (br s, 1H), 5.20 (br s, 1H), 5.17 (br s, 1H), 5.11 (d, 1H, J=4.0 Hz), 4.92 (br s, 1H), 4.90 (br s, 1H), 4.77 (br s, 1H), 4.73 (br s, 1H), 4.45 (d, 1H, J=7.5 Hz), 4.36 (d, 1H, J=8.0 Hz), 4.09 (br s, 2H), 3.97–3.21 (m, 67H), 1.94 (s, 3H), 1.89 (s, 3H), 1.41 (m, 2H), 0.73 (t, 3H, J=7.3 Hz); MALDI-TOF mass m/z calcd for $C_{73}H_{124}N_2O_{56}Na$ 1947.7 (M+Na)$^+$, found 1947.5.

4.1.47. Compound 10 (G1M8B). Undecasaccharide Compound **66** (24.2 mg, 0.00564 mmol) was subjected to a series of reactions in a manner as described for **1**. The mixture was subjected to a Sep-Pak C18 cartridge (500 mg, Waters) (H_2O only to H_2O/MeOH, 20:1) to give **10** (6.3 mg, 58%) as a white powder: ^{1}H NMR (400 MHz, D_2O) δ 5.21 (br s, 1H), 5.17 (br s, 1H), 5.11 (d, 1H, J=3.9 Hz), 5.01 (br s, 1H), 4.95 (br s, 1H), 4.90 (br s, 2H), 4.68 (br s, 1H), 4.45 (d, 1H, J=6.8 Hz), 4.36 (d, 1H, J=7.2 Hz), 4.10 (br s, 2H), 3.01–3.26 (m, 67H), 1.93 (s, 3H), 1.89 (s, 3H), 1.40 (m, 2H), 0.72 (t, 3H, J=7.3 Hz); MALDI-TOF mass m/z calcd for $C_{73}H_{124}N_2O_{56}Na$ 1947.7 (M+Na)$^+$, found 1948.6.

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Synthesis and biological activity of mycalolide analogs

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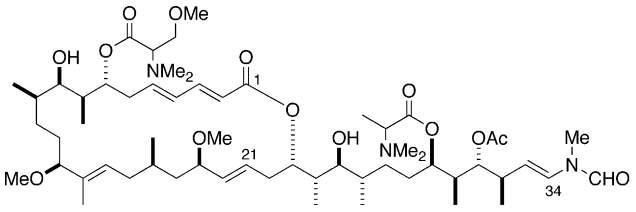
Abstract—Mycalolide analog **4**, consisting only of the side chain of mycalolide B (**2**), a trisoxazole macrolide of marine origin, was stereoselectively synthesized using Roush crotylboration, an Evans aldol reaction, and a Paterson aldol reaction as key steps. The analog **4** was found to have strong actin-depolymerizing activity.

1. Introduction

Actin-disrupting marine natural products are of interest to natural products chemists and pharmacologists.[1] These natural products consist of macrolides, cyclic peptides, and cyclodepsipeptides. Aplyronine A (**1**), an antitumor macrolide isolated from *Aplysia kurodai*,[2] interacts with actin, the major protein in cytoskeleton. Actin regulates various cell functions such as muscle contraction, cell motility, and cell division. Actin exists as a dynamic equilibrium mixture of two forms; one is polymeric F-actin and the other is monomeric G-actin. Aplyronine A (**1**) not only inhibits polymerization of actin by sequestering G-actin and forming a 1:1 complex, but also depolymerizes F-actin to G-actin by severing.[3] We achieved the total synthesis of **1** and investigated the structure–activity relationships of aplyronine A (**1**) using natural and synthetic analogs: the side chain in **1** is essential to actin-depolymerizing activity, and analog **3**, which consists only of the side-chain moiety of **1**, exhibits strong activity.[4] We recently determined the crystal structure



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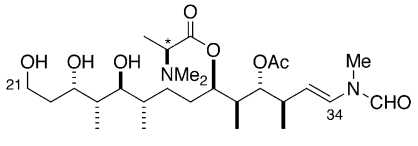
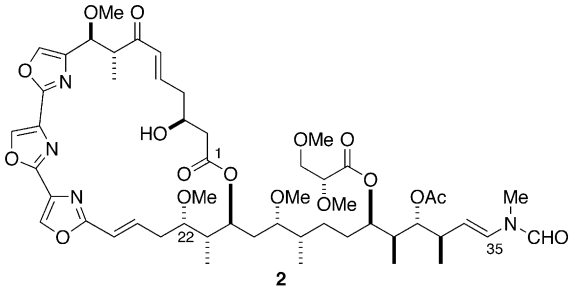


3 (* *S*/*R* = 9/1)



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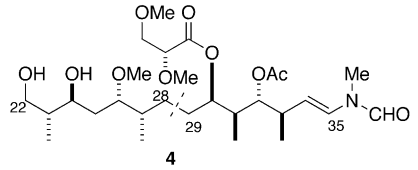
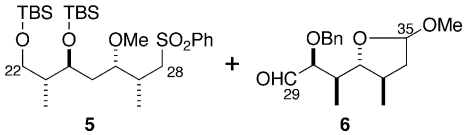


4



5 + **6**

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of actin–aplyronine A complex via synchrotron X-ray analysis[5] and obtained chemical evidence for the direct interaction between actin and the side-chain portion of aplyronine A by photoaffinity labeling experiments.[6] These results display the great importance of the side-chain moiety in the activity against actin.

Mycalolide B (**2**) is a cytotoxic and antifungal macrolide isolated from a sponge of the genus *Mycale* sp.[7] Mycalolide B (**2**) inhibits actomyosin Mg^{2+}-ATPase[8] and also interacts with actin in the same manner as **1**.[9] The total synthesis of mycalolide A, which lacks the 2,3-di-*O*-methyl-D-glyceroyl group at C30, has been achieved.[10] Recently, several crystal structures of trisoxazole macrolides with actin have been reported.[11] Since mycalolide B (**2**) possesses a similar side chain to that of **1**, analog **4** is expected to show actin-depolymerizing activity. We have previously reported the synthesis and actin-depolymerizing activity of analog **4**.[12] We describe herein details of the stereocontrolled synthesis of mycalolide analog **4** and its biological activities, including both its cytotoxicity and actin-depolymerizing activity, along with those of aplyronine analog **3**.

2. Results and discussion

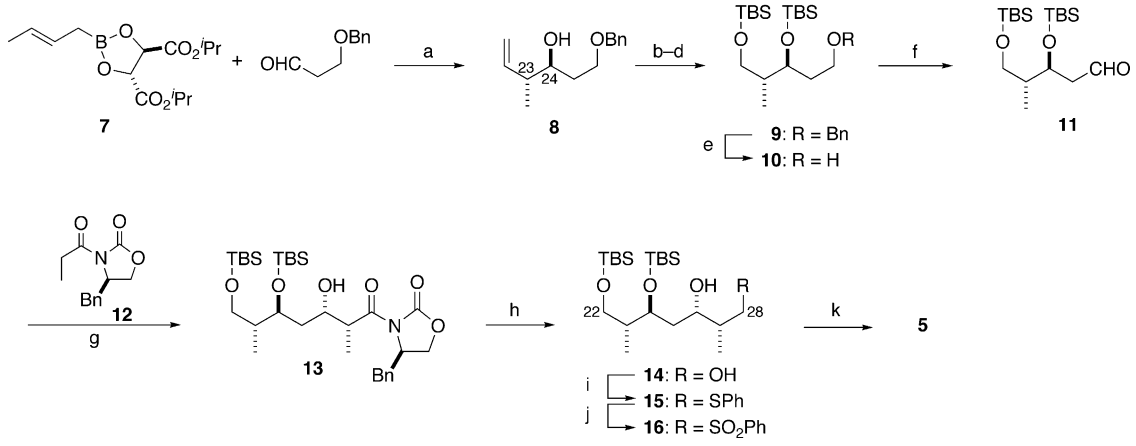
2.1. Chemical synthesis

The synthesis of mycalolide analog **4** has been carried out according to a convergent synthetic methodology connecting C22–C28 and C29–C35 segments, **5** and **6**.

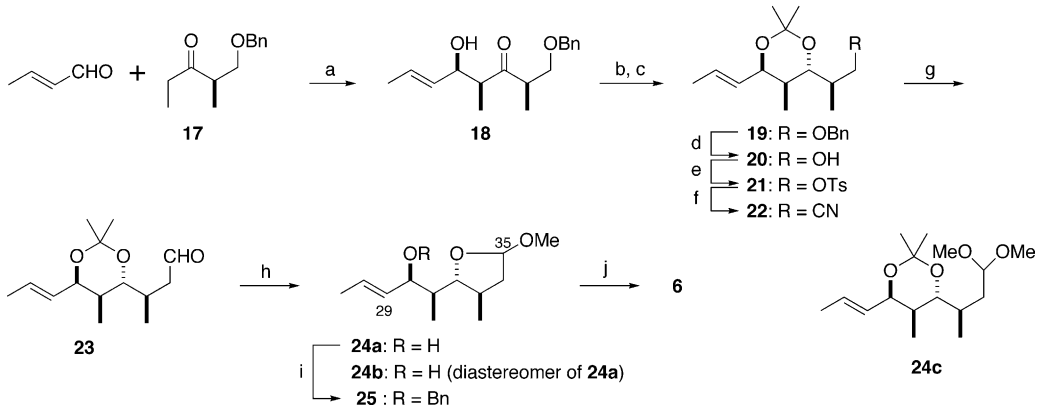
The synthesis of C22–C28 segment **5** is shown in Scheme 1. While *anti* stereocenters between C23 and C24 of **5** was previously constructed by using an anti-selective aldol reaction under Heathcock conditions,[13] the improved synthesis of **5** was developed by using Roush crotylboration[14] as the key step. Thus, the Roush crotylboration between boronate **7** and 3-benzyloxypropanal afforded homoallylic alcohol **8** (91%) as a single diastereomer (Scheme 1). Oxidative cleavage of the olefin moiety of **8**, reduction with $NaBH_4$, and silylation gave silyl ether **9**. The spectral data of **9** were identical to those of previously synthesized **9**,[12] this confirmed the stereochemistry. Cleavage of the benzyl-protecting group in **9** gave alcohol **10**, which was oxidized to aldehyde **11**. The Evans aldol reaction between aldehyde **11** and imide **12**[15] gave hydroxy imide **13** as a single diastereomer, which was converted into diol **14**. Diol **14** was transformed with $(PhS)_2$-Bu_3P[16] into sulfide **15**, which was oxidized with *m*-chloroperoxybenzoic acid to sulfone **16**. The secondary hydroxy group in **16** was methylated to afford C22–C28 segment **5** (57% from **7**).

The synthesis of C29–C35 segment **6** is shown in Scheme 2. While compound **6** with four contiguous *syn–anti–anti* stereocenters was previously prepared using the Evans aldol reaction and Sharpless epoxidation as the key steps,[4a–c] the improved synthesis of **6** was developed by using the Paterson aldol reaction[17] as the key step. Thus, the Paterson aldol reaction between ethyl ketone **17** and crotonaldehyde gave hydroxy ketone **18**.[17b] Stereoselective reduction of **18** with tetramethylammonium triacetoxyborohydride[18] afforded an *anti*-1,3-diol exclusively, which was transformed into acetonide **19**. Its stereochemistry was confirmed to be *anti* by the ^{13}C chemical shifts of two acetonide methyls (δ_C 25.8 and 23.7).[19] The benzyl-protecting group in **19** was removed with calcium in liquid ammonia to give alcohol **20**, which was converted into tosylate **21**. One carbon homologation with NaCN provided nitrile **22**, the reduction of which with DIBAL afforded aldehyde **23**. Aldehyde **23** was treated with PPTS in methanol to provide a separable mixture of diastereomeric acetals, **24a** and **24b**, and the dimethyl acetal **24c**.[20] After chromatographic separation, two minor products, **24b** and **24c**, were subjected to equilibration (PPTS in methanol) to afford a mixture of **24a**, **24b**, and **24c**, from which the major acetal **24a** was again obtained. By repeating this procedure, **24b** and **24c** could be transformed into **24a**. Protection of the hydroxy group in **24a** gave benzyl ether **25**, the double bond of which was cleaved oxidatively to afford the C29–C35 segment **6** (48% from **17**).

The Julia coupling reaction between **5** and **6** gave a hydroxy sulfone, which was converted into olefin **26** by reduction with sodium amalgam (Scheme 3). Removal of the benzyl-protecting group in **26** with calcium in liquid ammonia gave alcohol **27**, catalytic hydrogenation of which provided



Scheme 1. Reagents and conditions: (a) MS 4 Å, toluene, −78 °C, 91%; (b) OsO_4, NMO, THF–*t*-BuOH–H_2O, rt; then $NaIO_4$, rt; (c) $NaBH_4$, EtOH, rt; (d) TBSCl, imidazole, DMF, 50 °C, 79% (three steps); (e) H_2, 10% Pd–C, $NaHCO_3$, EtOAc, rt, 92%; (f) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C; Et_3N, −78 °C→0 °C, 89%; (g) **12**, Bu_2BOTf, Et_3N, CH_2Cl_2, −78 °C→0 °C, 100%; (h) $LiBH_4$, EtOH, Et_2O, −10 °C, 100%; (i) $(PhS)_2$, Bu_3P, DMF, rt, 96%; (j) *m*-CPBA, $NaHCO_3$, CH_2Cl_2, rt, 99%; (k) MeI, NaH, THF, rt, 94%.

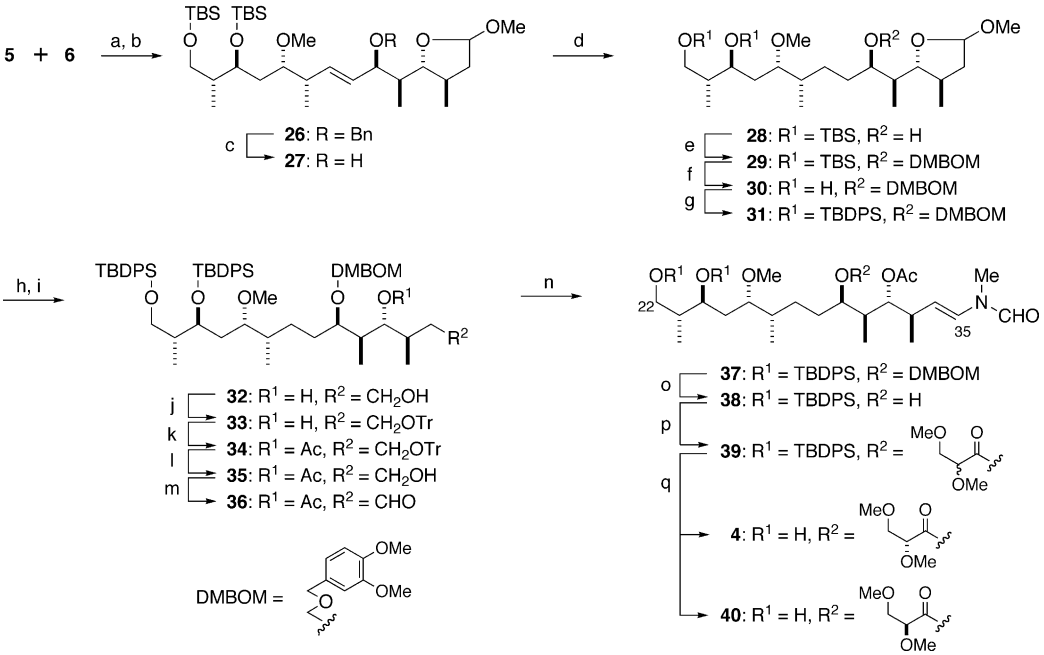


Scheme 2. Reagents and conditions: (a) $Sn(OTf)_2$, Et_3N, CH_2Cl_2, −78 °C→−60 °C, 85%; (b) $Me_4NBH(OAc)_3$, AcOH, MeCN, −25 °C; (c) $(MeO)_2CMe_2$, PPTS, acetone, rt, 84% (two steps); (d) Ca, liq. NH_3, *i*-PrOH, THF, −78 °C, 98%; (e) *p*-TsCl, pyridine, 0 °C, 100%; (f) NaCN, DMSO, 50 °C, 98%; (g) DIBAL, CH_2Cl_2, hexane, −78 °C, 95%; (h) PPTS, MeOH, rt, 82%; (i) BnBr, NaH, DMF, rt, 95%; (j) OsO_4, NMO, H_2O, acetone, rt; then $NaIO_4$, rt, 99%.

alcohol **28**.[4a] The hydroxyl group was protected to give 3,4-dimethoxybenzyloxymethyl ether **29**. At this stage, the TBS-protecting groups in **29** were changed to TBDPS groups, because TBS group was sensitive to acidic conditions at the later stage of the synthesis. Thus, deprotection of two TBS-protecting groups in **29** afforded diol **30**, which was converted into TBDPS ether **31**. The cyclic acetal moiety of **31** was hydrolyzed under acidic conditions to afford a hemiacetal, which was reduced with $NaBH_4$ to give diol **32**. Selective protection of the primary hydroxyl group in **32** provided trityl ether **33**, the secondary hydroxyl group of which was acetylated to afford acetate **34**. The trityl group of **34** was removed with formic acid to give alcohol **35**, which was oxidized to aldehyde **36**. Condensation between **36** and *N*-methylformamide under acidic conditions provided enamide **37**. Deprotection of the 3,4-dimethoxybenzyloxymethyl group of **37** gave alcohol **38**, which was esterified with 2,3-di-*O*-methyl-D-glyceric acid under Yamaguchi conditions to afford a mixture of diastereomeric esters **39**, which resulted from the racemization of 2,3-di-*O*-methyl-D-glyceric acid. After removal of the silyl groups in **39**, HPLC separation of the diastereomers provided analogs **4** and **40**.[21]

2.2. Biological activities

The actin-depolymerizing activity and cytotoxicity against HeLa S_3 cells of aplyronine A (**1**), mycalolide B (**2**), and their analogs **3**, **4**, and **40** are shown in Table 1. The mycalolide analog **3** exhibited strong activity comparable to that of



Scheme 3. Reagents and conditions: (a) BuLi, THF–hexane, −78 °C; (b) 5% Na–Hg, NaH_2PO_4, MeOH, 0 °C, 72% (two steps); (c) Ca, liq. NH_3, *i*-PrOH, THF, −78 °C, 89%; (d) H_2, 5% Pd–C, $NaHCO_3$, EtOH, 55 °C, 95%; (e) 3,4-dimethoxybenzyloxymethyl chloride, *i*-Pr_2NEt, CH_2Cl_2, rt, 84%; (f) Bu_4NF, THF, rt, 99%; (g) TBDPSCl, imidazole, DMF, rt, 75%; (h) 1 M HCl, DME, rt; (i) $NaBH_4$, EtOH, rt, 70% (two steps); (j) TrCl, pyridine, 50 °C, 95%; (k) Ac_2O, pyridine, DMAP, rt, 100%; (l) HCO_2H, Et_2O, rt, 77%; (m) Dess–Martin periodinane, pyridine, CH_2Cl_2, rt, 91%; (n) MeNHCHO, PPTS, hydroquinone, MS 3 Å, benzene, reflux, 55%; (o) DDQ, 1 M phosphate buffer (pH 6), *t*-BuOH, CH_2Cl_2, rt, 90%; (p) 2,3-di-*O*-methyl-D-glyceric acid, 2,4,6-trichlorobenzoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt, 74%; (q) HF·pyridine, pyridine, THF, rt; separation by HPLC, (**4**) 55%, (**40**) 34%.

Table 1. Cytotoxicity and actin-depolymerizing activity of mycalolide B, aplyronine A, and their analogs

Compounds	Actin-depolymerizing activity[a]		Cytotoxicity against HeLa S_3 cells	
	IC_{50} (μM)[b]	Relative potency[c]	IC_{50} (μg/mL)	Relative potency[c]
Aplyronine A (**1**)	1.6	100	0.00048[e]	100
Mycalolide B (**2**)[d]	nd	nd	0.0035	14
3	7.9	20	>10	<0.01
4	2.7	59	>10	<0.01
40	4.4	36	>10	<0.01

[a] Activity was monitored by measuring the fluorescent intensity of pyrenyl actin. For the conditions of assay, see Section 4.2.
[b] IC_{50} indicates the concentration required to depolymerize F-actin (3.7 μM) to 50% of its control amplitude.
[c] The relative potencies were calculated from the IC_{50} values of the compound (aplyronine A=100).
[d] Mycalolide B was purchased from Wako Pure Chemical Industries, Inc.
[e] Ref. 4c.

aplyronine A (**1**). This result revealed that the side-chain portion in mycalolide B (**2**) is responsible for the potent activity of **2**, as is the case with aplyronine A (**1**). Comparison of the activities of **3**, **4**, and **40** revealed that both the structure and stereochemistry of the acyl group influenced activity. In contrast, analogs **3**, **4**, and **40** showed no cytotoxicity at 10 μg/mL, thus indicating that the presence of the macrolide ring is essential to the strong cytotoxicity of **1** and **2**, and that actin-depolymerization is not directly related to the cytotoxicity.

3. Conclusion

The stereocontrolled synthesis of mycalolide analog **4**, consisting only of the side chain of mycalolide B (**2**), was carried out. The mycalolide analog **4** and aplyronine analog **3** were found to exhibit strong actin-depolymerizing activity. In contrast, the analogs did not show cytotoxicity against HeLa S_3 cells. These results clearly indicated that the side-chain portions of aplyronine A and mycalolide B are essential to the actin-depolymerizing activity and that the combination of the side-chain portion and the macrolactone portion is responsible for the cytotoxicity.

4. Experimental

4.1. General

Melting points are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter or a JASCO DIP-1000 polarimeter. ^{1}H NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) or a Bruker AVANCE-400M (400 MHz) instrument. Chemical shifts are reported in parts per million from internal standards [tetramethylsilane (0.00 ppm) for $CDCl_3$ and C_6D_5H (7.16 ppm) for C_6D_6] and J values are in hertz. ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 instrument (67.8 MHz) using $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million from the solvent peak (77.0 ppm). FAB mass spectra were recorded on a JEOL SX-102 instrument. ESI mass spectra were recorded on a QStar/Pulsar *i* spectrometer (Applied Biosystems). Both TLC analysis and preparative TLC were conducted on E. Merck precoated silica gel 60 F_{254} (0.25 mm layer thickness). Fuji Silysia silica gel BW-820 MH and FL-60D were used for column chromatography unless otherwise noted. Organic solvents for moisture-sensitive reactions were distilled from the following drying agents: THF and ether (Na-benzophenone ketyl), benzene (Na), acetonitrile and triethylamine (calcium hydride), DMSO (calcium hydride under reduced pressure), CH_2Cl_2 (P_2O_5), acetone (anhydrous K_2CO_3), and MeOH (Mg). All moisture-sensitive reactions were performed under an atmosphere of nitrogen, and the starting materials were azeotropically dried with benzene before use. All new compounds were determined to be >95% pure by ^{1}H NMR unless otherwise noted.

4.1.1. Homoallylic alcohol 8. Preparation of crotylboronate **7**: To a stirred solution of potassium *tert*-butylalkoxide (8.4 g, 75 mmol) in THF (60 mL) cooled at −78 °C was added liquefied *trans*-2-butene (7.2 mL, 78 mmol) cooled at −78 °C and then 1.56 M solution of BuLi in hexane (48 mL, 75 mmol) so as to maintain the reaction temperature below −65 °C for 2 h. The mixture was stirred at −50 °C for 15 min and re-cooled to −78 °C, triisopropyl borate (17.2 mL, 75.0 mmol) was added so as to keep the reaction temperature below −65 °C for 1 h. The mixture was stirred for 15 min and poured into 1 M aqueous HCl saturated with NaCl (220 mL). To the mixture D-(−)-diisopropyl tartrate (17.5 g, 74.7 mmol) was added, and the mixture was extracted with ether (4×50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated to give boronate **7** (25.4 g) as a colorless oil. Crotylboration: To a stirred mixture of boronate **7** (1.2 g, 9.7 mmol) and MS 4 Å (50 mg) in toluene (10 mL) cooled at −78 °C was added a solution of 3-benzyloxypropanal (517 mg, 3.15 mmol) in toluene (6 mL, 2×2 mL rinse). The mixture was stirred at −78 °C for 1.5 h and diluted with 2 M aqueous NaOH (50 mL). The mixture was stirred at 0 °C for 30 min and extracted with EtOAc (3×10 mL). The extracts were dried with $MgSO_4$ and concentrated. The residual oil was purified by column chromatography on silica gel (12 g, hexane–EtOAc 10:1) to give **8** (630 mg, 91%) as a colorless oil: TLC R_f 0.46 (hexane–EtOAc 3:1); $[\alpha]_D^{20}$ 3.38 (c 1.00, $CHCl_3$) ; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.38–7.24 (m, 5H), 5.81 (m, 1H), 5.11 (m, 1H), 5.05 (m, 1H), 4.56 (s, 2H), 3.77–3.61 (m, 3H), 2.47 (br s, 1H, OH), 2.24 (m, 1H), 1.78–1.71 (m, 2H), 1.05 (d, J=7.0 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 140.2, 137.8, 128.2, 127.5, 115.3, 74.0, 73.2, 69.1, 44.0, 33.6, 15.8.

4.1.2. Silyl ether 9. To a stirred solution of homoallylic alcohol **8** (5.00 g, 22.7 mmol) in THF (65 mL) were added a solution of *N*-methylmorpholine-*N*-oxide (4.05 g, 34.6 mmol) in H_2O (13 mL) and a 0.078 M solution of osmium tetroxide in *tert*-butyl alcohol (15 mL, 1.2 mmol). After being stirred at room temperature for 2.5 h, sodium periodate (10.5 g, 49.1 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h, diluted with saturated aqueous $Na_2S_2O_3$ (50 mL), and extracted with EtOAc (3×50 mL). The combined extracts were washed with saturated aqueous $Na_2S_2O_3$ (3×20 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated to give a crude aldehyde (8.0 g).

To a stirred solution of the crude aldehyde (8.0 g) in EtOH (230 mL) was added sodium borohydride (1.06 g, 28.0

mmol), and the mixture was stirred at room temperature for 20 min. The reaction was quenched by addition of acetone (50 mL) and the resulting mixture was stirred at room temperature for 10 min and concentrated. The mixture was diluted with H_2O (30 mL) and extracted with EtOAc (5×20 mL). The extracts were combined, washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (100 g, hexane–EtOAc 1:1) to give a crude diol (4.77 g).

To a stirred solution of the crude diol (4.77 g) and imidazole (12.7 g, 186 mmol) in DMF (10 mL) cooled at 0 °C was added *tert*-butyldimethylsilyl chloride (14.1 g, 93.6 mmol). The resulting solution was stirred at 50 °C for 11 h, cooled to room temperature, and diluted with cold water (50 mL). The mixture was extracted with EtOAc (3×50 mL). The combined extracts were washed with H_2O (3×30 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (FL60D 100 g, hexane–EtOAc 50:1) to give **9** (8.15 g, 79% in three steps) as a colorless oil : TLC, R_f 0.57 (hexane–EtOAc 4:1); $[\alpha]_D^{26}$ −7.2 (*c* 1.02, $CHCl_3$); IR (neat) 1471, 1255, 1092, 837 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.34–7.27 (m, 5H), 4.49 (s, 2H), 3.91 (dt, *J*=4.5, 7.3 Hz, 1H), 3.59–3.52 (m, 2H), 3.52 (dd, *J*=7.0, 9.9 Hz, 1H), 3.41 (dd, *J*=6.4, 9.9 Hz, 1H), 1.88–1.78 (m, 2H), 1.71 (m, 1H), 0.88 (s, 9H), 0.87 (s, 9H), 0.85 (d, *J*=7.0 Hz, 3H), 0.04 (s, 3H), 0.03 (s, 3H), 0.02 (s, 6H); HRMS (ESI) calcd for $C_{25}H_{49}O_3Si_2$ $(M+H)^+$ 453.3220, found 453.3204.

4.1.3. Alcohol 10. A mixture of silyl ether **9** (7.95 g, 17.6 mmol), $NaHCO_3$ (1.79 g, 21.3 mmol), and 10% Pd on carbon (1.32 g) in EtOAc (176 mL) was stirred under a hydrogen atmosphere at room temperature for 11 h. The mixture was filtered through a pad of Celite, and the residue was washed with EtOAc. The filtrate and the washings were combined and concentrated. The residual oil was purified by column chromatography on silica gel (100 g, hexane–EtOAc 20:1) to give **10** (5.88 g, 92%) as a colorless oil: TLC R_f 0.55 (hexane–EtOAc 5:1); $[\alpha]_D^{28}$ −8.7 (*c* 0.992, $CHCl_3$); IR ($CHCl_3$) 3425, 1473, 1255 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 3.95 (dt, *J*=5.3, 6.3 Hz, 1H), 3.72 (t, *J*=5.6 Hz, 2H), 3.42 (dd, *J*=6.6, 9.9 Hz, 1H), 3.24 (dd, *J*=6.0, 9.9 Hz, 1H), 2.29 (br, 1H, OH), 1.98–1.82 (m, 2H), 1.66 (m, 1H), 0.87 (s, 9H), 0.86 (s, 9H), 0.82 (d, *J*=6.9 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.01 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 72.4, 65.1, 60.7, 41.0, 33.8, 26.0, 25.9, 18.3, 18.1, 11.7, −4.4, −4.5, −5.3, −5.4; HRMS (ESI) calcd for $C_{18}H_{43}O_3Si_2$ $(M+H)^+$ 363.2751, found 363.2751.

4.1.4. Aldehyde 11. To a stirred solution of oxalyl chloride (0.44 mL, 5.0 mmol) in CH_2Cl_2 (12 mL) cooled at −78 °C was added a solution of DMSO (0.65 mL, 9.2 mmol) in CH_2Cl_2 (1.5 mL) dropwise. The resulting solution was stirred at −78 °C for 30 min, and a solution of alcohol **10** (1.22 g, 3.35 mmol) in CH_2Cl_2 (1.5 mL, 3×0.5 mL rinse) was added dropwise. The mixture was stirred at −78 °C for 40 min, and triethylamine (2.4 mL, 17.2 mmol) was added. The resulting mixture was stirred at −78 °C for 1 h, warmed to 0 °C, and stirred for 1 h. The mixture was diluted with H_2O (30 mL) and extracted with EtOAc (3×30 mL). The combined extracts were washed with H_2O (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL), successively, dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (50 g, hexane–ether 50:1→25:1) to give **11** (1.07 g, 89%) as a colorless oil: TLC, R_f 0.55 (hexane–EtOAc 5:1); $[\alpha]_D^{21}$ −4.56 (*c* 1.00, $CHCl_3$); IR (neat) 2854, 1732, 1257, 1086, 837 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 9.81 (t, *J*=2.6 Hz, 1H), 4.35 (dt, *J*=4.9, 5.6 Hz, 1H), 3.48 (dd, *J*=5.9, 10.0 Hz, 1H), 3.43 (dd, *J*=7.0, 10.0 Hz, 1H), 2.50 (dd, *J*=2.6, 5.9 Hz, 2H), 1.92 (m, 1H), 0.89 (s, 9H), 0.87 (s, 9H), 0.86 (d, *J*=7.0 Hz, 3H), 0.07 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 202.4, 68.9, 64.8, 47.8, 41.7, 26.0, 25.9, 18.3, 18.1, 11.6, −4.4, −4.5, −5.3, −5.4; HRMS (ESI) calcd for $C_{18}H_{40}NaO_3Si_2$ $(M+Na)^+$ 383.2414, found 383.2367.

4.1.5. Hydroxy imide 13. To a stirred solution of imide **12** (226 mg, 0.970 mmol) in CH_2Cl_2 (1.4 mL) cooled at 0 °C were added 1 M solution of dibutylboron triflate in CH_2Cl_2 (0.97 mL, 0.97 mmol) and triethylamine (0.19 mL, 1.3 mmol), successively. The reaction mixture was stirred at 0 °C for 40 min and cooled to −78 °C. A solution of aldehyde **11** (212 mg, 0.587 mmol) in CH_2Cl_2 (0.3 mL, 2×0.2 mL rinse) was added, and the reaction mixture was stirred at −78 °C for 2 h and at 0 °C for 20 min. After the reaction was quenched by addition of 0.5 M phosphate buffer (pH 7, 2 mL) and MeOH (3 mL), 30% aqueous hydrogen peroxide (1.5 mL) in MeOH (3 mL) was added slowly, and the resulting solution was stirred at 0 °C for 1 h. The organic solvents were evaporated, and the mixture was cooled to 0 °C. Saturated aqueous $Na_2S_2O_3$ (3 mL) was added slowly, and the mixture was extracted with ether (3×5 mL). The extracts were combined, washed with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (60 g, hexane–EtOAc 15:1→10:1→5:1) to give **13** (358 mg, 100%) as a colorless oil along with recovered **12** (64 mg). Compound **13**: TLC, R_f 0.40 (hexane–EtOAc 5:1); $[\alpha]_D^{22}$ −31.9 (*c* 1.00, $CHCl_3$); IR (neat) 3525, 1783, 1697, 1387, 1251, 1209, 1091 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.37–7.15 (m, 5H), 4.69 (m, 1H), 4.26–4.14 (m, 3H), 3.98 (dt, *J*=4.0, 6.6 Hz, 1H), 3.75 (dq, *J*=4.0, 7.0 Hz, 1H), 3.61 (dd, *J*=6.0, 10.0 Hz, 1H), 3.53 (br s, 1H, OH), 3.47 (dd, *J*=6.0, 10.0 Hz, 1H), 3.28 (dd, *J*=3.3, 13.5 Hz, 1H), 2.77 (dd, *J*=9.6, 13.5 Hz, 1H), 1.96 (dt, *J*=6.0, 6.6 Hz, 1H), 1.67 (ddd, *J*=4.0, 10.0, 14.0 Hz, 1H), 1.55 (ddd, *J*=2.6, 6.6, 14.0 Hz, 1H), 1.27 (d, *J*=7.0 Hz, 3H), 0.89 (s, 18H), 0.88 (d, *J*=7.0 Hz, 3H), 0.10 (s, 3H), 0.08 (s, 3H), 0.04 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 176.2, 153.0, 135.1, 129.3, 128.9, 127.3, 68.6, 66.1, 65.9, 65.0, 55.3, 43.4, 40.7, 37.9, 36.2, 26.0, 26.9, 18.4, 18.1, 12.6, 11.3, −4.3, −4.6, −5.2, −5.3; HRMS (ESI) calcd for $C_{31}H_{55}NaO_6Si_2$ $(M+Na)^+$ 616.3466, found 616.3466.

4.1.6. Alcohol 14. To a stirred solution of hydroxy imide **13** (878 mg, 1.48 mmol) in ether (26 mL) in the presence of anhydrous ethanol (0.10 mL, 1.8 mmol) cooled at −10 °C was added a 2.0 M solution of lithium borohydride in THF (0.90 mL, 1.8 mmol), and the solution was stirred at −10 °C for 30 min. The reaction was quenched by addition of 1 M aqueous NaOH (4 mL), and the mixture was stirred at 0 °C for 15 min. The mixture was diluted with saturated aqueous $Na_2S_2O_3$ (40 mL) and extracted with

ether (4×20 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (60 g, hexane–EtOAc 3:1→1:1→1:3) to give **14** (628 mg, 100%) as a colorless oil: TLC, R_f 0.54 (hexane–ether 1:3); $[\alpha]_D^{19}$ +7.9 (*c* 1.00, $CHCl_3$); IR (neat) 3392, 1471, 1255, 1064, 836 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 4.07 (ddd, *J*=2.4, 2.4, 10.5 Hz, 1H), 3.89 (dt, *J*=4.6, 7.3 Hz, 1H), 3.70–3.55 (m, 2H), 3.61 (dd, *J*=5.4, 9.7 Hz, 1H), 3.45 (dd, *J*=5.4, 9.7 Hz, 1H), 3.14 (br s, 1H, OH), 1.94 (m, 1H), 1.77 (m, 1H), 1.72 (ddd, *J*=4.6, 10.5, 12.4 Hz, 1H), 1.50 (ddd, *J*=2.4, 4.6, 12.4 Hz, 1H), 0.89 (d, *J*=7.0 Hz, 3H), 0.84 (s, 9H), 0.83 (s, 9H), 0.80 (d, *J*=7.0 Hz, 3H), 0.07 (s, 3H), 0.03 (s, 3H), 0.00 (s, 3H), −0.01 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 72.6, 72.0, 66.8, 65.9, 64.9, 39.9, 39.8, 35.1, 26.0, 25.4, 18.4, 18.1, 13.4, 11.2, −4.1, −4.7, −5.2, −5.3; HRMS (ESI) calcd for $C_{21}H_{48}NaO_4Si_2$ $(M+Na)^+$ 443.2989, found 443.2995.

4.1.7. Sulfide 15. To a stirred solution of alcohol **14** (215 mg, 0.511 mmol) and diphenyl disulfide (197 mg, 0.904 mmol) in DMF (2 mL) cooled at 0 °C was added tributylphosphine (0.25 mL, 1.0 mmol), and the resulting solution was stirred at room temperature for 11 h. Water (0.5 mL) was added, and the resulting mixture was concentrated. The residual oil was purified by column chromatography on silica gel (60 g, hexane–ether 10:1) to give **15** (254 mg, 99%) as a colorless oil: TLC, R_f 0.46 (hexane–ether 5:1); $[\alpha]_D^{22}$ +11.1 (*c* 1.00, $CHCl_3$); IR (neat) 3496, 1471, 1254, 1063, 837 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.35–7.11 (m, 5H), 4.05 (m, 1H), 3.93 (m, 1H), 3.63 (dd, *J*=5.8, 9.8 Hz, 1H), 3.50 (dd, *J*=5.4, 9.8 Hz, 1H), 3.18 (dd, *J*=6.1, 12.7 Hz, 1H), 2.78 (dd, *J*=7.8, 12.7 Hz, 1H), 2.01 (m, 1H), 1.73 (m, 1H), 1.68 (ddd, *J*=3.6, 10.8, 14.7 Hz, 1H), 1.51 (ddd, *J*=1.9, 5.4, 14.7 Hz, 1H), 1.02 (d, *J*=6.8 Hz, 3H), 0.91 (s, 9H), 0.90 (s, 9H), 0.85 (d, *J*=7.0 Hz, 3H), 0.11 (s, 3H), 0.08 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 137.1, 129.2, 128.9, 128.7, 128.5, 125.4, 72.3, 70.0, 64.9, 40.0, 38.9, 37.2, 36.3, 26.0, 25.9, 18.4, 18.1, 13.7, 13.2, −4.2, −4.7, −5.2, −5.3; HRMS (ESI) calcd for $C_{27}H_{52}NaO_3SSi_2$ $(M+Na)^+$ 535.3073, found 535.3054.

4.1.8. Sulfone 16. To a stirred solution of sulfide **15** (1.36 g, 2.66 mmol) in CH_2Cl_2 (26 mL) cooled at 0 °C were added $NaHCO_3$ (1.67 g, 19.9 mmol) and 77% *m*-chloroperoxybenzoic acid (1.61 g, 7.18 mmol). After 5 min, the mixture was warmed to room temperature and stirred at room temperature for 15 min. The reaction mixture was diluted with saturated aqueous $Na_2S_2O_3$ (10 mL) and H_2O (30 mL), stirred at room temperature for 30 min, and extracted with ether (3×50 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (43 g, hexane–EtOAc 6:1) to give **16** (1.44 g, 99%) as a colorless oil: TLC, R_f 0.4 (hexane–ether 1:1); $[\alpha]_D^{22}$ +7.4 (*c* 1.00, $CHCl_3$); IR (neat) 3519, 1471, 1306, 1255, 1147, 1086, 837 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.97–7.94 (m, 2H), 7.70–7.55 (m, 3H), 4.00–3.90 (m, 2H), 3.67 (dd, *J*=5.7, 9.7 Hz, 1H), 3.52 (dd, *J*=5.7, 9.7 Hz, 1H), 3.52 (br s, 1H, OH), 3.45 (dd, *J*=4.1, 14.0 Hz, 1H), 3.01 (dd, *J*=7.8, 14.0 Hz, 1H), 2.25 (m, 1H), 1.99 (m, 1H), 1.64 (ddd, *J*=4.9, 10.3, 14.3 Hz, 1H), 1.55 (ddd, *J*=2.7, 4.9, 14.3 Hz, 1H), 1.11 (d, *J*=7.0 Hz, 3H), 0.98 (s, 9H), 0.94 (s, 9H), 0.88 (d, *J*=7.0 Hz, 3H), 0.18 (s, 3H), 0.15 (s, 3H), 0.12 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 140.0, 133.4, 129.1, 127.7, 72.3, 70.4, 64.8, 59.4, 39.7, 35.7, 34.5, 26.0, 25.9, 18.4, 18.0, 14.1, 13.4, −4.2, −4.7, −5.2, −5.4; HRMS (ESI) calcd for $C_{27}H_{52}NaO_5Si_2$ $(M+Na)^+$ 567.2972, found 567.2980.

4.1.9. C22–C28 segment 5. To a stirred solution of sulfone **16** (1.44 g, 2.64 mmol) in THF (26 mL) cooled at 0 °C were added methyl iodide (0.84 mL, 13.5 mmol) and NaH (401 mg of 60% dispersion in mineral oil, 10.0 mmol), successively. The mixture was stirred at room temperature for 16 h, and the reaction was quenched by addition of ice (2 g) and saturated aqueous NH_4Cl (30 mL). The mixture was extracted with ether (3×30 mL). The combined extracts were washed with saturated aqueous $Na_2S_2O_3$ (30 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (75 g, benzene–ether 100:1→50:1) to give **5** (1.39 g, 95%) as a colorless oil: TLC, R_f 0.58 (benzene–ether 10:1); $[\alpha]_D^{26}$ −39.3 (*c* 1.00, $CHCl_3$); IR (neat) 1471, 1306, 1255, 1149, 1088, 837 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.97–7.94 (m, 2H), 7.70–7.55 (m, 3H), 3.87 (ddd, *J*=1.9, 3.5, 5.1 Hz, 1H), 3.38 (d, *J*=7.0 Hz, 2H), 3.35 (dd, *J*=1.5, 14.0 Hz, 1H), 3.23 (ddd, *J*=1.9, 3.8, 10.0 Hz, 1H), 3.09 (s, 3H), 2.78 (dd, *J*=10.3, 14.0 Hz, 1H), 2.53 (m, 1H), 1.89 (dtq, *J*=3.5, 7.0, 7.0 Hz, 1H), 1.31 (ddd, *J*=1.9, 10.0, 14.0 Hz, 1H), 1.08 (d, *J*=7.0 Hz, 3H), 1.07 (m, 1H), 0.89 (s, 9H), 0.88 (s, 9H), 0.80 (d, *J*=7.0 Hz, 3H), 0.08 (s, 3H), 0.07 (s, 3H), 0.04 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 139.9, 133.5, 129.2, 128.0, 80.3, 69.5, 65.2, 57.3, 56.3, 42.2, 31.8, 29.0, 26.1, 26.0, 18.3, 16.3, 10.6, −3.8, −4.4, −5.2, −5.3; HRMS (ESI) calcd for $C_{28}H_{54}NaO_5SSi_2$ $(M+Na)^+$ 581.3128, found 581.3095; Anal. Calcd for $C_{28}H_{54}O_5SSi_2$: C, 60.17; H, 9.74. Found: C, 60.05; H, 9.68.

4.1.10. Hydroxy ketone 18. To a mixture of $Sn(OTf)_2$ (5.8 g, 14 mmol) and triethylamine (2.4 mL, 17 mmol) in CH_2Cl_2 (130 mL) cooled at −78 °C was added a solution of ethylketone **17** (2.2 g, 11 mmol), and the mixture was stirred at −78 °C for 2 h. A 2.3 M solution of crotonaldehyde in CH_2Cl_2 (6.6 mL, 16 mmol) was added, and the reaction mixture was stirred at −78 °C for 2 h and −60 °C for 1 h. The mixture was warmed to room temperature and diluted with 0.5 M phosphate buffer (pH 7.0, 130 mL). The organic layer was separated, and the aqueous layer was extracted with ether (3×130 mL). The organic layer and the extracts were combined, washed with 0.5 M phosphate buffer (pH 7.0, 130 mL) and brine (130 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified twice by column chromatography on silica gel (100 g, hexane–ether 5:1→4:1→3:1) and (FL60D 100 g, hexane–ether 5:1→4:1→3:1) to give **18** (2.5 g, 85%) as a colorless oil: TLC, R_f 0.41 (hexane–ether 1:1); $[\alpha]_D^{26}$ −0.07 (*c* 1.00, $CHCl_3$); IR (neat) 3446 (br), 1701, 1655 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.36–7.24 (m, 5H), 5.65 (ddq, *J*=1.1, 15.1, 6.5 Hz, 1H), 5.41 (ddq, *J*=6.2, 15.1, 1.4 Hz, 1H), 4.49 (d, *J*=11.9 Hz, 1H), 4.43 (d, *J*=11.9 Hz, 1H), 4.42 (m, 1H), 3.64 (dd, *J*=8.6, 8.6 Hz, 1H), 3.45 (dd, *J*=5.1, 8.6 Hz, 1H), 3.15 (m, 1H), 2.84 (dq, *J*=3.5, 7.0 Hz, 1H), 2.60 (br, 1H, OH), 1.67 (dd, *J*=1.4, 6.5 Hz, 3H), 1.08

(d, J=7.3 Hz, 3H), 1.03 (d, J=7.0 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 217.4, 137.5, 130.3, 128.3, 127.6, 127.5, 127.5, 73.4, 72.9, 72.3, 51.3, 45.4, 17.8, 15.6, 10.2; HRMS (ESI) calcd for $C_{17}H_{24}NaO_3$ $(M+Na)^+$ 299.1623, found 299.1625.

4.1.11. Acetonide 19. To a solution of tetramethylammonium triacetoxyborohydride (24.8 g, 94.2 mmol) in acetonitrile (88 mL) and acetic acid (93 mL) cooled at –25 °C was added a solution of hydroxy ketone **18** (5.14 g, 18.6 mmol) in acetonitrile (3 mL, 2×1 mL rinse). The reaction mixture was stirred at −25 °C for 1 h and at −15 °C for 34 h. The mixture was diluted with 0.5 M aqueous Na/K tartrate (350 mL) and vigorously stirred at room temperature for 1 h. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3×50 mL). The organic layer and the extracts were combined, washed with H_2O (200 mL), saturated aqueous $NaHCO_3$ (3×200 mL), and brine (200 mL), respectively, dried (Na_2SO_4), and concentrated to give a crude diol (5.73 g).

To a solution of the diol (5.73 g) in acetone (77 mL) and 2,2-dimethoxypropane (77 mL) was added pyridinium *p*-toluenesulfonate (487 mg, 1.94 mmol). The mixture was stirred at room temperature for 1.5 h and diluted with saturated aqueous $NaHCO_3$ (150 mL). The organic layer was separated, and the aqueous layer was extracted with ether (3×100 mL). The organic layer and the extracts were combined, washed with brine (100 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (100 g, hexane–ether 10:1) to give **19** (4.97 g, 84%) as a colorless oil: TLC, R_f 0.89 (hexane–ether 1:1); $[\alpha]_D^{26}$ −10.9 (c 0.862, $CHCl_3$); IR (neat) 1222, 735, 698 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.34–7.24 (m, 5H), 5.67 (ddq, J=0.8, 15.4, 5.9 Hz, 1H), 5.45 (ddq, J=7.0, 15.4, 1.6 Hz, 1H), 4.52 (d, J=11.9 Hz, 1H), 4.47 (d, J=11.9 Hz, 1H), 4.28 (m, 1H), 3.60 (dd, J=4.9, 9.2 Hz, 1H), 3.36 (dd, J=7.0, 9.2 Hz, 1H), 3.28 (dd, J=5.1, 7.3 Hz, 1H), 1.96 (m, 1H), 1.89 (m, 1H), 1.71 (dd, J=1.6, 5.9 Hz, 3H), 1.32 (s, 6H), 1.02 (d, J=7.0 Hz, 3H), 0.87 (d, J=7.0 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 138.7, 128.6, 128.2, 127.5, 127.4, 127.3, 100.3, 76.2, 73.1, 72.2, 70.9, 38.0, 37.7, 25.8, 23.7, 18.0, 14.4, 13.4; MS (FAB) m/z 341 $(M+Na)^+$; HRMS (ESI) calcd for $C_{20}H_{30}NaO_3$ $(M+Na)^+$ 341.2093, found 341.2094.

4.1.12. Alcohol 20. Calcium (1.37 g, 34.2 mmol) was added to a stirred solution of acetonide **19** (4.96 g, 15.6 mmol) in THF (250 mL), isopropyl alcohol (85 mL), and liquid NH_3 (170 mL) cooled at −78 °C. After the mixture was stirred at −78 °C for 2 h, NH_4Cl (14.3 g) and $Fe(NO_3)_3\cdot 9H_2O$ (2.5 g) were added. The mixture was stirred at −78 °C for 1 h and allowed to warm to room temperature. The residue was diluted with H_2O (400 mL), and the mixture was stirred at room temperature for 1 h and extracted with EtOAc (5×100 mL). The combined extracts were washed with brine (100 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (150 g, hexane–ether 3:1→2:1) to give **20** (3.49 g, 98%) as a colorless oil: TLC, R_f 0.40 (hexane–ether 1:1); $[\alpha]_D^{32}$ −33.2 (c 1.25, $CHCl_3$); IR (neat) 3455 (br), 1678, 1225 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.64 (m, 1H), 5.39 (ddq, J=7.3, 15.4, 1.6 Hz, 1H), 4.31 (m, 1H), 3.69 (dd, J=3.2, 11.1 Hz, 1H), 3.52 (dd, J=5.9, 11.1 Hz, 1H), 3.26 (dd, J=5.7, 7.6 Hz, 1H), 2.68 (br, 1H), 1.82 (dd, J=2.2, 6.9 Hz, 1H), 1.75 (m, 1H), 1.65 (dd, J=1.6, 6.2 Hz, 1H), 1.32 (s, 3H), 1.31 (s, 3H), 0.96 (d, J=6.9 Hz, 3H), 0.83 (d, J=6.9 Hz, 3H); MS (FAB) m/z 251 $(M+Na)^+$; HRMS (ESI) calcd for $C_{13}H_{24}NaO_3$ $(M+Na)^+$ 251.1623, found 251.1607.

4.1.13. Tosylate 21. To a stirred solution of alcohol **20** (1.00 g, 4.38 mmol) in pyridine (2.2 mL) cooled at 0 °C was added *p*-toluenesulfonyl chloride (1.50 g, 7.87 mmol), and the mixture was stirred at 0 °C for 6 h. The mixture was diluted with H_2O (15 mL), stirred at room temperature for 30 min, and extracted with ether (3×15 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (50 g, hexane–ether 5:1→3:1) to give **21** (1.67 g, 100%) as a colorless oil: TLC, R_f 0.60 (hexane–ether 1:1); $[\alpha]_D^{26}$ −3.87 (c 1.08, $CHCl_3$); IR (neat) 1598, 1495, 1224, 794, 706 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.79 (d, J=7.9 Hz, 2H), 7.34 (d, J=7.9 Hz, 2H), 5.65 (ddq, J=0.8, 15.1, 6.2 Hz, 1H), 5.40 (ddq, J=7.0, 15.1, 1.6 Hz, 1H), 4.23 (m, 1H), 4.15 (dd, J=9.5, 4.0 Hz, 1H), 3.98 (dd, J=6.8, 9.5 Hz, 1H), 3.16 (dd, J=6.2, 7.6 Hz, 1H), 2.45 (s, 3H), 1.92 (m, 1H), 1.74 (m, 1H), 1.70 (dd, J=1.6, 6.2 Hz, 3H), 1.24 (s, 6H), 0.97 (d, J=6.8 Hz, 3H), 0.84 (d, J=6.8 Hz, 3H); MS (FAB) m/z 405 $(M+Na)^+$; HRMS (ESI) calcd for $C_{20}H_{30}NaO_5S$ $(M+Na)^+$ 405.1712, found 405.1720.

4.1.14. Nitrile 22. To a stirred solution of tosylate **21** (1.04 g, 2.72 mmol) in DMSO (15 mL) was added sodium cyanide (656 mg, 13.4 mmol) at room temperature, and the reaction mixture was stirred at 50 °C for 2 h. After cooling, the mixture was diluted with H_2O (38 mL) and extracted with ether (4×40 mL). The combined extracts were washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (25 g, hexane–ether 10:1→5:1) to give **22** (633 mg, 98%) as a colorless oil: TLC, R_f 0.63 (hexane–ether 1:1); $[\alpha]_D^{26}$ −19.5 (c 0.91, $CHCl_3$); IR (neat) 2246, 1224 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.69 (m, 1H), 5.44 (ddq, J=7.0, 15.4, 1.2 Hz, 1H), 4.30 (m, 1H), 3.15 (dd, J=7.0, 7.0 Hz, 1H), 2.45 (m, 2H), 1.95 (m, 1H), 1.74 (m, 1H), 1.71 (dd, J=1.2, 6.7 Hz, 3H), 1.35 (s, 3H), 1.33 (s, 3H), 1.14 (d, J=6.7 Hz, 3H), 0.92 (d, J=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 127.9, 127.9, 118.9, 100.6, 76.8, 70.5, 39.1, 35.1, 25.6, 23.6, 20.6, 18.0, 16.2, 13.5; MS (FAB) m/z 260 $(M+Na)^+$; HRMS (ESI) calcd for $C_{14}H_{23}NaO_2$ $(M+Na)^+$ 260.1626, found 260.1605.

4.1.15. Aldehyde 23. To a stirred solution of nitrile **22** (456 mg, 1.92 mmol) in CH_2Cl_2 (7.7 mL) cooled at −78 °C was added a 1.0 M solution of diisobutylaluminum hydride in hexane (2.4 mL, 2.4 mmol). The solution was stirred at −78 °C for 70 min, and the reaction was quenched by addition of MeOH (3 mL). After the mixture was warmed to room temperature, 0.5 M aqueous Na/K tartrate (25 mL) was added. The resulting mixture was vigorously stirred at room temperature for 1 h, and the organic layer was separated. The aqueous layer was extracted with ether (3×30 mL). The organic layer and the extracts were combined, washed with brine (10 mL), dried (Na_2SO_4), and

concentrated. The residual oil was purified by column chromatography on silica gel (FL60D 15 g, benzene–ether 100:1→80:1→20:1) to give **23** (439 mg, 95%) as a colorless oil: TLC, R_f 0.52 (benzene–ether 20:1); $[\alpha]_D^{26}$ −26.8 (*c* 0.81, $CHCl_3$); IR (neat) 2725, 1726, 1225 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 9.74 (dd, *J*=2.2, 2.2 Hz, 1H), 5.69 (ddq, *J*=1.0, 15.4, 6.3 Hz, 1H), 5.42 (ddq, *J*=7.0, 15.4, 0.8 Hz, 1H), 4.30 (m, 1H), 3.11 (dd, *J*=5.9, 7.0 Hz, 1H), 2.52 (ddd, *J*=2.2, 5.4, 15.9 Hz, 1H), 2.31 (ddd, *J*=2.2, 6.8, 15.9 Hz, 1H), 2.21 (m, 1H), 1.75 (m, 1H), 1.71 (dd, *J*=0.8, 6.3 Hz, 3H), 1.31 (s, 6H), 1.03 (d, *J*=6.8 Hz, 3H), 0.90 (d, *J*=7.0 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 202.2, 128.2, 127.7, 100.5, 78.4, 70.7, 47.7, 39.1, 33.2, 25.6, 23.6, 18.0, 17.1, 13.5; MS (FAB) *m/z* 263 $(M+Na)^+$; HRMS (ESI) calcd for $C_{14}H_{25}O_3$ $(M+H)^+$ 241.1804, found 241.1817.

4.1.16. Methyl acetal 24a. Aldehyde **23** (1.06 g, 4.41 mmol) was dissolved in a 0.009 M solution of pyridinium *p*-toluenesulfonate (16.2 mL, 0.146 mmol) in MeOH, and the solution was stirred at room temperature for 30 min. The reaction was quenched by addition of triethylamine (3 mL), and the resulting mixture was concentrated. The residual oil was purified by column chromatography on silica gel (FL60D 100 g, hexane–ether 10:1→7:1→6:1→5:1→4:1) to give **24a** (409 mg, 43%), **24b** (319 mg), and dimethyl acetal **24c** (199 mg) as a colorless oil, respectively. Acetals **24b** (319 mg) and **24c** (199 mg) were dissolved in a 0.009 M solution of pyridinium *p*-toluenesulfonate (8.0 mL, 0.072 mmol) in MeOH. After the mixture was stirred at room temperature for 1 h, triethylamine (1.5 mL) was added, and the resulting mixture was concentrated. The residual oil was purified by column chromatography on silica gel (FL60D 50 g, hexane–ether 7:1→6:1→5:1→4:1) to give **24a** (225 mg, 24%), **24b** (167 mg) and **24c** (46.7 mg) as colorless oil, respectively. Further, from acetals **24b** (167 mg) and **24c** (46.7 mg), acetals **24a** (89 mg, 9%), **24b** (84 mg), and **24c** (19 mg, 2%) were obtained by repeating the procedure described above. Further from **24b** (84 mg) and **24c** (19 mg), acetals **24a** (49 mg, 5%), **24b** (38 mg, 4%), and **24c** (4 mg, 0.3%) were obtained again. In total, methyl acetal **24a** (772 mg, 82%) was obtained from aldehyde **23** (1.06 g). Compound **24a**: TLC, R_f 0.37 (hexane–ether 1:1); $[\alpha]_D^{26}$ +77.2 (*c* 0.97, $CHCl_3$); IR ($CHCl_3$) 3490 (br), 1670, 1455, 1380, 1235 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 5.67 (ddq, *J*=1.1, 15.1, 6.5 Hz, 1H), 5.52 (ddq, *J*=6.2, 15.1, 1.6 Hz, 1H), 4.90 (d, *J*=4.9 Hz, 1H), 4.25 (br, 1H), 3.54 (dd, *J*=8.1, 8.1 Hz, 1H), 3.31 (s, 3H), 3.21 (m, 1H), 2.29 (m, 1H), 2.05 (dd, *J*=7.0, 12.7 Hz, 1H), 1.79 (m, 1H), 1.70 (dd, *J*=1.6, 6.5 Hz, 3H), 1.57 (ddd, *J*=4.9, 10.8, 12.7 Hz, 1H), 1.04 (d, *J*=6.8 Hz, 3H), 0.87 (d, *J*=7.3 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 131.4, 126.3, 104.9, 89.0, 74.4, 55.0, 44.0, 41.8, 35.8, 18.7, 17.9, 12.0; MS (FAB) *m/z* 237 $(M+Na)^+$; HRMS (ESI) calcd for $C_{12}H_{23}O_3$ $(M+H)^+$ 215.1647, found 215.1651. Compound **24b**: TLC, R_f 0.43 (hexane–ether 1:1); $[\alpha]_D^{29}$ +112 (*c* 0.995, $CHCl_3$); IR ($CHCl_3$) 3490 (br), 1675, 1450, 1380, 1230 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 5.70 (ddq, *J*=1.0, 15.5, 6.3 Hz, 1H), 5.55 (ddq, *J*=6.3, 15.5, 1.3 Hz, 1H), 4.99 (d, *J*=2.3, 5.6 Hz, 1H), 4.15 (m, 1H), 3.60 (dd, *J*=7.3, 7.3 Hz, 1H), 3.48 (br m, 1H), 3.34 (s, 3H), 2.30 (m, 1H), 2.04 (m, 1H), 1.81 (m, 1H), 1.73 (dd, *J*=1.3, 6.3 Hz, 3H), 1.51 (ddd, *J*=2.3, 5.6, 13.5 Hz, 1H), 1.10 (d, *J*=6.6 Hz, 3H), 0.95 (d, *J*=7.2 Hz, 3H); MS (FAB) *m/z* 237 $(M+Na)^+$. Compound **24c**: TLC, R_f 0.76 (hexane–ether 1:1); $[\alpha]_D^{28}$ −17 (*c* 0.34, $CHCl_3$); IR ($CHCl_3$) 1675, 1455, 1380, 1225 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 5.67 (ddq, *J*=0.7, 15.5, 6.3 Hz, 1H), 5.45 (ddq, *J*=7.3, 15.5, 1.3 Hz, 1H), 4.48 (dd, *J*=4.3, 7.6 Hz, 1H), 4.29 (ddd, *J*=0.7, 5.0, 7.3 Hz, 1H), 3.34 (s, 3H), 3.30 (s, 3H), 3.13 (dd, *J*=4.6, 7.3 Hz, 1H), 1.89 (ddd, *J*=3.3, 7.6, 14.2 Hz, 1H), 1.81–1.70 (m, 2H), 1.71 (dd, *J*=1.3, 6.3 Hz, 3H), 1.40 (ddd, *J*=4.3, 9.6, 14.2 Hz, 1H), 1.32 (s, 6H), 0.99 (d, *J*=6.6 Hz, 3H), 0.87 (d, *J*=7.2 Hz, 3H); MS (FAB) *m/z* 309 $(M+Na)^+$; HRMS (ESI) calcd for $C_{16}H_{30}NaO_4$ $(M+Na)^+$ 309.2042, found 309.2048.

4.1.17. Benzyl ether 25. To a stirred solution of methyl acetal **24a** (771 mg, 3.60 mmol) in DMF (11 mL) cooled at 0 °C were added benzyl bromide (1.3 mL, 11 mmol) and sodium hydride (438 mg of 60% dispersion in mineral oil, 11 mmol), successively. The mixture was stirred at room temperature for 3 h, cooled to 0 °C, and diluted with H_2O (20 mL) and saturated aqueous NH_4Cl (40 mL). The mixture was extracted with ether (3×30 mL), and the combined extracts were washed with saturated aqueous $NaHCO_3$ (2×30 mL) and brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (FL60D 50 g, hexane–ether 40:1→20:1) to give **25** (1.04 g, 95%) as a colorless oil: TLC, R_f 0.56 (hexane–ether 5:1); $[\alpha]_D^{26}$ −26.8 (*c* 0.81, $CHCl_3$); IR (neat) 2933, 1207, 734 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.27–7.11 (m, 5H), 5.57 (m, 1H), 5.42 (ddq, *J*=7.3, 15.1, 1.1 Hz, 1H), 4.81 (d, *J*=4.9 Hz, 1H), 4.49 (d, *J*=11.9 Hz, 1H), 4.21 (d, *J*=11.9 Hz, 1H), 4.13 (m, 1H), 3.60 (dd, *J*=6.8, 9.4 Hz, 1H), 3.21 (s, 3H), 2.14 (m, 1H), 1.99 (m, 1H), 1.64 (dd, *J*=1.1, 6.2 Hz, 3H), 1.56 (m, 1H), 1.52 (m, 1H), 0.99 (d, *J*=6.5 Hz, 3H), 0.85 (d, *J*=7.0 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 139.5, 131.0, 128.0, 127.6, 127.1, 126.9, 104.6, 87.3, 80.1, 70.4, 54.3, 46.5, 42.5, 35.5, 19.9, 17.9, 9.6; MS (FAB) *m/z* 327 $(M+Na)^+$; HRMS (ESI) calcd for $C_{19}H_{28}NaO_3$ $(M+Na)^+$ 327.1936, found 327.1935.

4.1.18. C29–C35 segment 6. To a stirred solution of benzyl ether **25** (69.2 mg, 0.227 mmol) in acetone (1.5 mL) and H_2O (0.5 mL) were added *N*-methylmorpholine-*N*-oxide (40.0 mg, 0.341 mmol) and a 2.4% solution of osmium tetroxide in *tert*-butyl alcohol (0.15 mL, 0.011 mmol). After being stirred at room temperature for 2 h, sodium periodate (133 mg, 0.622 mmol) was added. The reaction mixture was stirred at room temperature for 30 min, diluted with saturated aqueous $Na_2S_2O_3$ (10 mL), and extracted with EtOAc (3×13 mL). The combined extracts were washed with saturated aqueous $Na_2S_2O_3$ (13 mL) and brine (13 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (3 g, hexane–ether 3:1→1:1) to give **6** (66.9 mg, 99%) as a colorless oil: TLC, R_f 0.51 (hexane–ether 1:1); $[\alpha]_D^{26}$ +24.7 (*c* 1.34, $CHCl_3$); IR (neat) 2829, 2698, 1732, 1207, 739, 698 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 9.73 (d, *J*=1.3 Hz, 1H), 7.41–7.25 (m, 5H), 4.92 (d, *J*=4.9 Hz, 1H), 4.72 (d, *J*=11.3 Hz, 1H), 4.61 (d, *J*=11.3 Hz, 1H), 4.23 (dd, *J*=1.3, 3.0 Hz, 1H), 3.60 (dd, *J*=6.8, 10.0 Hz, 1H), 3.31 (s, 3H), 2.28 (m, 1H), 2.12 (dd, *J*=6.8, 12.7 Hz, 1H), 2.10 (m, 1H), 1.66 (ddd, *J*=4.9, 9.9, 12.7 Hz, 1H), 1.11 (d, *J*=6.5 Hz, 3H), 0.92 (d, *J*=7.0 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 204.4,

137.8, 128.3, 127.7, 127.7, 104.9, 86.4, 85.2, 73.3, 54.8, 42.9, 42.4, 35.9, 20.0, 10.7; MS (FAB) *m/z* 315 $(M+Na)^+$; HRMS (FAB) calcd for $C_{17}H_{24}NaO_4$ $[(M+Na)^+]$ 315.1572, found 315.1593; Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27. Found: C, 69.43; H, 8.31.

4.1.19. Olefin 26. To a stirred solution of C22–C28 segment **5** (744 mg, 1.30 mmol) in THF (4.3 mL) cooled at −78 °C was added a 1.52 M solution of BuLi in hexane (0.73 mL, 1.7 mmol) dropwise. The mixture was stirred at −78 °C for 30 min, and then a solution of C29–C35 segment **6** (115.3 mg, 0.394 mmol) in THF (4 mL) was added dropwise, and the resulting mixture was stirred at −78 °C for 3 h. The reaction was quenched by addition of saturated aqueous NH_4Cl (5 mL), and the mixture was extracted with ether (3×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (20 g, hexane–ether 10:1→4:1→2:1→1:1) to give a diastereomeric mixture of hydroxy sulfones (343 mg) as a colorless oil along with recovered **5** (498 mg, 67%). The hydroxy sulfones were employed in the next experiment without separation of the diastereomers. To a vigorously stirred solution of the diastereomeric mixture of hydroxy sulfones (343 mg) in MeOH (13 mL) cooled at 0 °C were added Na_2HPO_4 (965 mg, 6.80 mmol) and 5% sodium amalgam (2.2 g, 4.8 mmol). The mixture was stirred at 0 °C for 2 h, diluted with saturated aqueous NH_4Cl (10 mL), then stirred at room temperature for 30 min, and extracted with ether (3×20 mL). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (6 g, hexane–ether 10:1→5:1) to give olefin **26** (196 mg, 72% from **6**) as a colorless oil: TLC, R_f 0.51 (hexane–ether 4:1); $[\alpha]_D^{25}$ −19.6 (*c* 1.00, $CHCl_3$); IR (neat) 1470, 1254, 1092 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.36–7.20 (m, 5H), 5.62 (dd, *J*=7.0, 16.0 Hz, 1H), 5.46 (dd, *J*=7.0, 16.0 Hz, 1H), 4.88 (d, *J*=4.9 Hz, 1H), 4.57 (d, *J*=11.9 Hz, 1H), 4.35 (d, *J*=11.9 Hz, 1H), 4.20 (dd, *J*=2.7, 7.0 Hz, 1H), 4.01 (dt, *J*=9.5, 2.7 Hz, 1H), 3.67 (dd, *J*=7.0, 8.9 Hz, 1H), 3.47–3.24 (m, 3H), 3.32 (s, 3H), 3.28 (s, 3H), 2.54 (m, 1H), 2.21 (m, 1H), 2.06 (dd, *J*=7.6, 12.4 Hz, 1H), 1.88 (m, 1H), 1.67–1.57 (m, 2H), 1.42–1.24 (m, 2H), 1.07 (d, *J*=7.0 Hz, 3H), 0.97 (d, *J*=7.0 Hz, 3H), 0.92 (d, *J*=7.0 Hz, 3H), 0.88 (s, 9H), 0.86 (s, 9H), 0.77 (d, *J*=7.0 Hz, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.00 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 139.4, 135.2, 129.5, 128.0, 127.2, 127.7, 104.5, 87.3, 31.2, 80.3, 70.5, 69.8, 65.3, 56.6, 54.3, 46.4, 42.6, 42.4, 38.8, 35.4, 33.7, 26.1, 26.0, 19.9, 18.3, 18.2, 16.0, 10.7, 9.7, −3.8, −4.4, −5.3, −5.4; HRMS (ESI) calcd for $C_{39}H_{72}NaO_6Si_2$ $(M+Na)^+$ 715.4765, found 715.4727.

4.1.20. Alcohol 27. Calcium (1.13 g, 28.1 mmol) was added to a stirred solution of olefin **26** (616 mg, 0.956 mmol) in THF (30 mL), isopropyl alcohol (10 mL), and liquid NH_3 (20 mL) cooled at −78 °C. After the mixture was stirred at −78 °C for 1.5 h, NH_4Cl (6.0 g) and $Fe(NO_3)_3\cdot 9H_2O$ (1.3 g) were added. The mixture was stirred at −78 °C for 1 h and allowed to warm to room temperature. The residue was diluted with H_2O (70 mL), and the mixture was stirred at room temperature for 1.5 h and extracted with EtOAc (3×50 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (30 g, hexane–ether 5:1→1:1) to give **27** (512 mg, 89%) as a colorless oil: TLC, R_f 0.08 (hexane–ether 4:1); $[\alpha]_D^{28}$ −9.8 (*c* 1.00, $CHCl_3$); IR (neat) 3460 (br), 1458, 1253, 1089 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 5.67 (dd, *J*=6.5, 15.7 Hz, 1H), 5.48 (dd, *J*=5.7, 15.7 Hz, 1H), 4.90 (d, *J*=5.1 Hz, 1H), 4.29 (m, 1H), 3.98 (dt, *J*=7.0, 3.2 Hz, 1H), 3.55 (t, *J*=8.1 Hz, 1H), 3.44–3.20 (m, 3H), 3.32 (s, 3H), 3.31 (s, 3H), 2.57 (m, 1H), 2.26 (m, 1H), 2.05 (dd, *J*=7.0, 12.4 Hz, 1H), 1.93–1.73 (m, 2H), 1.68 (br s, OH), 1.57 (ddd, *J*=5.1, 11.1, 12.4 Hz, 1H), 1.31 (ddd, *J*=2.4, 9.5, 13.8 Hz, 1H), 1.25 (ddd, *J*=2.4, 9.7, 13.8 Hz, 1H), 1.04 (d, *J*=6.5 Hz, 3H), 0.97 (d, *J*=7.0 Hz, 3H), 0.89 (s, 9H), 0.87 (d, *J*=7.0 Hz, 3H), 0.86 (s, 9H), 0.77 (d, *J*=6.8 Hz, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.00 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 133.1, 130.3, 105.1, 89.1, 81.4, 74.8, 69.8, 65.5, 56.6, 55.2, 44.3, 42.5, 41.9, 38.1, 36.0, 33.6, 26.2, 26.1, 18.9, 18.4, 18.3, 15.9, 12.3, 10.9, −3.7, −4.2, −5.1, −5.2; HRMS (ESI) calcd for $C_{32}H_{66}NaO_6Si_2$ $(M+Na)^+$ 625.4296, found 625.4288.

4.1.21. Alcohol 28. A mixture of alcohol **27** (369 mg, 0.612 mmol), $NaHCO_3$ (206 mg, 2.45 mmol), and 5% Pd on carbon (121 mg) in ethanol (6 mL) was stirred under a hydrogen atmosphere at room temperature for 18 h. The mixture was filtered through a pad of Celite, and the residue was washed with EtOAc. The filtrate and the washings were combined and concentrated. The residual oil was purified by column chromatography on silica gel (20 g, hexane–ether 5:1→1:1) to give **28** (353 mg, 95%) as a colorless oil: TLC R_f 0.63 (hexane–ether 1:1); $[\alpha]_D^{26}$ +1.8 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 3481, 1462, 1255, 1095 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 4.88 (d, *J*=5.1 Hz, 1H), 3.96 (m, 1H), 3.83 (m, 1H), 3.54 (t, *J*=7.8 Hz, 1H), 3.45 (dd, *J*=7.6, 10.3 Hz, 1H), 3.33 (dd, *J*=6.5, 10,3 Hz, 1H), 3.21 (m, 1H), 3.30 (s, 3H), 3.28 (s, 3H), 2.24 (m, 1H), 2.05 (dd, *J*=7.0, 12.3 Hz, 1H), 1.90–1.35 (m, 8H), 1.33–1.27 (m, 2H), 1.03 (d, *J*=6.5 Hz, 3H), 0.93 (d, *J*=7.0 Hz, 3H), 0.86 (s, 9H), 0.86 (s, 9H), 0.83 (d, *J*=7.0 Hz, 3H), 0.79 (d, *J*=6.8 Hz, 3H), 0.04 (s, 3H), 0.04 (s, 3H), 0.00 (s, 6H); HRMS (ESI) calcd for $C_{32}H_{69}O_6Si_2$ $(M+H)^+$ 605.4633, found 605.4649.

4.1.22. 3,4-Dimethoxybenzyloxymethyl ether 29. To a stirred solution of alcohol **28** (607 mg, 1.00 mmol) in CH_2Cl_2 (8 mL) cooled at 0 °C were added diisopropylethylamine (8.8 mL, 52 mmol) and the 1 M solution of (3,4-dimethoxybenzyloxy)methyl chloride in CH_2Cl_2 (13 mL, 13 mmol) prepared from 3,4-dimethoxybenzyl (methylthio)methyl ether according to Ref. 4c. The mixture was stirred at room temperature for 3 h, and the reaction was quenched by addition of MeOH (40 mL) and $NaHCO_3$ (500 mg). The resulting mixture was stirred at room temperature for 1.5 h, and H_2O (20 mL) was added. The organic layer was separated, and the aqueous layer was extracted with hexane (5×20 mL). The organic layer and the extracts were combined, washed with saturated aqueous $NaHCO_3$ (20 mL), H_2O (20 mL), and brine (20 mL), successively, dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on alumina (50 g, hexane–EtOAc 2:1) and silica gel (FL60D 25 g, benzene–acetone 100:1→50:1→20:1) to give **29** (663 mg, 84%) as a colorless oil: TLC, R_f 0.59 (hexane–EtOAc 3:1); $[\alpha]_D^{25}$ +8.7 (*c* 1.00,

$CHCl_3$); IR (neat) 1516, 1463, 1380, 1257, 1097, 1032 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 6.86–6.73 (m, 3H), 4.81 (d, J=4.6 Hz, 1H), 4.75 (s, 2H), 4.52 (s, 2H), 4.01 (m, 1H), 3.94 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.50 (dd, J=6.5, 9.7 Hz, 1H), 3.45–3.10 (m, 3H), 3.17 (s, 3H), 3.17 (s, 3H), 2.16 (m, 1H), 2.02 (dd, J=7.6, 12.7 Hz, 1H), 1.81 (m, 1H), 1.66 (m, 1H), 1.63–1.33 (m, 6H), 1.27–1.15 (m, 2H), 1.03 (d, J=6.5 Hz, 3H), 0.83 (d, J=7.0 Hz, 3H), 0.81 (s, 9H), 0.80 (s, 9H), 0.77 (d, J=7.0 Hz, 3H), 0.74 (d, J=7.0 Hz, 3H), 0.01 (s, 3H), 0.00 (s, 3H), −0.01 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 148.9, 148.5, 130.8, 120.6, 111.4, 110.9, 104.7, 94.5, 87.3, 81.8, 78.6, 70.2, 69.5, 65.4, 57.0, 56.1, 56.0, 54.6, 43.6, 42.7, 42.5, 36.1, 35.5, 33.1, 30.8, 27.7, 26.3, 26.2, 26.1, 20.4, 18.4, 18.4, 15.6, 11.1, 9.1, −3.7, −4.2, −5.1, −5.2; HRMS (ESI) calcd for $C_{42}H_{80}NaO_9Si_2$ $(M+Na)^+$ 807.5239, found 807.5221.

4.1.23. Diol 30. To a stirred solution of 3,4-dimethoxybenzyloxymethyl ether **29** (664 mg, 0.846 mmol) in THF (42 mL) was added a 1.0 M solution of tetrabutylammonium fluoride in THF (2.0 mL, 2.0 mmol). The mixture was stirred at room temperature for 2 h, diluted with saturated aqueous NH_4Cl (9 mL), and extracted with ether (3×30 mL). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (20 g, hexane–EtOAc 1:1→EtOAc) to give **30** (466 mg, 99%) as a colorless oil: TLC, R_f 0.5 (EtOAc); $[\alpha]_D^{26}$ +6.9 (c 1.07, $CHCl_3$); IR (neat) 3428, 1516, 1265, 1095, 1029 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 6.83–6.71 (m, 3H), 4.78 (d, J=4.6 Hz, 1H), 4.75 (d, J=9.2 Hz, 1H), 4.72 (d, J=9.2 Hz, 1H), 4.49 (s, 2H), 3.95 (m, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.87–3.39 (m, 5H), 3.32 (m, 1H), 3.26 (s, 3H), 3.19 (s, 3H), 2.12 (m, 1H), 1.99 (m, 1H), 1.85–1.27 (m, 10H), 1.00 (d, J=6.5 Hz, 3H), 0.82 (d, J=6.8 Hz, 3H), 0.78 (d, J=7.3 Hz, 3H), 0.71 (d, J=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 148.9, 148.5, 130.7, 120.5, 111.3, 110.9, 104.8, 94.6, 87.2, 83.6, 78.4, 75.2, 69.5, 68.1, 57.5, 56.3, 56.0, 55.9, 54.7, 43.6, 42.5, 40.1, 36.1, 35.0, 30.3, 27.5, 20.4, 15.9, 13.9, 9.0; HRMS (ESI) calcd for $C_{30}H_{52}NaO_9$ $(M+Na)^+$ 579.3509, found 579.3496.

4.1.24. TBDPS ether 31. To a stirred solution of diol **30** (80.8 mg, 0.145 mmol) in DMF (1.5 mL) cooled at 0 °C were added imidazole (749 mg, 8.71 mmol) and *tert*-butyldiphenylsilyl chloride (1.1 mL, 4.4 mmol). The mixture was stirred at room temperature for 5 h, diluted with cold water (1 mL), and extracted with ether (3×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, benzene–ether 50:1→20:1) to give **31** (113 mg, 75%) as a colorless oil: TLC, R_f 0.7 (hexane–EtOAc 3:1); $[\alpha]_D^{26}$ +6.9 (c 1.00, $CHCl_3$); IR (neat) 1516, 1462, 1427, 1109, 1028 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.70–7.46 (m, 8H), 7.45–7.24 (m, 12H), 6.82–6.68 (m, 3H), 4.87 (d, J=4.6 Hz, 1H), 4.84 (d, J=7.0 Hz, 1H), 4.81 (d, J=7.0 Hz, 1H), 4.59 (s, 2H), 4.16 (m, 1H), 3.92 (m, 1H), 3.76 (s, 3H), 3.76 (s, 3H), 3.47 (dd, J=6.5, 10.0 Hz, 1H), 3.36 (dd, J=7.8, 10.0 Hz, 1H), 3.25 (dd, J=6.7, 10.0 Hz, 1H), 3.07 (s, 3H), 2.92 (m, 1H), 2.76 (s, 3H), 2.11 (m, 1H), 2.00 (m, 1H), 1.97 (dd, J=7.0, 12.2 Hz, 1H), 1.56–1.15 (m, 9H), 1.11 (d, J=6.5 Hz, 3H), 1.06 (s, 9H), 1.00 (s, 9H), 0.90 (d, J=7.0 Hz, 3H), 0.88 (d, J=7.0 Hz, 3H), 0.75 (d, J=7.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.1, 148.6, 136.3, 136.2, 135.8, 135.7, 134.7, 134.6, 134.0, 133.8, 130.9, 129.6, 129.6, 129.5, 127.7, 127.7, 127.5, 120.7, 111.4, 111.0, 104.7, 94.5, 87.3, 81.5, 78.6, 72.0, 69.5, 66.1, 56.8, 56.1, 55.9, 54.5, 43.5, 42.7, 42.0, 36.0, 35.6, 33.9, 30.7, 27.7, 27.4, 27.3, 26.9, 20.3, 19.7, 19.3, 15.1, 11.3, 8.9; HRMS (ESI) calcd for $C_{62}H_{88}NaO_9Si_2$ $(M+Na)^+$ 1055.5865, found 1055.5845; Anal. Calcd for $C_{62}H_{88}O_9Si_2$: C, 72.05; H, 8.58. Found: C, 72.53; H, 9.01.

4.1.25. Diol 32. To a stirred solution of TBDPS ether **31** (178 mg, 0.198 mmol) in 1,2-dimethoxyethane (5.0 mL) was added 1 M aqueous HCl (1.2 mL). The solution was stirred at room temperature for 7 h, cooled to 0 °C, and diluted with ether (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic layer was separated, and the aqueous layer was extracted with ether (3×10 mL). The organic layer and the extracts were combined, washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (6 g, hexane–EtOAc 5:1→4:1→2:1) to give a diastereomeric mixture of hemiacetals (143 mg) as a colorless oil along with recovered **31** (54 mg, 29%). Using the same procedure as described above, a diastereomeric mixture of hemiacetals (399 mg) was obtained from **31** (626 mg, 0.606 mmol). To a stirred solution of the hemiacetals (542 mg) in ethanol (10 mL) was added sodium borohydride (71.7 mg, 1.89 mmol). The mixture was stirred at room temperature for 1.5 h, diluted with saturated aqueous NH_4Cl (10 mL), and extracted with ether (5×20 mL). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (20 g, hexane–EtOAc 4:1→2:1→1:1→EtOAc) to give **32** (590 mg, 70%) as a colorless oil: TLC, R_f 0.2 (hexane–EtOAc 1:1); $[\alpha]_D^{28}$ −19.6 (c 1.00, $CHCl_3$); IR (neat) 3434, 1516, 1462, 1427, 1109, 1028 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.72–7.56 (m, 8H), 7.45–7.26 (m, 12H), 6.88–6.78 (m, 3H), 4.78 (d, J=7.0 Hz, 1H), 4.71 (d, J=7.0 Hz, 1H), 4.62 (d, J=11.6 Hz, 1H), 4.50 (d, J=11.6 Hz, 1H), 4.26 (m, 1H), 3.91 (m, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.72 (m, 1H), 3.60–3.32 (m, 5H), 2.80 (s, 3H), 2.12 (m, 1H), 1.99–1.82 (m, 2H), 1.76–1.36 (m, 9H), 1.06 (s, 9H), 1.01 (d, J=7.0 Hz, 3H), 0.98 (s, 9H), 0.90 (d, J=7.0 Hz, 3H), 0.85 (d, J=6.5 Hz, 3H), 0.74 (d, J=6.2 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 149.1, 148.8, 136.1, 136.1, 135.7, 135.6, 134.5, 134.5, 133.8, 133.8, 129.8, 129.5, 129.5, 129.4, 127.6, 127.6, 127.5, 127.5, 120.5, 111.1, 111.0, 94.4, 81.1, 81.1, 77.4, 72.1, 70.4, 66.1, 59.7, 56.9, 56.1, 56.0, 42.0, 37.8, 35.4, 33.3, 32.7, 29.9, 29.7, 28.1, 27.4, 27.0, 19.8, 19.4, 17.4, 15.4, 11.6, 11.4; HRMS (ESI) calcd for $C_{61}H_{88}NaO_9Si_2$ $(M+Na)^+$ 1043.5865, found 1043.5869.

4.1.26. Trityl ether 33. To a stirred solution of diol **32** (63.5 mg, 0.0614 mmol) in pyridine (0.7 mL) was added trityl chloride (86.6 mg, 0.307 mmol) at room temperature. The solution was stirred at 50 °C for 12 h and was cooled to room temperature. Saturated aqueous $NaHCO_3$ (3 mL) was added, and the mixture was stirred at room temperature for 2 h and extracted with ether (3×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column

chromatography on silica gel (2 g, hexane–ether–Et_3N 5:1:0.1→2:1:0→1:1:0) to give **33** (73.5 mg, 95%) as a colorless oil: TLC, R_f 0.8 (hexane–EtOAc 3:1); $[\alpha]_D^{28}$ −2.3 (*c* 1.00, $CHCl_3$); IR (neat) 3500, 1516, 1462, 1427, 1109, 1029 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.75–7.53 (m, 8H), 7.48–7.15 (m, 27H), 6.86–6.75 (m, 3H), 4.78 (d, *J*=7.0 Hz, 1H), 4.71 (d, *J*=7.0 Hz, 1H), 4.60 (d, *J*=11.6 Hz, 1H), 4.49 (d, *J*=11.6 Hz, 1H), 4.26 (m, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.82 (m, 1H), 3.55–3.30 (m, 4H), 3.21 (m, 1H), 3.03 (m, 1H), 2.85 (s, 3H), 2.11 (m, 1H), 1.97–1.70 (m, 2H), 1.66–1.31 (m, 6H), 1.05 (s, 9H), 0.97 (s, 9H), 1.13–0.88 (m, 3H), 0.89 (d, *J*=6.8 Hz, 3H), 0.86 (d, *J*=8.1 Hz, 3H), 0.84 (d, *J*=6.5 Hz, 3H), 0.75 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 149.1, 148.8, 144.6, 136.3, 136.2, 135.8, 135.7, 134.6, 134.6, 133.9, 133.8, 130.0, 129.6, 129.6, 129.5, 128.8, 127.8, 127.7, 127.7, 127.6, 127.0, 120.6, 111.2, 111.0, 94.6, 86.6, 81.8, 81.1, 77.6, 72.1, 70.3, 66.0, 62.0, 56.9, 56.0, 55.9, 41.9, 38.0, 35.4, 34.1, 31.9, 29.5, 29.4, 28.2, 27.3, 26.9, 19.7, 19.3, 17.5, 15.3, 11.7, 11.3; HRMS (ESI) calcd for $C_{80}H_{102}NaO_9Si_2$ $(M+Na)^+$ 1285.6960, found 1285.6974.

4.1.27. Acetate 34. To a stirred solution of trityl ether **33** (688 mg, 0.545 mmol) in pyridine (4.0 mL) were added acetic anhydride (2.0 mL) and 4-(dimethylamino)pyridine (30.0 mg, 0.246 mmol). The mixture was stirred at room temperature for 12 h and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, hexane–ether 4:1→2:1→1:1) to give **34** (725 mg, 100%) as a colorless oil: TLC, R_f 0.8 (hexane–EtOAc 3:1); $[\alpha]_D^{28}$ +5.1 (*c* 1.00, $CHCl_3$); IR (neat) 1732, 1516, 1462, 1427, 1240, 1109, 1031 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.75–7.55 (m, 8H), 7.48–7.18 (m, 27H), 6.87–6.77 (m, 3H), 4.99 (dd, *J*=2.7, 10.0 Hz, 1H), 4.75 (d, *J*=7.0 Hz, 1H), 4.67 (d, *J*=7.0 Hz, 1H), 4.60 (d, *J*=11.9 Hz, 1H), 4.46 (d, *J*=11.9 Hz, 1H), 4.26 (m, 1H), 3.85 (s, 6H), 3.48 (dd, *J*=7.6, 10.3 Hz, 1H), 3.43–3.34 (m, 2H), 3.20 (m, 1H), 3.13–2.92 (m, 2H), 2.81 (s, 3H), 2.36–1.75 (m, 4H), 1.97 (s, 3H), 1.70–1.20 (m, 5H), 1.06 (s, 9H), 0.97 (s, 9H), 1.13–0.80 (m, 3H), 0.93 (d, *J*=7.0 Hz, 3H), 0.89 (d, *J*=7.0 Hz, 3H), 0.75 (d, *J*=6.6 Hz, 3H), 0.70 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.8, 148.9, 148.5, 144.3, 136.2, 136.1, 135.7, 135.6, 134.6, 134.6, 133.8, 133.7, 130.8, 129.5, 128.7, 127.8, 127.6, 127.6, 127.5, 126.9, 120.4, 111.3, 110.9, 95.4, 86.6, 81.5, 78.8, 77.4, 72.2, 69.7, 66.1, 61.5, 56.6, 56.1, 56.0, 42.0, 37.0, 34.9, 33.7, 31.2, 30.8, 29.8, 27.4, 27.1, 27.0, 21.3, 19.8, 19.4, 17.2, 15.3, 11.5, 9.8; HRMS (ESI) calcd for $C_{82}H_{104}NaO_{10}Si_2$ $(M+Na)^+$ 1327.7066, found 1327.7075.

4.1.28. Alcohol 35. To a stirred solution of acetate **34** (52.4 mg, 0.0401 mmol) in ether (0.6 mL) was added formic acid (0.4 mL), and the mixture was stirred at room temperature (23 °C) for 15 min. The mixture was poured into saturated aqueous $NaHCO_3$ (4 mL) cooled at 0 °C, and the resulting mixture was extracted with ether (3×3 mL). The combined extracts were washed with brine (3 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (2 g, hexane–ether 2:1→1:1→1:2→1:5) to give **35** (33.0 mg, 77%) as a colorless oil: TLC, R_f 0.4 (hexane–ether 1:5); $[\alpha]_D^{28}$ −1.1 (*c* 1.00, $CHCl_3$); IR (neat) 3497, 1731, 1516, 1240, 1109, 1031 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.69–7.58 (m, 8H), 7.43–7.27 (m, 12H), 6.88–6.78 (m, 3H), 4.98 (dd, *J*=2.7, 9.4 Hz, 1H), 4.74 (d, *J*=7.0 Hz, 1H), 4.65 (d, *J*=7.0 Hz, 1H), 4.59 (d, *J*=11.6 Hz, 1H), 4.46 (d, *J*=11.6 Hz, 1H), 4.25 (m, 1H), 3.86 (s, 3H), 3.86 (s, 3H), 3.86 (m, 1H), 3.74 (m, 1H), 3.67–3.32 (m, 3H), 3.02 (m, 1H), 2.80 (s, 3H), 2.18–1.90 (m, 2H), 2.04 (s, 3H), 1.86 (m, 1H), 1.80–1.15 (m, 6H), 1.05 (s, 9H), 0.97 (s, 9H), 1.03–0.75 (m, 3H), 0.91 (d, *J*=7.0 Hz, 3H), 0.89 (d, *J*=6.8 Hz, 3H), 0.88 (d, *J*=7.3 Hz, 3H), 0.77 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 148.9, 148.5, 136.1, 136.1, 135.7, 135.6, 134.6, 134.4, 133.7, 133.7, 130.6, 129.5, 129.4, 127.6, 127.5, 127.4, 120.4, 111.1, 110.7, 95.1, 81.3, 78.3, 78.2, 71.8, 69.5, 65.9, 60.7, 56.4, 55.9, 55.8, 41.7, 36.4, 34.4, 32.7, 30.9, 30.3, 29.7, 27.1, 26.7, 26.5, 21.1, 19.6, 19.1, 16.8, 15.1, 11.1, 9.4; HRMS (ESI) calcd for $C_{63}H_{90}NaO_{10}Si_2$ $(M+Na)^+$ 1085.5970, found 1085.5970.

4.1.29. Aldehyde 36. To a stirred solution of alcohol **35** (19.0 mg, 0.0178 mmol) in CH_2Cl_2 (0.2 mL) were added pyridine (0.02 mL, 0.178 mmol) and the Dess–Martin periodinane (10.3 mg, 0.0243 mmol). The mixture was stirred at room temperature for 30 min and diluted with saturated aqueous $Na_2S_2O_3$ (2 mL) and saturated aqueous $NaHCO_3$ (1 mL). The resulting mixture was stirred at room temperature for 30 min and extracted with ether (3×2 mL). The combined extracts were washed with H_2O (2 mL) and brine (2 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (0.3 g, hexane–ether 1:1→1:2) to give **36** (17.3 mg, 91%) as a colorless oil: TLC, R_f 0.8 (hexane–EtOAc 1:1); $[\alpha]_D^{28}$ −2.3 (*c* 1.00, $CHCl_3$); IR (neat) 2719, 1730, 1516, 1238, 1169, 1031 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 9.73 (br s, 1H), 7.76–7.55 (m, 8H), 7.48–7.22 (m, 12H), 6.89–6.79 (m, 3H), 5.01 (dd, *J*=3.0, 9.5 Hz, 1H), 4.76 (d, *J*=7.0 Hz, 1H), 4.66 (d, *J*=7.0 Hz, 1H), 4.61 (d, *J*=11.9 Hz, 1H), 4.47 (d, *J*=11.9 Hz, 1H), 4.27 (m, 1H), 3.88 (s, 6H), 3.52–3.35 (m, 3H), 3.03 (m, 1H), 2.80 (s, 3H), 2.48 (m, 1H), 2.43 (m, 1H), 2.39–1.97 (m, 3H), 1.77 (m, 1H), 1.70–1.23 (m, 6H), 1.07 (s, 9H), 0.97 (s, 9H), 0.96 (d, *J*=6.8 Hz, 3H), 0.91 (d, *J*=6.8 Hz, 3H), 0.89 (d, *J*=7.3 Hz, 3H), 0.75 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.8, 170.7, 149.1, 148.7, 136.3, 136.2, 135.8, 135.7, 134.7, 134.6, 133.9, 133.9, 130.8, 129.6, 129.6, 129.6, 129.5, 127.7, 127.7, 127.6, 120.5, 111.3, 111.0, 95.2, 81.5, 78.4, 77.8, 72.1, 69.7, 66.1, 56.6, 56.1, 55.9, 45.0, 41.9, 37.0, 34.7, 33.4, 30.7, 29.9, 29.7, 27.3, 26.9, 21.1, 19.7, 19.3, 18.2, 15.3, 11.3, 9.8; HRMS (ESI) calcd for $C_{68}H_{92}NaO_{10}Si_2$ $(M+Na)^+$ 1083.5814, found 1083.5833.

4.1.30. Enamide 37. A solution of aldehyde **36** (112 mg, 0.106 mmol), *N*-methylformamide (1.1 mL, 19 mmol), hydroquinone (46.3 mg, 0.421 mmol), and pyridinium *p*-toluenesulfonate (79.4 mg, 0.316 mmol) in benzene (50 mL) was heated to reflux for 5 h under a stream of nitrogen with continuous removal of water using molecular sieves of 3 Å. The mixture was cooled to room temperature, diluted with triethylamine (2 mL) and saturated aqueous $NaHCO_3$ (5 mL), and extracted with ether (3×10 mL). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (20 g, hexane–EtOAc 4:1→2:1→1:1→1:2) to give **37** (64.6 mg, 55%) as a colorless

oil: TLC, R_f 0.5 (hexane–EtOAc 1:1); $[\alpha]_D^{28}$ −10.3 (*c* 1.00, $CHCl_3$); IR (neat) 1733, 1654, 1515, 1238, 1109, 1030 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.24 [7.92] (s, 1H), 7.72–7.54 (m, 8H), 7.45–7.22 (m, 12H), 6.45 [7.14] (d, *J*=14.0 Hz, 1H), 6.88–6.78 (m, 3H), 4.99 (dd, *J*=9.2, 14.0 Hz, 1H), 4.75 (d, *J*=7.0 Hz, 1H), 4.75 (m, 1H), 4.64 [4.65] (d, *J*=7.0 Hz, 1H), 4.69 (d, *J*=11.9 Hz, 1H), 4.46 [4.48] (d, *J*=11.9 Hz, 1H), 4.29 (m, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.52–3.32 (m, 3H), 3.01 (m, 1H), 2.87 (s, 3H), 2.80 [2.81] (s, 3H), 2.76 (m, 1H), 2.06 (s, 3H), 2.19–1.97 (m, 2H), 2.84–1.22 (m, 4H), 1.06 (s, 9H), 0.97 (s, 9H), 1.16–0.78 (m, 3H), 0.88 (d, *J*=6.8 Hz, 3H), 0.86 (d, *J*=8.1 Hz, 3H), 0.74 (d, *J*=6.8 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets; HRMS (ESI) calcd for $C_{65}H_{91}NNaO_{10}Si_2$ $(M+Na)^+$ 1124.6079, found 1124.6060.

4.1.31. Alcohol 38. To a stirred solution of enamide **37** (30.0 mg, 0.0272 mmol) in CH_2Cl_2 (7.6 mL), *tert*-butyl alcohol (0.4 mL), and 1 M phosphate buffer (pH 6, 0.4 mL) cooled at 0 °C was added 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (8.3 mg, 0.030 mmol). The mixture was warmed to room temperature and stirred at room temperature for 30 min. To the mixture cooled at 0 °C was added DDQ (7.0 mg, 0.025 mmol), and the mixture was stirred at room temperature for 40 min. Further, the mixture was cooled to 0 °C and DDQ (8.0 mg, 0.029 mmol) was added. After the mixture was stirred at room temperature for 40 min, DDQ (7.5 mg, 0.027 mmol) was added to the mixture cooled at 0 °C. The mixture was stirred at room temperature for 20 min and diluted with 1 M phosphate buffer (pH 6, 5 mL) was added. The mixture was stirred at room temperature for 1 h and extracted with ether (3×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (3 g, hexane–ether–acetone 15:15:1) to give **38** (22.7 mg, 90%) as a colorless oil: TLC, R_f 0.4 (benzene–ether–acetone 10:1:1); $[\alpha]_D^{21}$ +28.3 (*c* 0.97, $CHCl_3$); IR (neat) 3514, 1696, 1657, 1427, 1249, 1109 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 8.29 [8.02] (s, 1H), 7.78–7.54 (m, 8H), 7.47–7.22 (m, 12H), 6.50 [7.17] (d, *J*=14.3 Hz, 1H), 5.00 [5.01] (dd, *J*=9.5, 14.3 Hz, 1H), 4.83 (m, 1H), 4.28 (m, 1H), 3.51–3.33 (m, 3H), 3.04 (m, 1H), 2.98 [2.96] (s, 3H), 2.81 (s, 3H), 2.57 (m, 1H), 2.50 (m, 1H), 2.10 (m, 1H), 2.15 (s, 3H), 1.44–1.17 (m, 4H), 1.06 (s, 9H), 1.05 (d, *J*=6.5 Hz, 3H), 0.97 (s, 9H), 1.16–0.78 (m, 3H), 0.88 (d, *J*=6.8 Hz, 3H), 0.85 (d, *J*=6.0 Hz, 3H), 0.73 (d, *J*=6.5 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets; HRMS (ESI) calcd for $C_{55}H_{79}NNaO_7Si_2$ $(M+Na)^+$ 944.5293, found 944.5297.

4.1.32. Ester 39. To a stirred solution of alcohol **38** (3.2 mg, 0.0035 mmol) and 2,3-di-*O*-methyl-D-glyceric acid (12.8 mg, 0.0955 mmol) were added triethylamine (0.027 mL, 0.019 mmol), 2,4,6-trichlorobenzoyl chloride (0.023 mL, 0.014 mmol), and 4-(dimethylamino)pyridine (0.7 mg, 0.006 mmol). The mixture was stirred at room temperature for 1.5 h, diluted with 10% citric acid (5 mL), and extracted with ether (5×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (2 g, benzene–acetone 30:1→20:1→15:1→10:1) to give a diastereomeric mixture of **39** (2.7 mg, 74%) as a colorless oil, which was employed in the next experiment without separation of the diastereomers: TLC, R_f 0.3 (benzene–acetone 8:1); ^1H NMR (400 MHz, $CDCl_3$) δ 8.26 [7.95, 8.25, 7.93][a] (s, 1H), 7.69–7.56 (m, 8H), 7.43–7.27 (m, 12H), 6.47 [7.14][b] (d, *J*=14.0 Hz, 1H), 5.07 [5.05][c] (m, 1H), 4.94 (dd, *J*=6.2, 14.0 Hz, 1H), 4.76 [4.80][c] (m, 1H), 4.25 (m, 1H), 3.92 (m, 1H), 3.80–3.58 (m, 2H), 3.42 [3.43][b] (s, 3H), 3.38 [3.38, 3.35][d] (s, 3H), 3.51–3.36 (m, 2H), 3.00 (m, 1H), 2.91 [2.86, 2.89, 2.63][a] (s, 3H), 2.54 (m, 1H), 2.07 [2.08][c] (s, 3H), 1.67–1.18 (m, 9H), 1.04 (s, 9H), 1.00 [0.99][c] (d, *J*=6.8 Hz, 3H), 0.96 (s, 9H), 0.94 [0.93, 0.91, 0.90][a] (d, *J*=7.3 Hz, 3H), 0.86 [0.86][b] (d, *J*=7.3 Hz, 3H), 0.72 (d, *J*=6.8 Hz, 3H). The minor counterparts of doubled signals in the ratios of 9:6:3:2 (superscript a), 3:2 (superscript b), 2:1 (superscript c), and 3:4:2 (superscript d) are in brackets.

4.1.33. Analogs 4 and 40. A solution of esters **39** (2.7 mg, 0.0026 mmol) in a 5:3:8 mixture of HF·pyridine, pyridine, and THF (0.32 mL) was stirred at room temperature for 7 h. The mixture was diluted with EtOAc (5 mL) and poured into saturated aqueous $NaHCO_3$ (5 mL) cooled at 0 °C, and the resulting mixture was extracted with EtOAc (3×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (FL60D 1 g, benzene–acetone 2:1→1:1) followed by reversed-phase HPLC [Develosil Ph-UG-5 (20×250 mm), 50% aqueous MeOH, 5.0 mL/min, detection at UV 215 nm] to give **4** (t_R=91 min, 0.8 mg, 55%) and **40** (t_R=97 min, 0.5 mg, 34%) as a colorless oil, respectively. Compound **4**: TLC, R_f 0.5 (benzene–acetone 1:1); $[\alpha]_D^{28}$ +88.5 (*c* 0.067, $CHCl_3$); IR (neat) 3675, 1575, 1488, 1237, 1202, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.30 [8.08] (s, 1H), 6.49 [7.17] (d, *J*=14.0 Hz, 1H), 5.11 (m, 1H), 4.97 [4.99] (dd, *J*=9.6, 14.0 Hz, 1H), 4.80 (dd, *J*=2.8, 10.0 Hz, 1H), 3.93 (m, 1H), 3.79–3.29 (m, 5H), 3.50 (s, 3H), 3.40 (s, 3H), 3.37 (s, 3H), 3.20 (m, 1H), 3.03 [3.07] (s, 3H), 2.51 (m, 1H), 2.09 [2.08] (s, 3H), 1.90–1.72 (m, 2H), 1.76 (m, 1H), 1.69–1.36 (m, 6H), 1.02 [1.01] (d, *J*=6.8 Hz, 3H), 0.98 (d, *J*=6.8 Hz, 3H), 0.89 (d, *J*=6.8 Hz, 3H), 0.82 (d, *J*=6.8 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets; HRMS (ESI) calcd for $C_{28}H_{51}NNaO_{10}$ $(M+Na)^+$ 584.3411, found 584.3398. Compound **40**: TLC, R_f 0.5 (benzene–acetone 1:1); $[\alpha]_D^{28}$ +103 (*c* 0.042, $CHCl_3$); IR (neat) 3648, 1575, 1488, 1237, 1202, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.29 [8.08] (s, 1H), 6.49 [7.16] (d, *J*=14.0 Hz, 1H), 5.13 (m, 1H), 4.98 [4.99] (m, 1H), 4.82 (dd, *J*=2.8, 10.8 Hz, 1H), 3.94 (m, 1H), 3.77–3.09 (m, 6H), 3.49 (s, 3H), 3.38 (s, 3H), 3.37 (s, 3H), 3.03 [3.07] (s, 3H), 2.54 (m, 1H), 2.17 [2.09] (s, 3H), 1.90–1.39 (m, 9H), 1.02 [1.01] (d, *J*=7.2 Hz, 3H), 0.95 (d, *J*=6.8 Hz, 3H), 0.89 [0.89] (d, *J*=6.8 Hz, 3H), 0.83 [0.83] (d, *J*=7.2 Hz, 3H). The minor counterparts of doubled signals in the ratio of 2:1 are in brackets; HRMS (ESI) calcd for $C_{28}H_{51}NNaO_{10}$ $(M+Na)^+$ 584.3411, found 584.3398.

4.2. Actin-depolymerizing activity

The actin-depolymerizing activity of the compounds was measured as previously described.[3,6] To the 3.7 μM solution of actin (0.30 mL, containing 10% pyrenyl actin) in G-buffer

was added a 0.15 M solution of $MgCl_2$ (2.0 μL), and the mixture was stirred at room temperature for 1 h to polymerize G-actin to F-actin. To the solution of F-actin was added various concentrations of compounds in dimethyl sulfoxide (2.0 μL) with stirring, and the time course of depolymerization was continuously monitored by measuring fluorescence of pyrenyl actin (10% of total actin) with a fluorometer (HITACHI, F-4000, equipped with a magnetic stirrer) at 25 °C at 365-nm excitation and 407-nm emission wavelengths. The IC_{50} values were the concentrations required to depolymerize F-actin to 50% of its control amplitude.

4.3. Cytotoxicity

Growing cells of HeLa S_3 were suspended in Eagle's minimal essential medium containing 10% fetal bovine serum, penicillin (0.1 units/mL), streptomycin (0.1 mg/mL), and amphotericin B (0.25 μg/mL) at 3×10^4 cells/mL, and samples dissolved in DMSO were added. The mixture was incubated at 37 °C for 4 days in a CO_2 incubator with a humidified atmosphere containing 5% CO_2. The number of viable cells was assessed using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay.[22] The IC_{50} value (concentration required for 50% inhibition of cell growth) was determined using a growth curve.

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Facile stereoselective synthesis of *cis*- and *trans*-3-alkoxyazetidin-2-ones

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Abstract—A highly stereoselective synthesis of *cis*- and *trans*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones is described. The reaction of α-chlorosulfide-β-lactams with various alcohols catalyzed by a Lewis acid such as $ZnCl_2$ in the presence of molecular sieves (3–4 Å) leads to *cis*-3-alkoxy-3-phenyl/benzylthio-β-lactams whereas treatment of potassium 2-alkoxy-2-phenylthioethanoate with appropriate Schiff's base using $POCl_3$ in the presence of triethylamine leads to the formation of *trans*-3-alkoxy-3-phenylthioazetidin-2-ones as major products.

1. Introduction

Apart from being the sub-structure of widely used antibiotics[1–3] such as penicillins, cephalosporins and monobactams, β-lactams have emerged as an important class of heterocycles. There is a considerable activity directed at the stereocontrolled synthesis of this heterocycle.[4] Besides this, the unique feature of these strained molecules is that these heterocycles are also important building blocks for the stereoselective synthesis of a variety of biologically important compounds.[5] For example, suitably substituted hydroxy β-lactams have been used in the semi-synthesis of paclitaxel (Taxol) and docetaxel (Taxotere).[6] The need for potent effective β-lactam antibiotics as well as new β-lactamase inhibitors has motivated synthetic organic and medicinal chemists to design new functionalized azetidin-2-ones. Some of the synthetic azetidin-2-ones are reported to be biologically active as inhibitors of cholesterol acyl transferase,[7] thrombin,[8] human cytomegalovirus protease,[9] human leukocyte elastase[10] and cysteine protease.[11]

Further interest in the development of synthetic methodology for 3-alkoxy-β-lactams was sparked by the discoveries of 2-isocephem,[12] 2-oxa-isocephem,[12] 7-methoxycephalosporins[13] and PS-5,[14] possessing an alkoxy group at the C-3 position of azetidin-2-ones. The potential use of *cis*-3-alkoxy-β-lactams in the preparation of the Taxol C-13 side chain has also been well documented.[15] More recently, a novel 3-methoxy-β-lactam **1** (Fig. 1) has been found to have apoptotic activity against human leukaemia, breast, prostate and head–neck cancer cells, thus exhibiting antitumour activity.[16] Besides this, 3-methoxy spiro-β-lactam **2** (Fig. 1) has also been found to be an inhibitor of both poliovirus and human rhinovirus 3C-proteinases.[17]

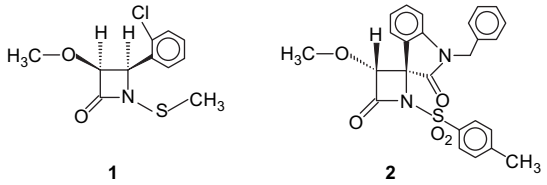

Figure 1. Biologically active 3-methoxyazetidin-2-ones.

The biological activity of the particular β-lactam ring is influenced by the type of substitution attached to the basic nucleus.[6–17] So, keeping in view the importance of relationship between biological activity and structural diversity as an essential component, we wish to report here a stereoselective synthesis of *cis*- and *trans*-3-alkoxyazetidin-2-ones. Synthesis of *cis*-3-alkoxy-3-phenyl/benzylthio-β-lactams has been achieved via transformation at C-3 of cationic β-lactam equivalents **3** using Lewis acid catalyzed reaction of various alcohols in the presence of silica gel and zinc chloride. However, both *trans*- and *cis*-3-alkoxy-3-phenylthio-β-lactams are obtained via direct annelation of potassium 2-alkoxy-2-phenylthioethanoate (**11**) with appropriate Schiff's base (**12**) using $POCl_3$ as the condensing reagent in the presence of triethylamine.

Keywords: Azetidin-2-ones; *cis*-3-Alkoxy-β-lactams; *trans*-3-Alkoxy-β-lactams; Lewis acid catalysis; Ethyl 2-alkoxy-2-phenylthioethanoate; Potassium 2-alkoxy-2-phenylthioethanoate.
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2. Results and discussion

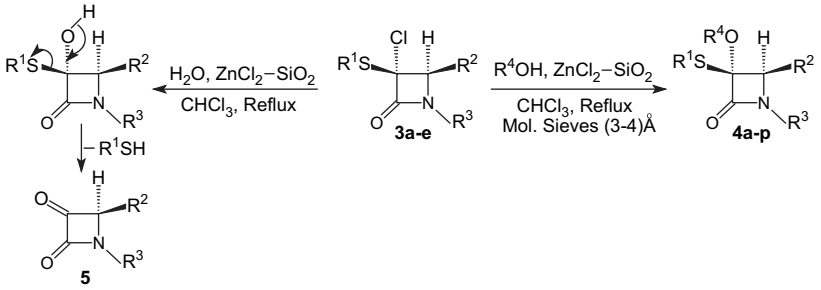
2.1. *cis*-3-Alkoxy-3-phenyl/benzylthio-β-lactams

In continuation of our efforts towards the synthesis of C-3 substituted β-lactams,[18–20] we became interested in studies towards the synthesis of C-3 alkoxy-β-lactams. Our earlier study, reporting[21] the preparation of α-methoxy-β-lactams was re-examined with a view to using it for the synthesis of other *cis*-3-alkoxy-β-lactams. We report here a modification of this procedure, which has now been successfully employed for the synthesis of various *cis*-3-alkoxy-β-lactams.

The starting substrates, *trans*-3-chloro-3-phenyl/benzylthioazetidin-2-ones (**3a–e**) were prepared from *trans*-3-phenyl/benzylthioazetidin-2-ones according to the procedure, reported in our earlier publication.[20] The reported reaction conditions,[21] when applied for the synthesis of other 3-alkoxy-β-lactams using various alcohols, invariably failed to produce the desired products. In some cases, the reaction did not even take place, whereas in others, it produced some amount of 3-keto-β-lactams (**5**)[22] only (Scheme 1). However, it was found that the addition of dry molecular sieves (3–4 Å) to the reaction mixture, containing anhydrous $ZnCl_2$, alcohol and SiO_2 in chloroform prior to the addition of substrate β-lactam (**3**) produced very satisfactory results and provided *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones (**4**) in very high yields (Scheme 1, Table 1).

Initial studies were carried out by treating **3a** with absolute ethyl alcohol as the nucleophile in the presence of dry molecular sieves (3–4 Å), SiO_2 and a sub-stoichiometric amount of anhydrous $ZnCl_2$ in refluxing chloroform. This reaction resulted in the exclusive formation of *cis*-3-ethoxy-3-phenylthioazetidin-2-one (**4b**) in quantitative yield (Scheme 1). The reaction was carried out successfully with a number of substrates (**3a–e**) using various alcohols (R^4OH) and the results are summarized in Table 1. However, this reaction failed to give the anticipated products with benzyl alcohol and chiral alcohols such as (*R*)-(+)-*sec*-phenethyl alcohol. Lewis acids such as $TiCl_4$ and $SnCl_4$ were found to give unsatisfactory results. Only anhydrous $ZnCl_2$ brought about this transformation effectively.

The structures of these *cis*-3-alkoxy-β-lactams **4** were confirmed on the basis of their spectral data (IR, ^1H NMR and



Scheme 1. Synthesis of *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones **4a–p**.

Table 1. *cis*-3-Alkoxy-3-phenyl/benzylthio-β-lactams **4a–p**

Entry	**3** (substrate)	R^4OH (nucleophile)	R^1	R^2	R^3	Product **4** (% yield)[a]
1	**3a**	CH_3OH	C_6H_5	C_6H_5	$C_6H_4OCH_3(p)$	**4a** (91)
2	**3a**		C_6H_5	C_6H_5	$C_6H_4OCH_3(p)$	**4b** (83)
3	**3a**		C_6H_5	C_6H_5	$C_6H_4OCH_3(p)$	**4c** (76)
4	**3a**		C_6H_5	C_6H_5	$C_6H_4OCH_3(p)$	**4d** (74)
5	**3a**		C_6H_5	C_6H_5	$C_6H_4OCH_3(p)$	**4e** (64)
6	**3a**		C_6H_5	C_6H_5	$C_6H_4OCH_3(p)$	**4f** (78)
7	**3b**	CH_3OH	C_6H_5	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4g** (81)
8	**3b**		C_6H_5	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4h** (80)
9	**3b**		C_6H_5	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4i** (70)
10	**3b**		C_6H_5	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4j** (66)
11	**3b**		C_6H_5	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4k** (61)
12	**3c**	CH_3OH	C_6H_5	C_6H_5	$CH_2C_6H_5$	**4l** (89)
13	**3d**	CH_3OH	$CH_2C_6H_5$	C_6H_5	$C_6H_4OCH_3(p)$	**4m** (90)
14	**3d**		$CH_2C_6H_5$	C_6H_5	$C_6H_4OCH_3(p)$	**4n** (81)
15	**3e**	CH_3OH	$CH_2C_6H_5$	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4o** (79)
16	**3e**		$CH_2C_6H_5$	$C_6H_4OCH_3(p)$	$C_6H_4OCH_3(p)$	**4p** (63)

[a] Yields quoted are for the isolated products.

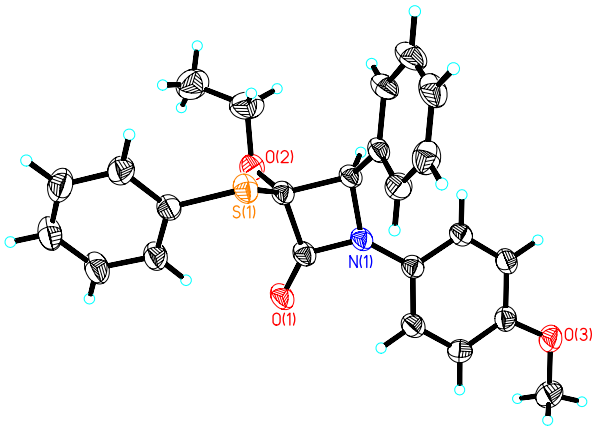


Figure 2. ORTEP diagram for compound **4b**.

^{13}C NMR). The stereochemistry at C-3 of *cis*-3-alkoxy-β-lactams was established through single crystal X-ray crystallographic studies of **4b**[23] (Fig. 2) and **4m**[24] (Fig. 3).

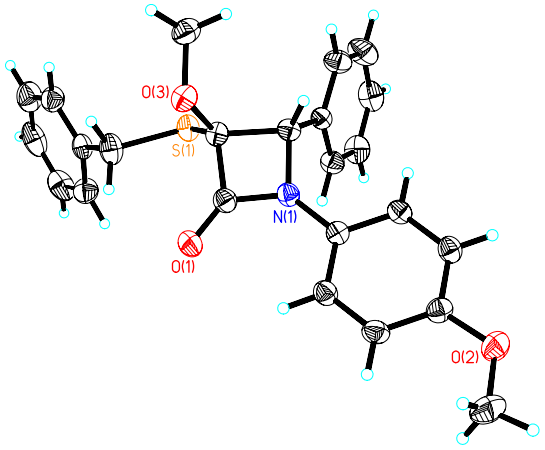
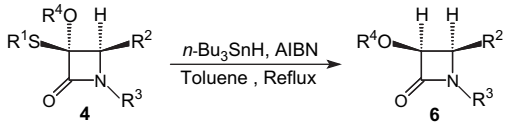


Figure 3. ORTEP diagram for compound **4m**.

In order to propose a plausible explanation for the transformation of *trans*-3-chloro-3-phenyl/benzylthioazetidin-2-ones (**3**) into *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones (**4**), a schematic reaction pathway is shown in Scheme 2.

It is likely that the reaction first involves the co-ordination by chlorine at C-3 to $ZnCl_2$ and the latter being a Lewis acid, it results in the formation of intermediate carbocation at C-3, which is further resonance stabilized by lone pair of electrons on sulfur. Subsequent approach of the nucleophile (R^4OH) to this carbocation from the side of hydrogen atom at C-4, which is less hindered, results in the formation of *cis*-β-lactam **4**.

In an effort to demonstrate the synthetic potential of this reaction and versatility of the products, the *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones were subjected to a desulfurization reaction. Initially, tri-*n*-butyltinhydride reduction of **4a**, catalyzed by AIBN in toluene at reflux temperature, led to stereoselective desulfurization to afford *cis*-3-methoxyazetidin-2-one (**6a**) (Scheme 3).



Scheme 3. *n*-Bu_3SnH desulfurization of *cis*-3-alkoxy-3-phenyl/benzylthio-β-lactams **4**.

The cis stereochemistry of the product **6a** was assigned on the basis of coupling constant (J=5.1 Hz, C3–H and C4–H) in the ^{1}H NMR spectrum.[20,25] The reaction was found to be general with several substrates and the results are summarized in Table 2. The exclusive formation of the cis product is due to the donation of hydrogen from the less hindered face of the intermediate radical.

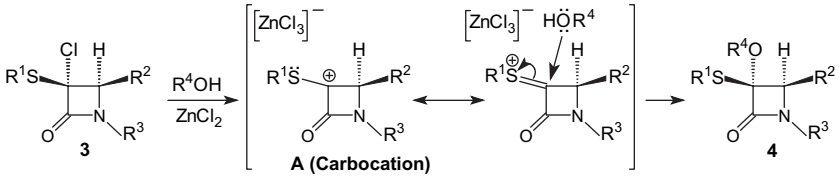
Table 2. *cis*-3-Alkoxyazetidin-2-ones **6**

Entry	**4** (substrate)	Product **6** (% yield)[a]
1	**4a**	**6a** (88)
2	**4b**	**6b** (83)
3	**4c**	**6c** (72)
4	**4g**	**6g** (71)
5	**4m**	**6a** (81)
6	**4o**	**6g** (74)

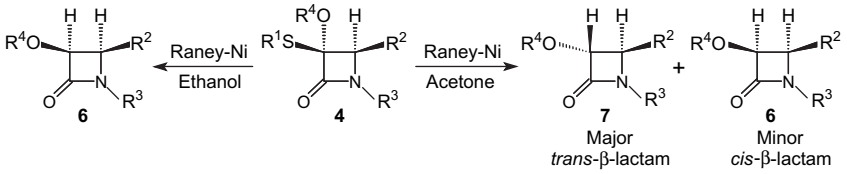
[a] Yields quoted are for the isolated products.

The stereospecific Raney-nickel desulfurization[26] of *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones (**4**) was carried out in different solvents to ascertain its effect on the product stereochemistry. Initially, treatment of **4a** with Raney-nickel in refluxing ethanol resulted in the exclusive formation of *cis*-3-methoxyazetidin-2-one (**6a**). However, when desulfurization was performed in acetone, it produced a mixture of two compounds, which were separated by column chromatography and identified as *trans*-3-methoxyazetidin-2-one (**7a**) and *cis*-3-methoxyazetidin-2-one (**6a**), respectively, in the ratio of 3:1 on the basis of their spectroscopic data (Scheme 4, Table 3).

Thereafter, the reaction was carried out successfully with a number of substrates using different solvents and the results are summarized in Table 3. Variable ratio of *trans*-3-alkoxyazetidin-2-ones (**7**) and *cis*-3-alkoxyazetidin-2-ones (**6**) was observed when desulfurization was performed



Scheme 2. Plausible reaction pathway for the formation of *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones (**4**).



Scheme 4. Raney-nickel desulfurization of *cis*-3-alkoxy-3-phenyl/benzylthio-β-lactams **4**.

Table 3. Raney-nickel desulfurization of azetidin-2-ones **4**

Entry	**4** (substrate)	Solvent	Products of type (% yield)[a]	
			7 (*trans*-β-lactam)	**6** (*cis*-β-lactam)
1	**4a**	Ethanol	—	**6a** (79)
2	**4b**	Ethanol	—	**6b** (74)
3	**4c**	Ethanol	—	**6c** (69)
4	**4d**	Ethanol	—	**6d** (70)
5	**4g**	Ethanol	—	**6g** (75)
6	**4m**	Ethanol	—	**6a** (68)
7	**4a**	Acetone	**7a** (63)	**6a** (20)
8	**4b**	Acetone	**7b** (55)	**6b** (28)
9	**4c**	Acetone	**7c** (49)	**6c** (30)
10	**4d**	Acetone	**7d** (65)	**6d** (22)
11	**4g**	Acetone	**7g** (46)	**6g** (27)
12	**4m**	Acetone	**7a** (52)	**6a** (16)
13	**4n**	Acetone	**7c** (53)	**6c** (21)
14	**4o**	Acetone	**7g** (43)	**6g** (24)

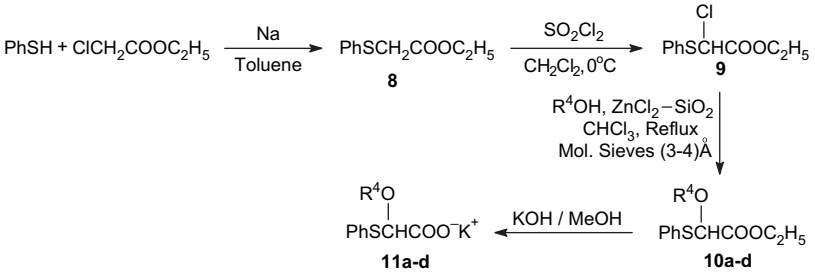
[a] Yields quoted are for the isolated products.

in acetone. The spatial juxtaposition of the C3–H and C4–H was assigned cis in product **6** and trans in product **7** on the basis of coupling constant values (J=4.8–5.1 Hz, C3–H and C4–H) and (J=1.8–2.1 Hz, C3–H and C4–H), respectively, in ^{1}H NMR spectrum.[20,25] The structures of these 3-alkoxy-β-lactams (**6** and **7**) were confirmed on the basis of their spectral data (IR, ^{1}H NMR and ^{13}C NMR).

2.2. *trans*-3-Alkoxy-3-phenylthio-β-lactams

Our interest in the stereodivergent construction of the β-lactam ring with alkoxy substituents at C-3 led us to examine the preparation of *trans*-3-alkoxy-3-phenylthio-β-lactams (**13**) also. A convenient procedure for the synthesis of the β-lactam ring skeleton is the [2+2] cyclocondensation of ketenes to imines, a process known as the Staudinger reaction.[28,29] In particular, this method has provided useful and economic entries to β-lactams, mainly due to ready availability of both Schiff's bases and ketenes. It was envisaged to study the synthesis of *trans*-3-alkoxy-β-lactams via direct annelation of alkoxy-substituted potassium phenylthioacetate (**11**) with appropriate Schiff's base (**12**) using phosphorus oxychloride ($POCl_3$) as the condensing reagent in the presence of triethylamine. The desired substrates **11**(**a**–**d**) were prepared from ethyl 2-chloro-2-phenylthioethanoate (**9**) (Scheme 5). To the best of our knowledge, no such alkoxy-substituted phenylthioacetates have been reported so far.

The reaction of ethyl chloroacetate with thiophenol in the presence of sodium in toluene at refluxing temperature gave a quantitative yield of thiophenoxy ethylacetate (**8**). This ester was further treated with 1 equiv of SO_2Cl_2 in methylene chloride at 0 °C, to yield ethyl 2-chloro-2-phenylthioethanoate (**9**). Treatment of ethyl 2-chloro-2-phenylthioethanoate



Scheme 5. Synthesis of potassium 2-alkoxy-2-phenylthioethanoate **11a**–**d**.

The variation in the stereochemistry of 3-alkoxy-β-lactams formed with Raney-nickel desulfurization in acetone or ethanol can be rationalized on the basis of the availability of surface hydrogen on the catalyst. Thus, the greater the hydrogen availability, the greater is the tendency for an inversion[27] to take place at C-3. Retention of configuration at C-3 in acetone solvent may be attributed to depletion in the supply of surface bound hydrogen due to the reducible character of the carbonyl function of acetone. In contrast, ethanol as a solvent enhances the supply of surface hydrogen due to its capability of initiating dehydrogenation in the presence of Raney-nickel under reflux conditions and thus favours the inversion of configuration at C-3.

(**9**) with various alcohols catalyzed by $ZnCl_2$–SiO_2, resulted in the formation of ethyl 2-alkoxy-2-phenylthioethanoate (**10a**–**d**) efficiently and the results are summarized in Table 4. Ethyl 2-alkoxy-2-phenylthioethanoate (**10a**–**d**) on hydrolysis

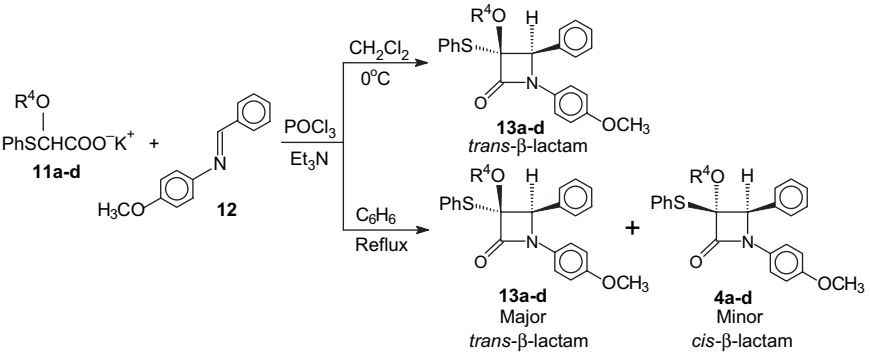
Table 4. Ethyl 2-alkoxy-2-phenylthioethanoate **10a**–**d**

Entry	R^4OH (nucleophile)	Product **10** (% yield)[a]
1	CH_3OH	**10a** (90)
2		**10b** (87)
3		**10c** (81)
4		**10d** (84)

[a] Yields quoted are for the isolated products.

using KOH in methanol afforded potassium 2-alkoxy-2-phenylthioethanoate (**11a–d**).

Initial studies were carried out by treating **11a** with appropriate Schiff's base (**12**) using methylene chloride at 0 °C. This reaction resulted in the exclusive formation of *trans*-3-methoxy-3-phenylthioazetidin-2-one (**13a**) in quantitative yield (Scheme 6). The reaction was carried out successfully with a number of substrates **11b–d** and the results are summarized in Table 5. Interestingly, all the substrates produced exclusively *trans*-3-alkoxy-3-phenylthio-β-lactams.



Scheme 6. Synthesis of *trans*- and *cis*-3-alkoxy-3-phenylthioazetidin-2-ones.

Table 5. Synthesis of *trans*-3-alkoxy-3-phenylthio-β-lactams (**13a–d**) using CH_2Cl_2 at 0 °C

Entry	**11** (substrate)	Product **13** (% yield)[a]
1	**11a**	**13a** (85)
2	**11b**	**13b** (80)
3	**11c**	**13c** (71)
4	**11d**	**13d** (74)

[a] Yields quoted are for the isolated products.

On the other hand, when this reaction was performed in refluxing benzene (Scheme 6), instead of leading to the exclusive formation of the expected *trans*-3-methoxy-3-phenylthioazetidin-2-one (**13a**), a mixture of *trans*- and *cis*-3-alkoxy-β-lactams was formed in a ratio of 3:1, respectively, and the β-lactams were separated by column chromatography. The reaction was found to be general for various substrates (**11b–d**) and the results are summarized in Table 6. The structures of these *trans*-3-alkoxy-β-lactams (**13a–d**) and *cis*-3-alkoxy-β-lactams (**4a–d**) were confirmed on the basis of their spectral data (IR, ^{1}H NMR and ^{13}C NMR).

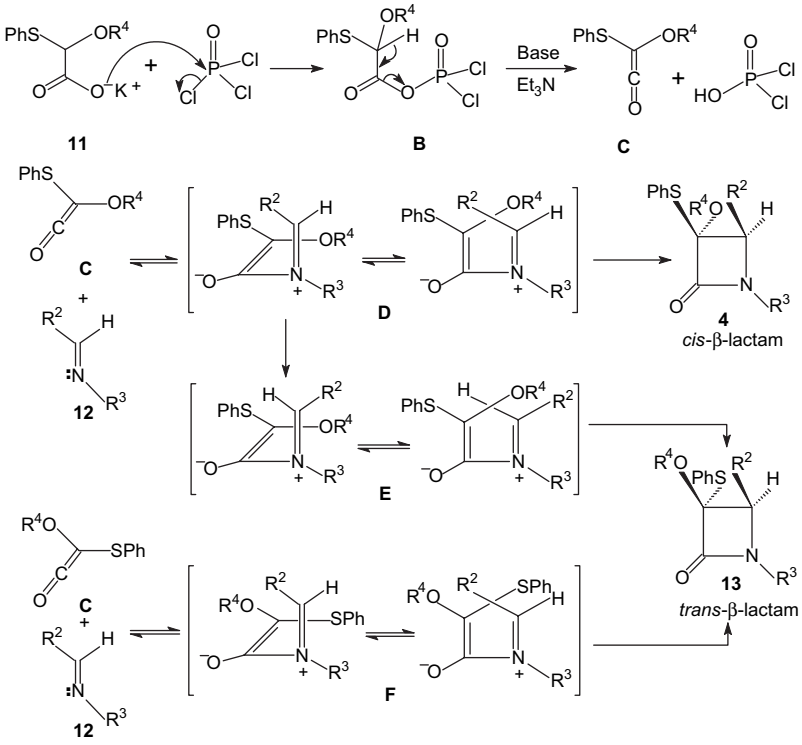
A variety of factors, such as structure and size of the substituents of the acid and imine components, sequence of addition of reactants, nature of solvent and temperature play an important role in the stereochemical outcome of the Staudinger reaction.[30,31] The formation of *trans*- and *cis*-3-alkoxy-β-lactams in this case can be rationalized on the basis of the mechanism, which is presented in Scheme 7.

Here, first an active ester **B** is formed by the reaction of potassium 2-alkoxy-2-phenylthioethanoate (**11**) and $POCl_3$, which furnishes the ketene **C** by undergoing elimination under the influence of a base. It has been postulated that LUMO of the ketene carbonyl group, which is coplanar to the substituents of the ketene, is attacked by imine in an orthogonal approach.[30] *E* imines gave preferentially *cis*-β-lactams and *Z* imines gave predominantly the corresponding *trans*-β-lactams.[31] The literature studies reveal that most of the starting acyclic imines employed in the Staudinger reaction exist in *E* configuration exclusively.[31,32] Considering this, the formation of *trans*-β-lactams can be proposed initially by the *exo* attack of the *E* imine to the ketene **C**, generating the zwitterionic intermediate **D**. Further, the isomerization of the *E* imine to less favoured *Z* imine gives the zwitterionic intermediate **E**, which, on conrotatory electrocyclization generates the thermodynamically more stable *trans*-3-alkoxy-β-lactams (**13**). The literature findings conclude that the reaction of ketene with cyclic imines also gives *trans*-β-lactams exclusively.[31] Since the cyclic imines cannot undergo the isomerization in the reaction,[30,31,33] the possibility of formation of *trans*-β-lactams through isomerization of the *E* imine moiety to less favoured *Z* imine or reaction with inversion of imine configuration is not feasible. So, in this case, it is believed that the attack of the *E* imine on the face of the ketene **C** brings the PhS group closure to imine, thus generating the zwitterionic intermediate **F**, which on direct ring closure or conrotatory electrocyclization produces exclusively the *trans*-3-alkoxy-β-lactams (**13**).[34] It has been proposed that the competition between the direct ring closure and the isomerization controls the relative stereoselectivity of β-lactam formation, which can be further explained in terms of rate constant. It has been reported[31] that the lower rate constant for direct ring closure process is the real reason for the exclusive formation of the *trans*-β-lactams. The rate constant of the direct ring closure process has been found to be quite small when the R^1 in ketene **C** is PhS.[31] This can also be rationalized through the experimental classification as proposed by Georg and Ravikumar.[30] 'Moore ketenes' possessing very weak electron-donating substituents R^1 (such as *S*-alkyl, *S*-aryl, alkyl and

Table 6. Synthesis of *trans*- and *cis*-3-alkoxy-3-phenylthio-β-lactams using C_6H_6 at reflux temperature

Entry	**11** (substrate)	Products of type (% yield)[a]	
		13 (*trans*-β-lactam)	**4** (*cis*-β-lactam)
1	**11a**	**13a** (69)	**4a** (21)
2	**11b**	**13b** (60)	**4b** (18)
3	**11c**	**13c** (54)	**4c** (15)
4	**11d**	**13d** (57)	**4d** (17)

[a] Yields quoted are for the isolated products.



Scheme 7. A plausible mechanism for the formation of *trans*- and *cis*-3-alkoxy-3-phenylthioazetidin-2-ones.

aryl) have a strong preference for *trans*-β-lactam formation due to small rate constant of the direct ring closure (k_1, $_{rel}<1$). However, the formation of almost 30% *cis*-3-alkoxy-β-lactams (**4**) at high temperature in refluxing benzene indicates the involvement of high energy zwitterionic intermediate **D** as shown, which undergoes further conrotatory electrocyclization to form *cis*-β-lactam.

The *trans*-3-alkoxy-3-phenylthioazetidin-2-ones (**13a–d**) were also subjected to stereospecific Raney-nickel desulfurization[25] in different solvents. When **13a** was treated with Raney-nickel in refluxing acetone, *cis*-3-methoxyazetidin-2-one **6a** was formed exclusively, whereas, it undergoes reductive desulfurization with inversion of configuration in ethanol, leading to the exclusive formation of *trans*-3-methoxyazetidin-2-one **7a** (Scheme 8). The reaction was found to be general with several substrates (**13b–d**) and the results are summarized in Table 7.

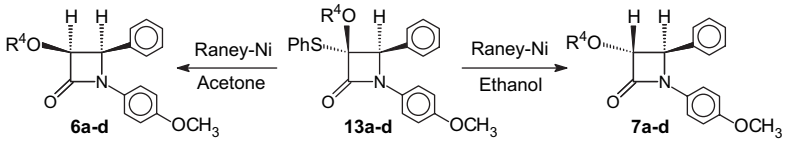
The assignment of stereochemistry to *trans*- and *cis*-3-alkoxy-β-lactams is also based on ^{1}H NMR spectroscopy. The alkoxy signal in the ^{1}H NMR spectrum of *trans*-3-alkoxy-β-lactams is shifted to higher field by about 0.35–0.50 ppm as compared to *cis*-3-alkoxy-β-lactams.[21] This upfield shift is due to the shielding effect of the *cis*-4-phenyl group on the 3-alkoxy protons. Support for this

Table 7. Reductive desulfurization of *trans*-3-alkoxy-3-phenylthio-β-lactams **13**

Entry	**13** (substrate)	Solvent	Products of type (% yield)[a]	
			6 (*cis*-β-lactam)	**7** (*trans*-β-lactam)
1	**13a**	Acetone	**6a** (85)	—
2	**13b**	Acetone	**6b** (82)	—
3	**13c**	Acetone	**6c** (78)	—
4	**13d**	Acetone	**6d** (81)	—
5	**13a**	Ethanol	—	**7a** (75)
6	**13b**	Ethanol	—	**7b** (72)
7	**13c**	Ethanol	—	**7c** (63)
8	**13d**	Ethanol	—	**7d** (68)

[a] Yields quoted are for the isolated products.

configurational assignment is provided by taking into consideration the comparison of spectroscopic data of **13a** and **4a**. The stereochemistry of **4a** was confirmed by single crystal X-ray crystallographic studies of **4b**[23] (Fig. 2) and **4m**[24] (Fig. 3) and further, the comparison of spectroscopic data of **13a** with **4a**, confirmed the stereochemistry of **13a** as well. The methoxy group in *trans*-β-lactam **13a**, in which, it is cis to phenyl group, resonates at higher field (3.41 ppm) than the methoxy group in *cis*-β-lactam **4a**, in which, it is trans to phenyl group, resonating at 3.77 ppm (Fig. 4).



Scheme 8. Raney-nickel desulfurization of *trans*-3-alkoxy-3-phenylthio-β-lactams **13a–d**.

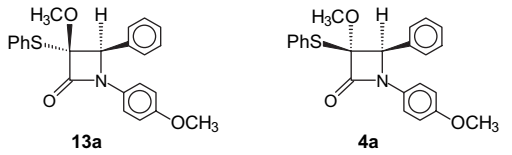


Figure 4. *trans*- and *cis*-3-Methoxy-azetidin-2-ones (**13a** and **4a**).

3. Conclusion

In conclusion, it is thus possible to achieve the stereoselective synthesis of *cis*-3-alkoxy-3-phenyl/benzylthioazetidin-2-ones by reacting *trans*-3-chloro-3-phenyl/benzylthioazetidin-2-ones (**3a–e**) with various alcohols in silica gel mediated by Lewis acid such as $ZnCl_2$ using molecular sieves (3–4 Å) in refluxing chloroform. The X-ray crystallographic analysis of compounds **4b** and **4m** allowed establishment of the stereochemistry at C-3 of *cis*-3-alkoxy-β-lactams **4**. Additionally, we have also shown that direct annelation of potassium 2-alkoxy-2-phenylthioethanoate (**11a–d**) and appropriate Schiff's base (**12**) using phosphorous oxychloride ($POCl_3$) as condensing reagent in the presence of triethylamine as the base provides an easy access to *trans*-3-alkoxy-3-phenylthioazetidin-2-ones. Further elaboration of these products to potentially useful building blocks is underway in our laboratory.

4. Experimental

4.1. General

General experimental has been described previously.[20] Crystallographic data (excluding structure factors) of compounds **4b**[23] and **4m**[24] in CIF format have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internet.) +44 1223/336 033; e-mail: deposit@ccdc.cam.ac.uk]. All other relevant information regarding the data and supplementary publication CCDC number is presented in respective references.

Compounds **3a–e**[20] were prepared by the procedures described in the cited reference. The spectroscopic data of compounds **3a–e**[20] were also reported in the cited reference.

4.2. General procedure for the synthesis of *cis*-3-alkoxy-3-phenyl/benzylthio-β-lactams (4a–p)

A mixture containing silica gel (1.00 g, 100–200 mesh), alcohol (4.68 mmol), anhydrous chloroform (10 mL), molecular sieves (3–4 Å) and anhydrous zinc chloride (0.03 mmol) was stirred for 25–30 min, followed by the addition of a solution of *trans*-3-chloro-3-phenylthio-β-lactam (**3**) (0.13 mmol) in 1 mL of $CHCl_3$. The reaction mixture was refluxed for 2 h with constant stirring. The progress of the reaction was monitored by TLC. Disappearance of the starting β-lactam was considered as the completion of the reaction. The reactants were filtered, washed with water (2×5 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and filtered. The residue after solvent evaporation in vacuo, was purified by silica gel column chromatography (10% EtoAc/hexane).

4.2.1. *cis*-1-(4′-Methoxyphenyl)-3-methoxy-3-phenylthio-4-phenylazetidin-2-one (4a). Colourless crystalline solid (0.045 g, 91%); mp 141–142 °C [Found: C, 70.49; H, 5.36; N, 3.52. $C_{23}H_{21}NO_3S$ requires C, 70.57; H, 5.40; N, 3.58%]; IR (cm^{-1}, KBr): 1755 (C=O); δ_H (300 MHz, $CDCl_3$) 7.33–6.76 (14H, m, Ph), 5.10 (1H, s, C4-*H*), 3.77 (6H, s, 2×OCH_3); δ_C (75 MHz, $CDCl_3$) 160.8, 156.4, 133.7, 132.2, 130.6, 130.5, 128.6, 128.3, 128.2, 128.1, 127.2, 118.9, 114.3, 99.9, 68.5, 55.1, 53.4.

4.2.2. *cis*-1-(4′-Methoxyphenyl)-3-ethoxy-3-phenylthio-4-phenylazetidin-2-one (4b). Colourless crystalline solid (0.042 g, 83%); mp 113–114 °C [Found: C, 71.05; H, 5.67; N, 3.34. $C_{24}H_{23}NO_3S$ requires C, 71.09; H, 5.71; N, 3.45%]; IR (cm^{-1}, KBr): 1757 (C=O); δ_H (300 MHz, $CDCl_3$) 7.65–6.75 (14H, m, Ph), 5.15 (1H, s, C4-*H*), 4.16 (1H, m, OCH_aH$_b$), 4.05 (1H, m, OCH$_a$$H_b$), 3.71 (3H, s, OC$H_3$), 1.35 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 162.0, 156.4, 132.9, 130.3, 128.6, 128.4, 128.2, 128.1, 127.5, 119.1, 114.3, 96.7, 68.4, 61.7, 55.4, 14.9.

4.2.3. *cis*-1-(4′-Methoxyphenyl)-3-isopropyloxy-3-phenylthio-4-phenylazetidin-2-one (4c). Colourless crystalline solid (0.040 g, 76%); mp 112–113 °C [Found: C, 77.54; H, 6.03; N, 3.24. $C_{25}H_{25}NO_3S$ requires C, 77.57; H, 6.00; N, 3.33%]; IR (cm^{-1}, KBr): 1750 (C=O); δ_H (300 MHz, $CDCl_3$) 7.23–6.61 (14H, m, Ph), 5.00 (1H, s, C4-*H*), 4.52 (1H, m, OC*H*), 3.62 (3H, s, OCH_3), 1.23 (3H, d, *J* 6.0 Hz, CH_3), 1.13 (3H, d, *J* 6.0 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 161.7, 156.4, 133.2, 132.1, 131.4, 130.5, 128.6, 128.4, 128.2, 127.9, 126.5, 119.0, 114.3, 98.9, 70.0, 69.8, 55.1, 23.8, 23.7.

4.2.4. *cis*-1-(4′-Methoxyphenyl)-3-propyloxy-3-phenylthio-4-phenylazetidin-2-one (4d). White crystalline solid (0.039 g, 74%); mp 114–116 °C [Found: C, 77.52; H, 5.91; N, 3.24. $C_{25}H_{25}NO_3S$ requires C, 77.57; H, 6.00; N, 3.33%]; IR (cm^{-1}, KBr): 1756 (C=O); δ_H (300 MHz, $CDCl_3$) 7.25–6.69 (14H, m, Ph), 5.05 (1H, s, C4-*H*), 4.01 (1H, m, OCH_aH$_b$), 3.89 (1H, m, OCH$_a$$H_b$), 3.71 (3H, s, OC$H_3$), 1.67 (2H, m, C$H_2CH_3$), 0.91 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 161.2, 156.4, 133.2, 132.5, 130.9, 130.6, 128.6, 128.2, 128.1, 127.8, 118.9, 114.3, 99.0, 68.5, 67.7, 55.1, 22.9, 10.9.

4.2.5. *cis*-1-(4′-Methoxyphenyl)-3-butyloxy-3-phenylthio-4-phenylazetidin-2-one (4e). Yellow solid (0.035 g, 64%); mp 72–74 °C [Found: C, 71.95; H, 6.24; N, 3.17. $C_{26}H_{27}NO_3S$ requires C, 72.03; H, 6.27; N, 3.23%]; IR (cm^{-1}, $CHCl_3$): 1757 (C=O); δ_H (300 MHz, $CDCl_3$) 7.25–6.69 (14H, m, Ph), 5.04 (1H, s, C4-*H*), 4.04 (1H, m, OCH_aH$_b$), 3.92 (1H, m, OCH$_a$$H_b$), 3.70 (3H, s, OC$H_3$), 1.61 (2H, m, OCH$_2CH_2$), 1.39 (2H, m, CH$_2CH_2CH_3$), 0.91 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 161.2, 156.4, 133.2, 132.4, 130.9, 130.6, 128.6, 128.3, 128.2, 128.1, 127.0, 118.9, 114.3, 99.0, 68.5, 65.9, 55.1, 31.7, 19.4, 14.1.

4.2.6. *cis*-1-(4′-Methoxyphenyl)-3-(prop-2-ynyloxy)-3-phenylthio-4-phenylazetidin-2-one (4f). Yellow oil (0.041 g, 78%) [Found: C, 72.24; H, 5.02; N, 3.31.

$C_{25}H_{21}NO_3S$ requires C, 72.27; H, 5.09; N, 3.37%]; IR (cm^{-1}, $CHCl_3$): 1758 (C=O); δ_H (300 MHz, $CDCl_3$) 7.25–6.69 (14H, m, Ph), 5.27 (1H, s, C4-*H*), 4.87 (1H, dd, *J* 2.4, 2.4 Hz, OCH_aH$_b$), 4.57 (1H, dd, *J* 2.4, 2.4 Hz, OCH$_a$$H_b$), 3.71 (3H, s, OC$H_3$), 2.47 (1H, t, *H*C≡); δ_C (75 MHz, $CDCl_3$) 160.3, 156.5, 133.0, 132.9, 132.9, 130.4, 129.8, 128.7, 128.4, 128.3, 128.2, 127.5, 119.1, 119.0, 114.4, 99.2, 79.4, 75.4, 68.4, 55.1, 53.9; δ_C (DEPT-135) (75 MHz, $CDCl_3$) 133.0 (+), 132.9 (+), 132.9 (+), 128.7 (+), 128.4 (+), 128.3 (+), 128.2 (+), 127.5 (+), 119.1 (+), 119.0 (+), 114.4 (+), 79.4 (+), 75.4 (+), 68.4 (+), 55.1 (+), 53.9 (−).

4.2.7. *cis*-1-(4′-Methoxyphenyl)-3-methoxy-3-phenylthio-4-(4′-methoxyphenyl)azetidin-2-one (4g). Yellowish-brown oil (0.040 g, 81%) [Found: C, 68.36; H, 5.40; N, 3.28. $C_{24}H_{23}NO_4S$ requires C, 68.39; H, 5.49; N, 3.32%]; IR (cm^{-1}, $CHCl_3$): 1745 (C=O); δ_H (300 MHz, $CDCl_3$) 7.49–6.60 (13H, m, Ph), 4.98 (1H, s, C4-*H*), 3.76 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.70 (3H, s, OCH_3); δ_C (75 MHz, $CDCl_3$) 161.2, 159.8, 156.2, 132.5, 130.9, 130.5, 129.2, 128.1, 127.0, 124.7, 118.9, 114.5, 113.7, 98.8, 68.1, 55.0, 54.7, 52.8.

4.2.8. *cis*-1-(4′-Methoxyphenyl)-3-ethoxy-3-phenylthio-4-(4′-methoxyphenyl)azetidin-2-one (4h). Colourless crystalline solid (0.040 g, 80%); mp 114–115 °C [Found: C, 68.91; H, 5.71; N, 3.19. $C_{25}H_{25}NO_4S$ requires C, 68.95; H, 5.78; N, 3.22%]; IR (cm^{-1}, KBr): 1756 (C=O); δ_H (300 MHz, $CDCl_3$) 7.31–6.65 (13H, m, Ph), 4.99 (1H, s, C4-*H*), 4.09 (1H, m, OCH_aH$_b$), 3.99 (1H, m, OCH$_a$$H_b$), 3.73 (3H, s, OC$H_3$), 3.66 (3H, s, OC$H_3$), 1.23 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 161.3, 159.9, 156.2, 132.5, 130.9, 130.5, 129.3, 128.2, 127.0, 124.8, 118.9, 114.2, 113.5, 99.3, 68.1, 61.5, 55.0, 54.9, 15.1.

4.2.9. *cis*-1-(4′-Methoxyphenyl)-3-isopropyloxy-3-phenylthio-4-(4′-methoxyphenyl)azetidin-2-one (4i). Colourless crystalline solid (0.037 g, 70%); mp 87–88 °C [Found: C, 69.50; H, 6.01; N, 3.07. $C_{26}H_{27}NO_4S$ requires C, 69.47; H, 6.05; N, 3.12%]; IR (cm^{-1}, $CHCl_3$): 1755 (C=O); δ_H (300 MHz, $CDCl_3$) 7.29–6.59 (13H, m, Ph), 4.99 (1H, s, C4-*H*), 4.57 (1H, m, OC*H*), 3.73 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 1.29 (3H, d, *J* 6.0 Hz, CH_3), 1.19 (3H, d, *J* 6.0 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 161.6, 159.9, 156.3, 132.3, 131.3, 130.6, 129.5, 128.1, 126.5, 124.9, 119.1, 114.3, 113.5, 99.1, 69.9, 69.4, 55.1, 54.9, 23.8, 23.6.

4.2.10. *cis*-1-(4′-Methoxyphenyl)-3-propyloxy-3-phenylthio-4-(4′-methoxyphenyl)azetidin-2-one (4j). White solid (0.035 g, 66%); mp 75–76 °C [Found: C, 69.39; H, 6.00; N, 3.05. $C_{26}H_{27}NO_4S$ requires C, 69.47; H, 6.05; N, 3.12%]; IR (cm^{-1}, $CHCl_3$): 1756 (C=O); δ_H (300 MHz, $CDCl_3$) 7.27–6.66 (13H, m, Ph), 4.99 (1H, s, C4-*H*), 3.95 (1H, m, OCH_aH$_b$), 3.86 (1H, m, OCH$_a$$H_b$), 3.74 (3H, s, OC$H_3$), 3.70 (3H, s, OC$H_3$), 1.65 (2H, m, C$H_2CH_3$), 0.92 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 161.4, 160.0, 156.3, 132.4, 131.1, 130.6, 129.3, 128.2, 127.0, 124.9, 119.0, 114.3, 113.6, 99.3, 68.1, 67.6, 55.1, 55.0, 22.9, 10.8.

4.2.11. *cis*-1-(4′-Methoxyphenyl)-3-butyloxy-3-phenylthio-4-(4′-methoxyphenyl)azetidin-2-one (4k). Yellowish-brown oil (0.033 g, 61%) [Found: C, 69.91; H, 6.22; N, 2.93. $C_{27}H_{29}NO_4S$ requires C, 69.96; H, 6.30; N, 3.02%]; IR (cm^{-1}, $CHCl_3$): 1756 (C=O); δ_H (300 MHz, $CDCl_3$) 7.21–6.60 (13H, m, Ph), 4.92 (1H, s, C4-*H*), 3.94 (1H, m, OCH_aH$_b$), 3.83 (1H, m, OCH$_a$$H_b$), 3.69 (3H, s, OC$H_3$), 3.63 (3H, s, OC$H_3$), 1.55 (2H, m, OCH$_2CH_2$), 1.31 (2H, m, CH$_2CH_2CH_3$), 0.84 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 161.4, 160.0, 156.3, 132.4, 131.1, 130.6, 129.3, 128.2, 127.0, 125.0, 119.0, 114.3, 113.6, 99.3, 68.2, 65.8, 55.1, 55.0, 31.7, 19.4, 14.1.

4.2.12. *cis*-1-Benzyl-3-methoxy-3-phenylthio-4-phenylazetidin-2-one (4l). Colourless crystalline solid (0.044 g, 89%); mp 101–102 °C [Found: C, 73.55; H, 5.64; N, 3.70. $C_{23}H_{21}NO_2S$ requires C, 73.57; H, 5.63; N, 3.73%]; IR (cm^{-1}, KBr): 1752 (C=O); δ_H (300 MHz, $CDCl_3$) 7.22–6.94 (15H, m, Ph), 4.87 (1H, d, *J* 15.0 Hz, CH_aH$_b$Ph), 4.40 (1H, s, C4-*H*), 3.83 (1H, d, *J* 14.7 Hz, CH$_a$$H_b$Ph), 3.54 (3H, s, OC$H_3$); δ_C (75 MHz, $CDCl_3$) 164.5, 135.0, 133.3, 132.4, 130.6, 128.9, 128.6, 128.5, 128.2, 128.1, 127.9, 127.1, 100.6, 67.7, 53.4, 44.1.

4.2.13. *cis*-1-(4′-Methoxyphenyl)-3-methoxy-3-benzylthio-4-phenylazetidin-2-one (4m). Colourless crystalline solid (0.044 g, 90%); mp 128–129 °C [Found: C, 71.05; H, 5.62; N, 3.41. $C_{24}H_{23}NO_3S$ requires C, 71.09; H, 5.71; N, 3.45%]; IR (cm^{-1}, KBr): 1762 (C=O); δ_H (300 MHz, $CDCl_3$) 7.39–6.74 (14H, m, Ph), 5.13 (1H, s, C4-*H*), 4.02 (1H, d, *J* 12.0 Hz, CH_aH$_b$S), 3.77 (1H, d, *J* 12.0 Hz, CH$_a$$H_b$S), 3.75 (3H, s, OC$H_3$), 3.56 (3H, s, OC$H_3$); δ_C (75 MHz, $CDCl_3$) 161.1, 156.4, 137.6, 133.2, 130.6, 129.2, 128.8, 128.5, 128.3, 128.0, 126.9, 118.9, 114.4, 97.8, 67.9, 55.1, 52.5, 32.2.

4.2.14. *cis*-1-(4′-Methoxyphenyl)-3-isopropyloxy-3-benzylthio-4-phenylazetidin-2-one (4n). Colourless crystalline solid (0.043 g, 81%); mp 115–116 °C [Found: C, 72.10; H, 6.24; N, 3.20. $C_{26}H_{27}NO_3S$ requires C, 72.13; H, 6.27; N, 3.22%]; IR (cm^{-1}, KBr): 1760 (C=O); δ_H (300 MHz, $CDCl_3$) 7.35–6.70 (13H, m, Ph), 5.04 (1H, s, C4-*H*), 4.46 (1H, m, OC*H*), 3.96 (1H, d, *J* 12.0 Hz, CH_aH$_b$S), 3.71 (3H, s, OCH_3), 3.51 (1H, d, *J* 12.0 Hz, CH$_a$$H_b$S), 1.36 (3H, d, *J* 6.0 Hz, CH_3), 1.31 (3H, d, *J* 6.0 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 162.1, 156.3, 137.0, 133.4, 130.6, 129.2, 128.9, 128.5, 128.4, 128.3, 127.0, 118.9, 114.3, 97.6, 69.4, 69.1, 55.1, 32.5, 24.0, 23.8.

4.2.15. *cis*-1-(4′-Methoxyphenyl)-3-methoxy-3-benzylthio-4-(4′-methoxyphenyl)azetidin-2-one (4o). Colourless crystalline solid (0.039 g, 79%); mp 101–102 °C [Found: C, 68.90; H, 5.77; N, 3.19. $C_{25}H_{25}NO_4S$ requires C, 68.95; H, 5.78; N, 3.22%]; IR (cm^{-1}, KBr): 1762 (C=O); δ_H (300 MHz, $CDCl_3$) 7.12–6.62 (13H, m, Ph), 4.92 (1H, s, C4-*H*), 3.86 (1H, d, *J* 12.3 Hz, CH_aH$_b$S), 3.68 (3H, s, OCH_3), 3.64 (3H, s, OCH_3), 3.63 (1H, d, *J* 12.6 Hz, CH$_a$$H_b$S), 3.42 (3H, s, OC$H_3$); δ_C (75 MHz, $CDCl_3$) 162.0, 160.0, 156.3, 137.6, 130.5, 129.2, 128.3, 126.9, 124.7, 118.9, 114.3, 113.8, 98.6, 67.3, 55.1, 54.9, 52.4, 32.2.

4.2.16. *cis*-1-(4′-Methoxyphenyl)-3-isopropyloxy-3-benzylthio-4-(4′-methoxyphenyl)azetidin-2-one (4p). Yellow oil (0.033 g, 63%) [Found: C, 69.92; H, 6.27; N, 2.99. $C_{27}H_{29}NO_4S$ requires C, 69.96; H, 6.30; N, 3.02%]; IR

(cm^{-1}, $CHCl_3$): 1764 (C=O); δ_H (300 MHz, $CDCl_3$) 7.25–6.70 (13H, m, Ph), 4.90 (1H, s, C4-*H*), 4.43 (1H, m, OC*H*), 4.00 (1H, d, *J* 12.0 Hz, CH_aH$_b$S), 3.77 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.57 (1H, d, *J* 12.0 Hz, CH$_a$$H_b$S), 1.35 (3H, d, *J* 6.0 Hz, CH_3), 1.25 (3H, d, *J* 6.0 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 160.1, 156.3, 137.9, 130.8, 129.6, 129.3, 129.2, 128.4, 128.3, 126.9, 125.0, 119.0, 118.9, 114.3, 113.9, 113.8, 98.0, 67.7, 60.9, 55.1, 55.0, 32.4, 24.0, 23.8.

4.3. General procedure for *n*-Bu_3SnH reduction

n-Bu_3SnH (0.14 mmol) was added dropwise via a syringe in the mixture of **4** (0.13 mmol) and catalytic amount of AIBN in toluene (4 mL). The reaction mixture was refluxed for 1 h. The progress of the reaction was checked by TLC. After the completion of reaction, the solvent was evaporated in vacuo. The residue was redissolved in methylene chloride (20 mL), washed with water (2×5 mL) and dried over anhydrous Na_2SO_4. The residue after solvent evaporation in vacuo, was purified by silica gel column chromatography (8% EtOAc/hexane).

4.3.1. *cis*-1-(4′-Methoxyphenyl)-3-methoxy-4-phenylazetidin-2-one (6a). Colourless crystalline solid (0.027 g, 88%); mp 165–166 °C [Found: C, 85.62; H, 7.14; N, 5.84. $C_{17}H_{17}NO_3$ requires C, 85.68; H, 7.18; N, 5.87%]; IR (cm^{-1}, KBr): 1747 (C=O); δ_H (300 MHz, $CDCl_3$) 7.35–6.68 (9H, m, Ph), 5.09 (1H, d, *J* 5.1 Hz, C3-*H*), 4.73 (1H, d, *J* 5.1 Hz, C4-*H*), 3.73 (3H, s, OCH_3), 3.22 (3H, s, OCH_3); δ_C (75 MHz, $CDCl_3$) 163.5, 156.3, 133.5, 130.7, 128.6, 128.5, 128.0, 118.7, 114.3, 84.8, 61.8, 58.3, 55.3.

4.3.2. *cis*-1-(4′-Methoxyphenyl)-3-ethoxy-4-phenylazetidin-2-one (6b). White solid (0.030 g, 83%); mp 127–128 °C [Found: C, 72.60; H, 6.37; N, 4.65. $C_{18}H_{19}NO_3$ requires C, 72.71; H, 6.43; N, 4.71%]; IR (cm^{-1}, KBr): 1757 (C=O); δ_H (300 MHz, $CDCl_3$) 7.29–6.63 (9H, m, Ph), 5.04 (1H, d, *J* 4.8 Hz, C3-*H*), 4.79 (1H, d, *J* 4.8 Hz, C4-*H*), 3.63 (3H, s, OCH_3), 3.39 (1H, m, OCH_aH$_b$), 3.10 (1H, m, OCH$_a$$H_b$), 0.82 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 163.5, 156.2, 133.6, 130.7, 128.4, 128.3, 128.0, 118.6, 114.2, 83.6, 66.2, 62.0, 55.2, 14.7.

4.3.3. *cis*-1-(4′-Methoxyphenyl)-3-isopropyloxy-4-phenylazetidin-2-one (6c). Colourless crystalline solid (0.026 g, 72%); mp 122–123 °C [Found: C, 77.21; H, 6.72; N, 4.43. $C_{19}H_{21}NO_3$ requires C, 73.29; H, 6.79; N, 4.49%]; IR (cm^{-1}, KBr): 1750 (C=O); δ_H (300 MHz, $CDCl_3$) 7.28–6.65 (14H, m, Ph), 5.03 (1H, d, *J* 4.5 Hz, C3-*H*), 4.90 (1H, d, *J* 4.8 Hz, C4-*H*), 3.65 (3H, s, OCH_3), 3.39 (1H, m, OC*H*), 1.03 (3H, d, *J* 6.0 Hz, CH_3), 0.60 (3H, d, *J* 6.0 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 164.2, 156.2, 134.5, 130.8, 128.4, 128.3, 118.4, 114.3, 82.1, 72.7, 62.6, 55.3, 22.1, 21.3.

4.3.4. *cis*-1-(4′-Methoxyphenyl)-3-methoxy-4-(4′-methoxyphenyl)azetidin-2-one (6g). Colourless oil (0.026 g, 71%) [Found: C, 68.81; H, 5.98; N, 4.38. $C_{18}H_{19}NO_4$ requires C, 68.99; H, 6.10; N, 4.47%]; IR (cm^{-1}, $CHCl_3$): 1751 (C=O); δ_H (300 MHz, $CDCl_3$) 7.37–6.71 (8H, m, Ph), 4.78 (1H, d, *J* 5.1 Hz, C3-*H*), 4.41 (1H, d, *J* 5.1 Hz, C4-*H*), 3.72 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.31 (3H, s, OCH_3).

4.4. General procedure for Raney-nickel desulfurization

Compounds **6** and **7**[20] were prepared by the procedure described in the cited reference.

4.4.1. *cis*-1-(4′-Methoxyphenyl)-3-propyloxy-4-phenylazetidin-2-one (6d). Colourless crystalline solid (0.026 g, 70%); mp 112–113 °C [Found: C, 73.18; H, 6.82; N, 4.43. $C_{19}H_{21}NO_3$ requires C, 73.29; H, 6.89; N, 4.49%]; IR (cm^{-1}, $CHCl_3$): 1760 (C=O); δ_H (300 MHz, $CDCl_3$) 6.85–6.21 (9H, m, Ph), 4.61 (1H, d, *J* 4.8 Hz, C3-*H*), 4.34 (1H, d, *J* 4.8 Hz, C4-*H*), 3.21 (3H, s, OCH_3), 2.89 (1H, m, OCH_aH$_b$), 2.56 (1H, m, OCH$_a$$H_b$), 0.82 (2H, m, C$H_2CH_3$), 0.52 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 163.7, 156.3, 133.7, 130.7, 128.5, 128.4, 128.1, 118.7, 114.3, 83.8, 72.5, 62.2, 55.3, 22.5, 10.2.

4.4.2. *trans*-1-(4′-Methoxyphenyl)-3-methoxy-4-phenylazetidin-2-one (7a). Yellow oil (0.019 g, 63%) [Found: C, 85.60; H, 7.11; N, 5.81. $C_{17}H_{17}NO_3$ requires C, 85.68; H, 7.18; N, 5.87%]; IR (cm^{-1}, $CHCl_3$): 1757 (C=O); ^1H NMR ($CDCl_3$) δ: 7.36–6.45 (9H, m, Ph), 4.80 (1H, d, *J* 1.8 Hz, C3-*H*), 4.31 (1H, d, *J* 1.8 Hz, C4-*H*), 3.71 (3H, s, OCH_3), 3.57 (3H, s, OCH_3); δ_C (75 MHz, $CDCl_3$) 168.3, 156.3, 136.6, 130.7, 129.2, 128.7, 127.9, 126.0, 118.8, 114.3, 91.3, 63.2, 58.0, 55.3.

4.4.3. *trans*-1-(4′-Methoxyphenyl)-3-ethoxy-4-phenylazetidin-2-one (7b). Colourless oil (0.020 g, 55%) [Found: C, 72.58; H, 6.39; N, 4.61. $C_{18}H_{19}NO_3$ requires C, 72.71; H, 6.43; N, 4.71%]; IR (cm^{-1}, $CHCl_3$): 1761 (C=O); δ_H (300 MHz, $CDCl_3$) 7.33–6.56 (9H, m, Ph), 5.10 (1H, d, *J* 4.8 Hz, C3-*H*), 4.76 (1H, d, *J* 4.8 Hz, C4-*H*), 3.68 (1H, m, OCH_aH$_b$), 3.62 (3H, s, OCH_3), 3.57 (1H, m, OCH$_a$$H_b$), 1.06 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 163.7, 156.2, 133.4, 130.5, 129.1, 128.4, 128.0, 125.3, 118.7, 114.2, 80.2, 69.3, 61.8, 55.3, 14.9.

4.4.4. *trans*-1-(4′-Methoxyphenyl)-3-isopropyloxy-4-phenylazetidin-2-one (7c). Pinkish-yellow oil (0.018 g, 49%) [Found: C, 77.16; H, 6.66; N, 4.38. $C_{19}H_{21}NO_3$ requires C, 73.29; H, 6.79; N, 4.49%]; IR (cm^{-1}, $CHCl_3$): 1759 (C=O); δ_H (300 MHz, $CDCl_3$) 6.96–6.29 (14H, m, Ph), 4.35 (1H, d, *J* 2.1 Hz, C3-*H*), 4.01 (1H, d, *J* 1.8 Hz, C4-*H*), 3.43 (1H, m, OCH), 3.29 (3H, s, OCH_3), 0.89 (3H, d, *J* 6.0 Hz, CH_3), 0.77 (3H, d, *J* 6.0 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 164.2, 156.2, 133.0, 132.7, 131.2, 130.3, 128.6, 128.4, 128.2, 126.7, 126.1, 119.1, 114.3, 89.1, 70.1, 64.9, 55.3, 24.8, 24.6.

4.4.5. *trans*-1-(4′-Methoxyphenyl)-3-propyloxy-4-phenylazetidin-2-one (7d). Colourless oil (0.024 g, 65%) [Found: C, 73.15; H, 6.78; N, 4.37. $C_{19}H_{21}NO_3$ requires C, 73.29; H, 6.89; N, 4.49%]; IR (cm^{-1}, $CHCl_3$): 1767 (C=O); δ_H (300 MHz, $CDCl_3$) 7.28–6.66 (9H, m, Ph), 4.77 (1H, d, *J* 4.8 Hz, C3-*H*), 4.41 (1H, d, *J* 4.8 Hz, C4-*H*), 3.74 (1H, m, OCH_aH$_b$), 3.62 (3H, s, OCH_3), 3.54 (1H, m, OCH$_a$$H_b$), 1.62 (2H, m, C$H_2CH_3$), 0.91 (3H, t, C$H_3$); δ_C (75 MHz, $CDCl_3$) 163.9, 156.3, 133.9, 130.8, 128.5, 128.1, 126.0, 118.8, 114.3, 90.4, 72.7, 63.7, 55.3, 23.0, 10.5.

4.4.6. *trans*-1-(4′-Methoxyphenyl)-3-methoxy-4-(4′-methoxyphenyl)azetidin-2-one (7g). Brownish-yellow oil

(0.017 g, 46%) [Found: C, 68.83; H, 5.95; N, 4.36. $C_{18}H_{19}NO_4$ requires C, 68.99; H, 6.10; N, 4.47%]; IR (cm^{-1}, $CHCl_3$): 1762 (C=O); δ_H (300 MHz, $CDCl_3$) 7.37–6.51 (8H, m, Ph), 4.43 (1H, d, *J* 2.1 Hz, C3-*H*), 4.15 (1H, d, *J* 2.1 Hz, C4-*H*), 3.70 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 3.55 (3H, s, OCH_3).

4.5. General procedure for the preparation of ethyl 2-phenylthioethanoate (8)

A mixture of thiophenol (27.50 g, 250 mmol) and molecularized sodium (5.75 g, 250 mmol) in toluene (250 mL) was refluxed for 10 h. To the resulting sodium thiophenoxide (33.00 g, 250 mmol) was added dropwise ethyl chloroacetate (33.68 g, 275 mmol) and the reaction mixture was refluxed. Progress of the reaction was monitored by TLC. The reaction mixture was washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent in vacuo, the residue was vacuum distilled to furnish the *title compound* **8** (41.45 g, 85%) as colourless oil [Found: C, 61.12; H, 2.51. $C_{10}H_{12}O_2S$ requires C, 61.20; H, 2.58%]; IR (cm^{-1}, $CHCl_3$): 1755 (C=O); δ_H (300 MHz, $CDCl_3$) 7.37–7.13 (5H, m, Ph), 4.14 (2H, q, OCH_2), 3.54 (2H, s, CH_2), 1.19 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 166.8, 133.7, 132.1, 128.8, 128.7, 128.2, 128.1, 84.6, 61.0, 14.1.

4.6. General procedure for the synthesis of ethyl 2-chloro-2-phenylthioethanoate (9)

This compound was prepared by using the same method as for **3a–c**, starting from ethyl 2-phenylthioethanoate (**8**). Colourless oil (0.044 g, 75%) [Found: C, 51.96; H, 4.77. $C_{10}H_{11}O_2SCl$ requires C, 52.04; H, 4.80%]; IR (cm^{-1}, $CHCl_3$): 1744 (C=O); δ_H (300 MHz, $CDCl_3$) 7.51–7.27 (5H, m, Ph), 5.39 (1H, s, C*H*), 4.14 (2H, q, OCH_2), 1.19 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 165.4, 134.1, 133.2, 130.8, 129.4, 129.3, 129.2, 129.1, 65.5, 62.5, 14.0.

4.7. General procedure for the synthesis of ethyl 2-alkoxy-2-phenylthioethanoate (10a–d)

Compounds **10a–d** were prepared by using the same method as for **4**, starting from ethyl 2-chloro-2-phenylthioethanoate (**9**).

4.7.1. Ethyl 2-methoxy-2-phenylthioethanoate (10a). Colourless oil (0.044 g, 90%) [Found: C, 58.35; H, 6.18. $C_{11}H_{14}O_3S$ requires C, 58.39; H, 6.23%]; IR (cm^{-1}, $CHCl_3$): 1755 (C=O); δ_H (300 MHz, $CDCl_3$) 7.39–7.16 (5H, m, Ph), 4.96 (1H, s, C*H*), 4.03 (2H, q, OCH_2), 3.45 (3H, s, OCH_3), 1.09 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 166.3, 133.5, 131.7, 128.5, 128.3, 128.1, 128.0, 86.2, 60.8, 55.2, 14.0.

4.7.2. Ethyl 2-ethoxy-2-phenylthioethanoate (10b). Colourless oil (0.045 g, 87%) [Found: C, 59.91; H, 6.63. $C_{12}H_{16}O_3S$ requires C, 59.98; H, 6.70%]; IR (cm^{-1}, $CHCl_3$): 1753 (C=O); δ_H (300 MHz, $CDCl_3$) 7.42–7.19 (5H, m, Ph), 5.05 (1H, s, C*H*), 4.08 (2H, q, OCH_2), 3.97 (1H, m, OCH_aH_b), 3.53 (1H, m, OCH_aH_b), 1.25 (3H, t, CH_3), 1.15 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 167.1, 133.8, 131.9, 129.9, 128.8, 128.3, 84.8, 61.8, 61.2, 14.7, 14.1.

4.7.3. Ethyl 2-isopropyloxy-2-phenylthioethanoate (10c). Yellow oil (0.044 g, 81%) [Found: C, 61.03; H, 7.05. $C_{13}H_{18}O_3S$ requires C, 61.14; H, 7.12%]; IR (cm^{-1}, $CHCl_3$): 1756 (C=O); δ_H (300 MHz, $CDCl_3$) 7.40–7.20 (5H, m, Ph), 5.07 (1H, s, C*H*), 4.21 (2H, q, OCH_2), 4.07 (1H, m, OC*H*), 1.14 (3H, t, CH_3), 1.07 (3H, d, *J* 6.0 Hz, CH_3), 0.97 (3H, d, *J* 6.0 Hz, CH_3).

4.7.4. Ethyl 2-propyloxy-2-phenylthioethanoate (10d). Yellow oil (0.046 g, 84%) [Found: C, 61.09; H, 7.14. $C_{13}H_{18}O_3S$ requires C, 61.14; H, 7.12%]; IR (cm^{-1}, $CHCl_3$): 1753 (C=O); δ_H (300 MHz, $CDCl_3$) 7.46–7.21 (5H, m, Ph), 5.09 (1H, s, C*H*), 4.11 (2H, q, OCH_2), 3.85 (1H, m, OCH_aH_b), 3.47 (1H, m, OCH_aH_b), 1.70 (2H, m, CH_2CH_3), 1.18 (3H, t, CH_3), 0.95 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 167.1, 133.7, 132.0, 128.8, 128.7, 128.3, 128.2, 84.7, 61.7, 61.2, 22.5, 14.1, 10.7.

4.8. General procedure for the synthesis of *trans*-3-alkoxy-3-phenylthio-β-lactams (13a–d)

Compounds **13a–d** were prepared by using the same procedure as for *trans*-3-phenyl/benzylthioazetidin-2-ones,[20] reported in cited reference, starting from potassium 2-alkoxy-2-phenylthioethanoate (**11a–d**).

4.8.1. *trans*-1-(4′-Methoxyphenyl)-3-methoxy-3-phenylthio-4-phenylazetidin-2-one (13a). White solid (0.042 g, 85%); mp 112–114 °C [Found: C, 70.52; H, 5.32; N, 3.54. $C_{23}H_{21}NO_3S$ requires C, 70.57; H, 5.40; N, 3.58%]; IR (cm^{-1}, KBr): 1755 (C=O); δ_H (300 MHz, $CDCl_3$) 7.49–6.73 (14H, m, Ph), 4.97 (1H, s, C4-*H*), 3.64 (3H, s, OCH_3), 3.41 (3H, s, OCH_3); δ_C (75 MHz, $CDCl_3$) 160.6, 156.4, 133.6, 133.5, 131.9, 130.7, 128.9, 128.6, 128.3, 128.1, 118.8, 114.3, 97.2, 69.1, 55.1, 53.7.

4.8.2. *trans*-1-(4′-Methoxyphenyl)-3-ethoxy-3-phenylthio-4-phenylazetidin-2-one (13b). White solid (0.040 g, 80%); mp 114–115 °C [Found: C, 71.06; H, 5.74; N, 3.50. $C_{24}H_{23}NO_3S$ requires C, 71.09; H, 5.71; N, 3.45%]; IR (cm^{-1}, KBr): 1754 (C=O); δ_H (300 MHz, $CDCl_3$) 7.56–6.66 (14H, m, Ph), 4.98 (1H, s, C4-*H*), 3.92 (1H, m, OCH_aH_b), 3.67 (3H, s, OCH_3), 3.62 (1H, m, OCH_aH_b), 0.93 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 161.2, 156.3, 133.4, 133.1, 131.7, 130.5, 128.9, 128.7, 128.2, 118.9, 114.1, 98.9, 68.7, 61.7, 55.0, 14.7.

4.8.3. *trans*-1-(4′-Methoxyphenyl)-3-isopropyloxy-3-phenylthio-4-phenylazetidin-2-one (13c). White solid (0.037 g, 71%); mp 115–116 °C [Found: C, 77.51; H, 5.91; N, 3.28. $C_{25}H_{25}NO_3S$ requires C, 77.57; H, 6.00; N, 3.33%]; IR (cm^{-1}, KBr): 1757 (C=O); δ_H (300 MHz, $CDCl_3$) 7.08–6.18 (14H, m, Ph), 4.52 (1H, s, C4-*H*), 4.39 (1H, m, OC*H*), 3.57 (3H, s, OCH_3), 1.17 (3H, d, *J* 6.0 Hz, CH_3), 0.87 (3H, d, *J* 6.0 Hz, CH_3).

4.8.4. *trans*-1-(4′-Methoxyphenyl)-3-propyloxy-3-phenylthio-4-phenylazetidin-2-one (13d). White solid (0.039 g, 74%); mp 118–119 °C [Found: C, 77.50; H, 6.03; N, 3.29. $C_{25}H_{25}NO_3S$ requires C, 77.57; H, 6.00; N, 3.33%]; IR (cm^{-1}, KBr): 1753 (C=O); δ_H (300 MHz, $CDCl_3$) 7.13–6.25 (14H, m, Ph), 4.58 (1H, s, C4-*H*), 3.39 (1H, m, OCH_aH_b), 3.26 (3H, s, OCH_3), 3.17 (1H, m,

OCH_aH_b), 0.91 (2H, m, CH_2CH_3), 0.23 (3H, t, CH_3); δ_C (75 MHz, $CDCl_3$) 161.6, 156.4, 133.3, 133.2, 131.6, 130.5, 128.9, 128.6, 128.2, 128.1, 128.0, 118.9, 114.3, 96.3, 69.2, 67.8, 55.3, 22.5, 10.5.

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23. Crystal data for **4b**: monoclinic; $P2_1/n$; a=12.7209(2) Å, b=10.0018(2) Å, c=17.7900(3) Å; α=90°, β=110.1480(10)°, γ=90 °C; V=2124.95(6) Å^3; Z=4; ρ_{calcd}=1.267 mg/m^3; μ(Mo Kα)=0.177 mm^{-1}; full matrix least-square on F^2; R_1=0.0456, wR_2=0.1239 for 2805 reflections [$I>2\sigma$ (I)]; T=293(2) K; GOF=1.042. Crystallographic data (excluding structure factors) for the structure **4b** in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 292949.
24. Crystal data for **4m**: monoclinic; $P2_1/c$; a=10.2813(4) Å, b=18.5745(8) Å, c=11.9227(5) Å; α=90°, β=110.335(2)°, γ=90°; V=2134.98(15) Å^3; Z=4; ρ_{calcd}=1.262 mg/m^3; μ(Mo Kα)=0.176 mm^{-1}; full matrix least-square on F^2; R_1=0.0480, wR_2=0.1066 for 2447 reflections [$I>2\sigma$ (I)]; T=293(2) K; GOF=1.011. Crystallographic data (excluding structure factors) for the structure **4m** in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 292948.
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Enantiodivergent synthesis of both antipodes of hydroxy-*exo*-brevicomin from L-(+)-tartaric acid

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Abstract—An enantiodivergent approach to both antipodes of hydroxy-*exo*-brevicomin was achieved from a common chiral precursor L-(+)-tartaric acid. The strategy utilizes the elaboration of a keto-Weinreb amide and successive stereoselective reductions.

1. Introduction

Alkylated 6,8-dioxabicyclo[3.2.1]octane structural units are widespread in bioactive natural products. These compounds can have simple bicyclic structures such as the pine beetle pheromones or can be very complex systems that originate from marine sources. Brevicomin **1** and frontalin **2** were the first pheromones to be identified belonging to the 6,8-dioxabicyclo[3.2.1]octane.[1] Similar structures having varied bicyclooctane structures such as multistriatin **3** and *iso-exo*-brevicomin **4** were isolated in addition to a number of other compounds from different *Dendroctonus* species. Marine tunicates belonging to the genus *Didemnum* produce serinolipids such as didemniserinolipid B **6**, which possess the same bicyclic core.[2] Francke et al. reported the isolation and synthesis of hydroxy-*exo*-brevicomin **5**, 1-(5-methyl-6,8-dioxabicyclo[3.2.1]oct-7-yl)ethanol from the headspace extracts of *Dendroctonus ponderosae* (Fig. 1).[3] These pheromones play a crucial role in the communication system of these beetle species and their enantioselective synthesis is highly desirable in the study of structure–activity relationships and in pest management.[4] Recently we have accomplished a concise enantiospecific synthesis of hydroxy-*exo*-brevicomin from natural L-(+)-tartaric acid.[5] Herein, we report the full details delineating the synthesis of hydroxy-*exo*-brevicomin[6] including an efficient enantiodivergent approach for the access of both antipodes of the title compound starting from the same chiral entity, i.e., L-(+)-tartaric acid.

It was anticipated that the precursor triols **17** and *ent*-**17** for the synthesis of both antipodes of hydroxy-*exo*-brevicomin **5** can be accessed by elaboration of keto-Weinreb amides **8** and **9**, respectively. Synthesis of amides **8** and **9** can be accomplished by a controlled addition of the corresponding Grignard reagent to the bis-Weinreb amide **7** derived from tartaric acid (Scheme 1).

Thus, addition of 4-pentenylmagnesium bromide or MeMgBr to the bis-Weinreb amide[7] **7** afforded the keto amides **8** or **9** in 92 and 60% yields, respectively. The keto group in **8** and **9** was reduced with L-Selectride, yielding a single diastereomer of the alcohols,[8] which were subsequently

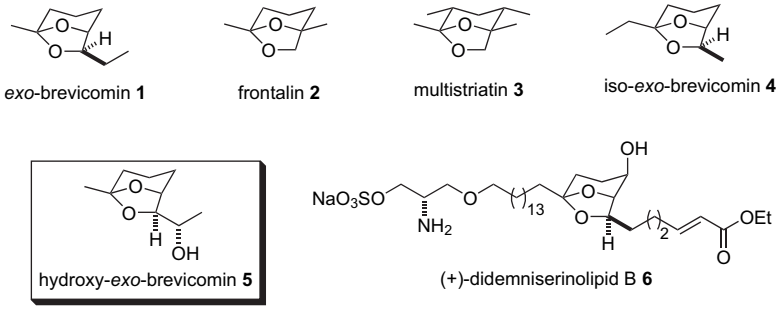

Figure 1. Bio-active bicyclic acetals possessing 6,8-dioxabicyclo[3.2.1]octane skeleton.

Keywords: Stereoselective reduction; L-(+)-Tartaric acid; Hydroxy-*exo*-brevicomin; 6,8-Dioxabicyclo[3.2.1]octane.
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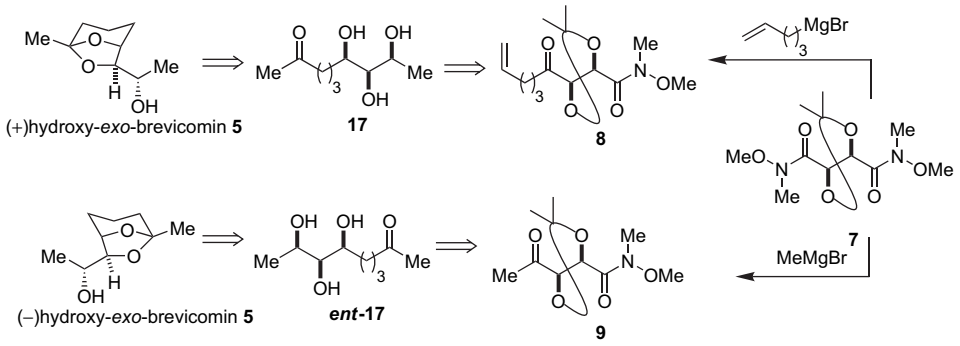
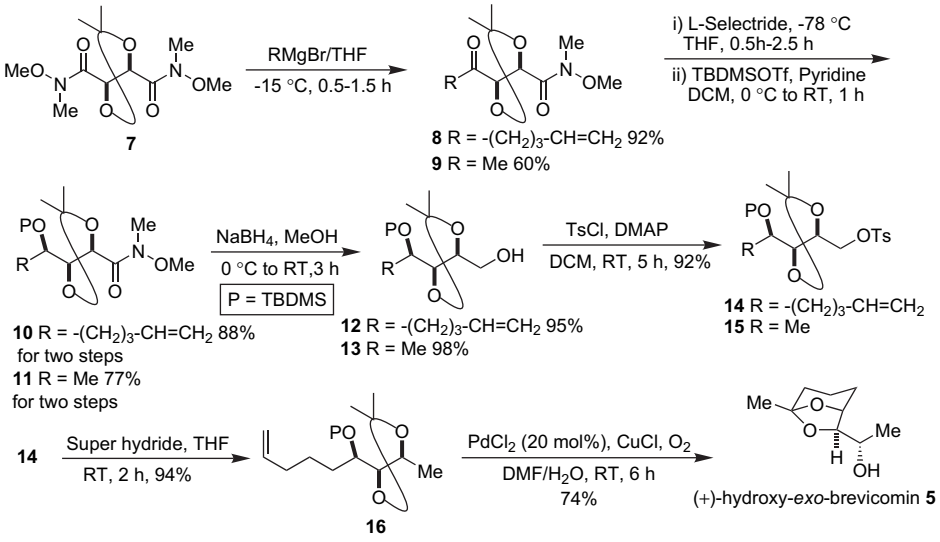


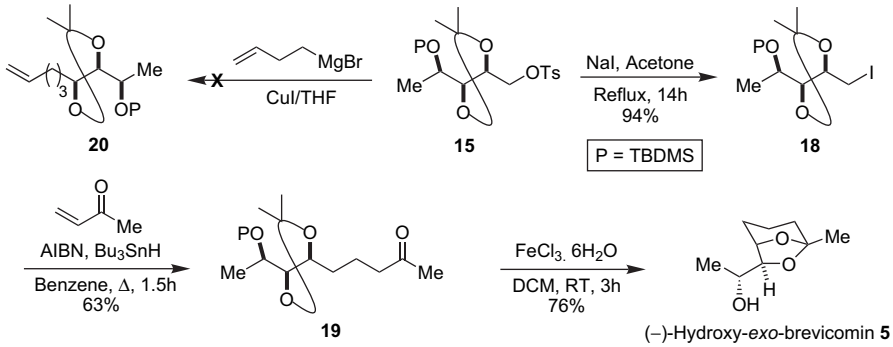
Scheme 1. Retrosynthesis for (+)- and (−)-hydroxy-*exo*-brevicomin from L-(+)-tartaric acid.



Scheme 2. Synthesis of (+)-hydroxy-*exo*-brevicomin.

protected as their silyl ethers **10** and **11**. High reactivity of the Weinreb amide in **10** and **11** was then exploited and the reduction of **10** and **11** with $NaBH_4$ resulted in alcohols **12** and **13**, respectively. Alcohols **12** and **13** were converted using standard conditions into the corresponding tosylates **14** and **15**, pivotal precursors for the access of triols **17** and *ent*-**17** (Scheme 2). Reduction of the tosylate **14** with superhydride[9] produced **16**, which under Wacker oxidation[10] conditions, produced (+)-hydroxy-*exo*-brevicomin ($[\alpha]_D^{25}$ +61.2 (*c* 2.4, $CHCl_3$), lit.[6a] $[\alpha]_D^{25}$ +61.3 (*c* 1.18, $CHCl_3$)), via the formation of the trihydroxy ketone **17**. The synthetic sample exhibited spectral data identical to that of an authentic sample.

The synthesis of *ent*-**17**, the precursor for (−)-hydroxy-*exo*-brevicomin, was envisaged by the coupling of the tosylate **15** with 3-butenylmagnesium bromide in the presence of CuI followed by transformation of alkene into *ent*-**17** under Wacker oxidation conditions. Efforts for the displacement of the tosylate **15** with the Grignard reagent under various conditions failed to yield the alkene **20**. To circumvent this problem, tosylate **15** was converted into the iodide **18**, which on Bu_3SnH/AIBN mediated radical addition[11] to methyl-vinyl ketone produced the ketone **19** in 63% yield (71% based on starting material consumption). Simultaneous deprotection of the silyl group and the acetonide[12] was achieved in a single pot with $FeCl_3$ yielding *ent*-**17**, which under the conditions spontaneously underwent ketalization to produce (−)-hydroxy-*exo*-brevicomin $[\alpha]_D^{25}$ −62.5 (*c* 0.8, $CHCl_3$). Spectral data are identical to that of the (+)-isomer prepared (vide supra).



In summary, an enantiodivergent approach to both antipodes of hydroxy-*exo*-brevicomin was accomplished starting from a single chiral entity, i.e., L-(+)-tartaric acid. (+)- and (−)-hydroxy-*exo*-brevicomin were prepared in 48 and 20% overall yields starting from the bis-Weinreb amide derived from L-(+)-tartaric acid. The synthetic transformations are simple, highly selective, and are applicable for the synthesis of a number of oxygen containing heterocycles.

2. Experimental

2.1. General

Column chromatography was performed on silica gel, Acme grade 100–200 mesh. TLC plates were visualized either with UV, in an iodine chamber, or with phosphomolybdic acid spray, unless noted otherwise. Unless stated otherwise, all reagents were purchased from commercial sources and used without additional purification. THF was freshly distilled over Na-benzophenone ketyl. Melting points were uncorrected. Unless stated otherwise, all the reactions were performed under inert atmosphere. Optical rotations were measured on a JASCO DIP-370 digital polarimeter at 25 °C.

2.1.1. Preparation of (4*R*,5*R*)-5-(hex-5-enoyl)-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide (8). In an oven dried two neck 50 mL round-bottom flask equipped with magnetic stirrer and argon inlet was placed the bis-Weinreb amide (**7**) (0.5 g, 1.8 mmol) dissolved in 6 mL of THF. This was cooled to −15 °C and a THF solution of 4-pentenylmagnesium bromide (3 mL, 1 M solution in THF, 3 mmol) was added dropwise under argon atmosphere. The reaction mixture was stirred for 0.5 h at the same temperature. After the reaction was complete (TLC), it was quenched with satd NH_4Cl (8 mL) and extracted with ether (3×10 mL). The combined ethereal extracts were washed with brine and dried (Na_2SO_4). The residue obtained after the evaporation of solvent was purified by column chromatography to yield **8** in 92% (0.47 g) as colorless oil. $[\alpha]_D$ +6.6 (*c* 1.8, $CHCl_3$); IR (neat): 2989, 1718, 1654, 1504, 1455, 1382, 1259, 1155, 1083, 997, 863, 804 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.77 (ddt, *J*=17.1, 10.2, 6.6 Hz, 1H), 5.06–4.97 (m, 3H), 4.82 (d, *J*=5.4 Hz, 1H), 3.71 (s, 3H), 3.23 (s, 3H), 2.77–2.55 (m, 2H), 2.11–2.04 (m, 2H), 1.76–1.66 (m, 2H), 1.49 (s, 3H), 1.44 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.1, 169.7, 137.7, 115.3, 112.7, 82.2, 73.9, 61.6, 38.4, 32.9, 32.5, 26.6, 26.2, 22.1; HRMS for $C_{14}H_{23}NO_5$+Na calcd 308.1474; found 308.1480.

2.1.2. Preparation of (4*R*,5*R*)-5-acetyl-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide (9). In an oven dried two neck 50 mL round-bottom flask equipped with magnetic stirrer and argon inlet was placed the bis-Weinreb amide (7) (0.4 g, 1.5 mmol) dissolved in 6 mL of THF. This was cooled to −15 °C and a THF solution of methylmagnesium bromide (0.6 mL, 3 M solution in THF, 1.8 mmol) was added dropwise under argon atmosphere. The reaction was stirred for 0.5 h at the same temperature. After the reaction was complete (TLC), it was quenched with satd NH_4Cl (7 mL) and extracted with ether (3×10 mL). The combined ethereal extracts were washed with brine and dried (Na_2SO_4). The residue obtained after the evaporation of solvent was purified by column chromatography to yield **9** in 60% (0.2 g) as colorless oil. $[\alpha]_D$ +5.5 (*c* 5.6, $CHCl_3$); IR (neat): 2987, 2854, 1720, 1671, 1459, 1375, 1255, 1182, 1085, 997, 923, 852, 721 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.04 (d, *J*=5.7 Hz, 1H), 4.83 (d, *J*=5.7 Hz, 1H), 3.73 (s, 3H), 3.24 (s, 3H), 2.31 (s, 3H), 1.50 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.0, 169.3, 112.4, 82.3, 73.6, 61.3, 32.1, 26.3, 25.9; HRMS for $C_{10}H_{17}NO_5$+Na calcd 254.1004; found 254.1005.

2.1.3. Preparation of (4*R*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyhex-5-enyl)-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide (10). To a solution of **8** (0.45 g, 1.5 mmol) in 4 mL of THF at −78 °C was added L-Selectride (3 mL, 1 M solution in THF, 3 mmol) dropwise over a period of 10 min, under argon atmosphere. The reaction mixture was stirred for 2.5 h at the same temperature, quenched with water (6 mL), and extracted with ether (3×10 mL). The combined ethereal extracts were washed with brine and dried over Na_2SO_4. Residue obtained after evaporation of solvent was subjected to column chromatography to afford (4*R*,5*S*)-5-((*R*)-1-hydroxyhex-5-enyl)-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide as colorless oil in 89% (0.41 g) yield. $[\alpha]_D$ −5.5 (*c* 1.8, $CHCl_3$); IR (neat): 3465, 2984, 1670, 1451, 1381, 1259, 1161, 1065, 990, 879 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddt, *J*=17.0, 10.2, 6.6 Hz, 1H), 5.03–4.93 (m, 2H), 4.75 (br s, 1H), 4.36 (br s, 1H), 3.74 (s, 3H), 3.62–3.60 (m, 1H), 3.23 (s, 3H), 2.08–2.04 (m, 2H), 1.68–1.42 (m, 4H), 1.48 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 138.5, 114.7, 111.1, 80.7, 73.8, 70.2, 61.6, 38.4, 34.0, 33.5, 27.0, 26.1, 25.1.

To a solution of alcohol prepared above (0.4 g, 1.4 mmol) in 3 mL of CH_2Cl_2 and 1 mL of pyridine at 0 °C was added TBDMSOTf (0.4 mL, 1.6 mmol) under argon atmosphere. The reaction mixture was stirred for 1 h and allowed to warm up to room temperature. Progress of the reaction was monitored by TLC and after the reaction was complete, it was poured into water (6 mL) and extracted with ether (3×10 mL). The combined ethereal extracts were washed with brine and dried (Na_2SO_4). Evaporation of solvent followed by column chromatography of the resulting residue afforded **10** as colorless oil in 98% (0.55 g) yield. $[\alpha]_D$ −6.2 (*c* 1.3, $CHCl_3$); IR (neat): 2935, 2858, 1674, 1471, 1380, 1371, 1255, 1162, 1072, 993, 912, 836, 811, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.72 (ddt, *J*=16.8, 10.2, 6.6 Hz, 1H), 5.10–4.75 (m, 2H), 4.63 (br s, 1H), 4.55–4.35 (m, 1H), 3.85–3.65 (m, 1H), 3.68 (s, 3H), 3.15 (s, 3H), 2.10–1.93 (m, 2H), 1.60–1.38 (m, 4H), 1.37 (s, 3H), 1.34 (s, 3H), 0.80 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 138.6, 114.5, 110.9, 80.2, 72.3, 72.0, 61.8, 33.7, 32.2, 26.9, 26.2, 26.1, 25.8, 24.9, 18.1, −4.5; HRMS for $C_{20}H_{39}NO_5Si$+Na calcd 424.2495; found 424.2462.

2.1.4. Preparation of (4*R*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyethyl)-*N*-methoxy-*N*,2,2-trimethyl-1,3-dioxolane-4-carboxamide (11). To a solution of **9** (0.15 g, 0.65 mmol) in 4 mL of THF at −78 °C was added L-Selectride (1.2 mL, 1 M solution in THF, 1.2 mmol) dropwise

over 10 min, under argon atmosphere. The reaction mixture was stirred for 0.5 h, quenched with water (3 mL) and extracted with ether (3×6 mL). The combined ethereal extracts were washed with brine and dried over Na_2SO_4. Residue obtained after evaporation of solvent was filtered through silica and evaporated to yield crude alcohol. Using a similar procedure described for the synthesis of **10**, the alcohol was converted to the silylether **11**. Alternately, the same reaction can be effected using TBDMSCl as described below.

To a solution of crude alcohol (obtained above) in 3 mL of DMF at room temperature were added imidazole (0.09 g, 1.38 mmol), DMAP (10 mg, 0.07 mmol), and TBDMSCl (0.21 g, 1.38 mmol) and was heated up to 80 °C. The reaction mixture was stirred for 2 h at same temperature. After the reaction was complete (TLC), it was cooled to room temperature, poured into water (5 mL) and extracted with ether (3×10 mL). The combined ethereal extracts were washed with brine and dried over Na_2SO_4. Evaporation of solvent followed by column chromatography of the resulting residue afforded **11** as colorless oil in 77% (0.17 g) yield. $[\alpha]_D$ −16 (*c* 2.3, $CHCl_3$); IR (neat): 2954, 2896, 1673, 1463, 1380, 1255, 1159, 1074, 1008, 979, 894, 836, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.74 (br s, 1H), 4.50 (dd, *J*=6.6, 4.5 Hz, 1H), 4.03 (qd, *J*=6.3, 4.5 Hz, 1H), 3.75 (s, 3H), 3.22 (s, 3H), 1.45 (s, 6H), 1.19 (d, *J*=6.3 Hz, 3H), 0.87 (s, 9H), 0.07 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 111.1, 81.2, 72.2, 67.7, 61.7, 32.2, 27.0, 26.3, 25.7, 18.7, 18.0, −4.5, −4.9; HRMS for $C_{16}H_{33}NO_5Si$+Na calcd 370.2026; found 370.2021.

2.1.5. Preparation of (4*S*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyhex-5-enyl)-4-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolane (12). To a solution of **10** (0.33 g, 0.82 mmol) in 3 mL of methanol at 0 °C was added $NaBH_4$ (0.078 g, 2.1 mmol) in portion wise. The reaction mixture was stirred for 1 h at the same temperature and slowly warmed up to room temperature and stirred at room temperature for 2 h. After the reaction was complete (indicated by TLC), it was poured into water (5 mL) and extracted with ether (3×10 mL). Combined ethereal extracts were washed with brine and dried over Na_2SO_4. Residue obtained after evaporation of solvent was purified by column chromatography to yield **12** in 95% (0.27 g) as colorless oil. $[\alpha]_D$ +12.1 (*c* 2.4, $CHCl_3$); IR (neat): 3469, 2985, 2857, 1471, 1461, 1378, 1253, 1164, 1101, 1004, 937, 836, 775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.70 (ddt, *J*=16.8, 10.2, 6.6 Hz, 1H), 4.94–4.84 (m, 2H), 3.93 (dt, *J*=8.1, 4.8 Hz, 1H), 3.77–3.55 (m, 4H), 2.37 (br s, 1H), 1.98–1.95 (m, 2H), 1.60–1.24 (m, 4H), 1.31 (s, 3H), 1.30 (s, 3H), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 114.7, 108.6, 80.5, 77.0, 71.9, 62.9, 33.7, 31.9, 27.0, 26.9, 25.8, 25.3, 18.1, −4.2, −4.7; HRMS for $C_{18}H_{36}O_4Si$+Na calcd 367.2281; found 367.2281.

2.1.6. Preparation of (4*S*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyethyl)-4-(hydroxylmethyl)-2,2-dimethyl-1,3-dioxolane (13). To a solution of **11** (0.15 g, 0.43 mmol) in 3 mL of methanol at 0 °C was added $NaBH_4$ (0.04 g, 1 mmol) in portions. The reaction mixture was stirred for 1 h at the same temperature and was slowly warmed up to room temperature and stirred for 1 h. After the reaction was complete (TLC), it was poured into water (5 mL) and extracted with ether (3×5 mL). Combined ethereal extracts were washed with brine and dried over Na_2SO_4. Residue obtained after evaporation of solvent was purified by column chromatography to yield **13** in 98% (0.12 g) as colorless oil. $[\alpha]_D$ +6.4 (*c* 2.8, $CHCl_3$); IR (neat): 3473, 2933, 2857, 1471, 1378, 1255, 1157, 1106, 835, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.02–3.91 (m, 2H), 3.74–3.57 (m, 3H), 2.69 (t, *J*=6.6 Hz, 1H), 1.31 (s, 6H), 1.12 (d, *J*=6.6 Hz, 3H), 0.80 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.7, 81.4, 76.6, 67.7, 63.1, 27.1, 26.9, 25.7, 18.1, 18.0, −4.7, −4.9.

2.1.7. Preparation of (4*S*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyhex-5-enyl)-4-(*p*-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane (14). To a solution of **12** (0.25 g, 0.73 mmol) and DMAP (0.22 g, 1.8 mmol) in 5 mL of CH_2Cl_2 at 0 °C was added *p*-toluenesulfonyl chloride (0.21 g, 1.1 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature for 5 h, poured into water (8 mL) and extracted with ether (3×10 mL). The ethereal extracts were washed with brine and dried over Na_2SO_4. Residue obtained after evaporation of solvent was subjected to column chromatography to yield **14** in 92% (0.33 g) as colorless oil. $[\alpha]_D$ −7.2 (*c* 1.1, $CHCl_3$); IR (neat): 2929, 2857, 1598, 1461, 1369, 1253, 1189, 1095, 983, 835, 775, 665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, *J*=8.4 Hz, 2H), 7.28 (d, *J*=8.4 Hz, 2H), 5.73 (ddt, *J*=16.8, 10.2, 6.6 Hz, 1H), 4.98–4.87 (m, 2H), 4.15 (dd, *J*=10.5, 3.0 Hz, 1H), 4.09–4.02 (m, 1H), 3.96 (dd, *J*=10.5, 5.7 Hz, 1H), 3.76–3.66 (m, 2H), 2.39 (s, 3H), 1.99–1.96 (m, 2H), 1.56–1.23 (m, 4H), 1.30 (s, 3H), 1.25 (s, 3H), 0.80 (s, 9H), −0.01 (s, 3H), −0.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.8, 138.4, 132.8. 129.8. 128.0, 114.7, 109.7, 79.2, 74.4, 71.5, 70.0, 33.6, 32.0, 26.9, 26.8, 25.8, 25.0, 21.6, 18.0, −4.2, −4.6. HRMS for $C_{25}H_{42}O_6SSi$+Na calcd 521.2371; found 521.2369.

2.1.8. Preparation of (4*S*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyethyl)-4-(*p*-toluenesulfonyloxymethyl)-2,2-dimethyl-1,3-dioxolane (15). To a solution of **13** (0.12 g, 0.41 mmol) and DMAP (0.12 g, 1 mmol) in 5 mL of CH_2Cl_2 at 0 °C was added *p*-toluenesulfonyl chloride (0.11 g, 0.6 mmol) under argon atmosphere. The reaction mixture was stirred at room temperature for 5 h, poured into water (5 mL), and extracted with ether (3×5 mL). The ethereal extracts were washed with brine and dried over Na_2SO_4. Residue obtained after evaporation of solvent was subjected to column chromatography to yield **15** in 92% (0.17 g) as colorless oil. $[\alpha]_D$ −19.1 (*c* 1.1, $CHCl_3$); IR (neat): 2931, 2857, 1598, 1461, 1369, 1255, 1178, 1097, 981, 835, 777, 665 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, *J*=8.1 Hz, 2H), 7.33 (d, *J*=8.1 Hz, 2H), 4.21 (dd, *J*=10.2, 2.7 Hz, 1H), 4.16–3.90 (m, 3H), 3.74 (dd, *J*=7.5, 4.5 Hz, 1H), 2.43 (s, 3H), 1.35 (s, 3H), 1.31 (s, 3H), 1.12 (d, *J*=6.3 Hz, 3H), 0.83 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.8, 132.8, 129.7, 127.9, 109.8, 79.8, 74.1, 70.2, 67.3, 26.9, 26.8, 25.7, 21.6, 18.2, 17.9, −4.7, −4.9; HRMS for $C_{21}H_{36}O_6SSi$+Na calcd 467.1900; found 467.1877.

2.1.9. Preparation of (4*S*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyhex-5-enyl)-4-methyl-2,2-dimethyl-1,3-dioxolane (16). To a solution of **14** (0.25 g, 0.5 mmol) in 3 mL

of THF was added superhydride (2.5 mL, 1 M solution in THF, 2.5 mmol) dropwise at room temperature, under argon atmosphere. The reaction mixture was stirred at the same temperature for 2 h. After the reaction was complete (TLC), it was cautiously quenched with water (3 mL), and extracted with ether (3×5 mL). Combined ethereal extracts were washed with brine and dried (Na_2SO_4). Evaporation of solvent under reduced pressure followed by column chromatography of resulting residue afforded **16** in 94% (0.31 g) as colorless oil. $[\alpha]_D^{25}$ +16.0 (*c* 1.5, $CHCl_3$); IR (neat): 2933, 2859, 1589, 1492, 1376, 1253, 1174, 1099, 1004, 958, 835, 808, 775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.80 (ddt, *J*=17.1, 10.5, 6.6 Hz, 1H), 5.05–4.92 (m, 2H), 3.99 (dq, *J*=8.4, 6.3 Hz, 1H), 3.80–3.69 (m, 1H), 3.53 (dd, *J*=8.4, 4.2 Hz, 1H), 2.06 (m, 2H), 1.59–1.38 (m, 4H), 1.39 (s, 3H), 1.37 (s, 3H), 1.29 (d, *J*=6.3 Hz, 3H), 0.89 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.6, 114.5, 107.7, 84.6, 72.7, 71.9, 33.7, 32.4, 27.3, 26.9, 25.9, 25.1, 18.8, 18.2, −4.1, −4.6.

2.1.10. Preparation of (+)-hydroxy-*exo*-brevicomin (5). A mixture of $PdCl_2$ (9 mg, 0.05 mmol) and CuCl (0.35 g, 3.6 mmol) in 10 mL of DMF and 2.5 mL of water were stirred under O_2 atmosphere at room temperature for 1.5 h. A solution of olefin **16** (0.09 g, 0.27 mmol) in minimum amount of DMF was added to the above mixture at room temperature. The reaction mixture was stirred for 5 h, under oxygen atmosphere at the same temperature. After the reaction was complete (TLC), it was poured into 3 N HCl (5 mL), and extracted with ether (3×5 mL). The combined ethereal extracts were washed with brine and dried over Na_2SO_4. Evaporation of solvent followed by column chromatography of the resulting residue yielded (+)-hydroxy-*exo*-brevicomin **5** as colorless oil (35 mg, 74%). $[\alpha]_D$ +61.2 (*c* 2.4, $CHCl_3$), lit.[6a] $[\alpha]_D$ +61.3 (*c* 1.18, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.23 (br s, 1H), 3.77 (d, *J*=7.2 Hz, 1H), 3.63 (dq, *J*=7.2, 6.3 Hz, 1H), 2.58 (br s, 1H), 1.95–1.75 (m, 2H), 1.72–1.61 (m, 3H), 1.52–1.46 (m, 1H), 1.45 (s, 3H), 1.14 (d, *J*=6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.4, 83.8, 76.5, 69.2, 34.6, 27.6, 24.8, 18.4, 17.1.

2.1.11. Preparation of (4*R*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyethyl)-4-(iodomethyl)-2,2-dimethyl-1,3-dioxolane (18). To a solution of **15** (0.15 g, 0.34 mmol) in 4 mL of acetone was added sodium iodide (0.15 g, 1 mmol) at room temperature, under argon atmosphere and the reaction mixture was refluxed for 14 h. After reaction was complete (TLC), it was cooled, poured into water (6 mL), and extracted with ether (3×6 mL). Combined ethereal extracts were washed with satd sodium thiosulphate, brine, and dried (Na_2SO_4). Evaporation of solvent followed by column chromatography of the resulting residue afforded **18** as colorless oil in 94% (0.128 g) yield. $[\alpha]_D$ −15 (*c* 1, $CHCl_3$); IR (neat): 2927, 2856, 1602, 1463, 1375, 1253, 1074, 835, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.02 (qd, *J*=6.3, 4.5 Hz, 1H), 3.90–3.82 (m, 1H), 3.71 (dd, *J*=7.5, 4.2 Hz, 1H), 3.41 (dd, *J*=10.8, 4.2 Hz, 1H), 3.28 (dd, *J*=10.8, 5.7 Hz, 1H), 1.45 (s, 3H), 1.40 (s, 3H), 1.19 (d, *J*=6.3 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 109.3, 83.7, 75.3, 67.5, 27.6, 27.3, 25.8, 18.7, 18.0, 7.9, −4.6, −4.7; HRMS for $C_{14}H_{29}IO_3Si$+Na calcd 423.0828; found 423.0807.

2.1.12. Preparation of (4*S*,5*R*)-5-((*R*)-1-*tert*-butyldimethylsilyloxyethyl)-4-(4-oxopentyl)-2,2-dimethyl-1,3-dioxolane (19). To a refluxing solution of **18** (0.12 g, 0.3 mmol) and methylvinyl ketone (0.25 mL, 3 mmol) in 6 mL benzene was added a benzene (3 mL) solution of Bu_3SnH (0.16 mL, 0.6 mmol) and AIBN (10 mg, 0.06 mmol) dropwise over a period of 10 min. The reaction mixture was refluxed for further 1.5 h. It was cooled, poured into 1% aq NH_3 (6 mL) and extracted with ether (3×10 mL). Combined ethereal extracts were washed with 1% aq NH_3, water, brine, and dried (Na_2SO_4). Residue obtained after evaporation of solvent was purified by column chromatography to yield **19** in 63% (0.065 g, 71% based on starting material recovery) as colorless oil. $[\alpha]_D$ −17.3 (*c* 1.5, $CHCl_3$); IR (neat): 2985, 2857, 1718, 1473, 1369, 1253, 1159, 1105, 1070, 950, 877, 809, 777 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.94–3.84 (m, 2H), 3.53 (dd, *J*=8.1, 4.2 Hz, 1H), 2.47 (t, *J*=6.9 Hz, 2H), 2.13 (s, 3H), 1.80–1.43 (m, 4H), 1.37 (s, 3H), 1.36 (s, 3H), 1.57 (d, *J*=6.6 Hz, 3H), 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.7, 108.2, 84.0, 76.4, 67.7, 43.5, 33.1, 29.8, 27.4, 26.9, 25.8, 20.6, 19.1, 18.1, −4.6, −4.8; HRMS for $C_{18}H_{36}O_4Si$+Na calcd 367.2281; found 367.2280.

2.1.13. Preparation of (−)-hydroxy-*exo*-brevicomin (5). To a solution of **19** (40 mg, 0.11 mmol) in 2 mL of CH_2Cl_2 was added $FeCl_3 \cdot 6H_2O$ (0.11 g, 0.4 mmol) at room temperature, under argon atmosphere. The reaction mixture was stirred for 3 h, filtered through a short pad of Celite and Celite pad was washed with ether (10 mL). The ethereal layer was washed with satd Na_2CO_3, brine, and dried over Na_2SO_4. The residue obtained after evaporation of solvent was purified by column chromatography to yield (−)-**5** in 76% (0.015 g) as colorless oil. $[\alpha]_D$ −62.5 (*c* 0.8, $CHCl_3$), lit. $[\alpha]_D$ +61.3 (*c* 1.18, $CHCl_3$) for the (+)-enantiomer (vide supra); ^1H NMR (300 MHz, $CDCl_3$) δ 4.23 (br s, 1H), 3.77 (d, *J*=7.2 Hz, 1H), 3.63 (dq, *J*=7.2, 6.3 Hz, 1H), 2.57 (br s, 1H), 1.90–1.76 (m, 2H), 1.70–1.57 (m, 3H), 1.52–1.44 (m, 1H), 1.45 (s, 3H), 1.14 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 108.5, 83.8, 76.5, 69.2, 34.6, 27.6, 24.8, 18.4, 17.1.

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Cobalt-mediated solid phase synthesis of 3-*O*-alkynylbenzyl galactosides and their evaluation as galectin inhibitors

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Abstract—Methyl β-D-galactoside was converted to the corresponding 3,4-*O*-stannylene acetal, which was selectively benzylated with 3-iodobenzyl bromide and coupled to a polymer-bound propargylic ether via a Sonogashira reaction. The polymer-bound carbohydrate substrate was cleaved from the resin with different carbon nucleophiles in a cobalt-mediated Nicholas reaction. The product 3-*O*-alkynylbenzyl galactosides were screened towards galectin-1, -3, -7, -8N and -9N in a competitive fluorescence polarisation assay. Particularly potent inhibitors were identified against galectin-7 with affinity enhancements up to one order of magnitude due to the 3-*O*-alkynylbenzyl moiety.

1. Introduction

Cells can communicate with the outer world by exchanging information via their surfaces. Cell surface glycoconjugates, e.g., glycoproteins and glycolipids, code for information such as cell identity and are involved in cell signalling pathways, which are the reasons why carbohydrate-recognising proteins, lectins, are potential targets for pharmaceutical research. The galectins are a sub-class of lectins defined by having an affinity for β-galactosides, a carbohydrate recognition domain (CRD) of approximately 130 amino acids and with a conserved amino acid sequence motif of about seven residues.[1] There are today 14 known galectins that can be found in mammals,[2] and they have a wide variety of biological functions, such as inducing apoptosis of T-cells, antiapoptotic and pro-inflammatory functions as well as modulation of cell adhesion and migration. The galectins can be found in almost all types of tissues and organs.[3] The biological importance of galectins discovered over the past few years makes the discovery of novel and potent galectin inhibitors more interesting than ever.[4] Among natural saccharide ligands, galectins bind lactose, Lac*N*Ac (Fig. 1) and related disaccharides. Most galectins also bind to longer saccharides more efficiently. These longer saccharides are typically characterised by having an additional sugar residue added to C-3 of Gal in lactose or Lac*N*Ac. X-ray studies of galectins[5] show highly conserved CRDs as well as extended binding sites that could accommodate the additional sugar residues at the galactose C-3. We recently showed that an affinity-enhancing effect can be achieved by derivatising Lac*N*Ac with 3- or 4-substitued benzamides or a 4-methoxybenzyl ether.[6] Hence, synthesis of galactosides carrying 3- or 4-substituted benzyl ethers at O-3 of galactose emerged as a route towards galectin inhibitors (Fig. 2). Within this context, the use of alkyne-substituted benzyl ethers appeared attractive, as alkyne derivatives can be exploited in various diversifying reactions, among others the Nicholas reaction.

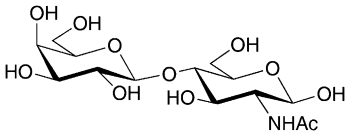

Figure 1. Lac*N*Ac (*N*-acetyllactosamine).

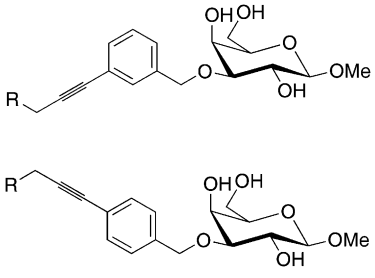

Figure 2. Targeted potential galectin inhibitors.

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An important tool for investigating biologically active compounds is parallel synthesis on solid phase.[7] By using solid phase techniques, many tedious purification steps can be avoided thus, accelerating access to new compounds. One disadvantage, however, is that extra steps are needed for initial attachment of the substrate to the resin as well cleavage from the solid support at the end of the sequence. If additional diversity could be introduced during cleavage, this reaction step could be seen as an advantage rather than an impediment, especially if new carbon–carbon bonds could be formed. Our group has recently developed a method to this effect using a solid phase variant of the Nicholas reaction[8] and we herein describe its use in the preparation of potential galectin inhibitors, using different carbon and oxygen nucleophiles to introduce diversity.

2. Results and discussion

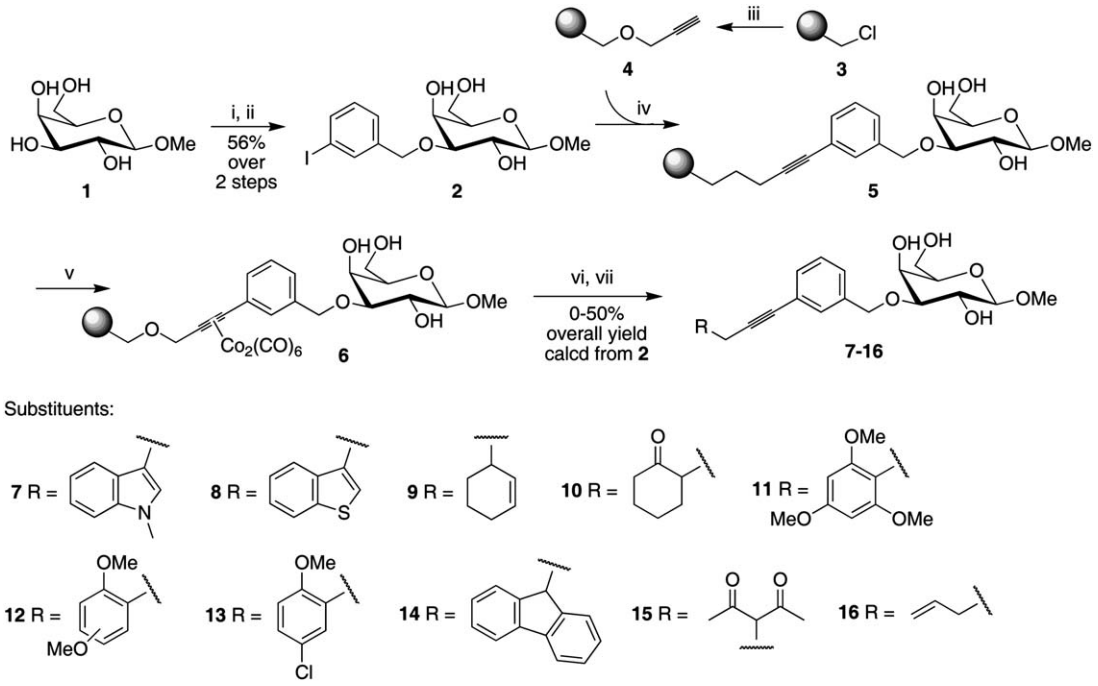
The enticing prospect of performing the synthesis without protecting the carbohydrate hydroxyl groups could be achieved by regioselective benzylation at O-3 of methyl β-D-galactoside **1**.[9] Functionalisation with *meta*- or *para*-iodobenzyl ether allows the use of the Sonogashira reaction to introduce an alkyne functionality on the aromatic ring. This approach would also allow us to connect the carbohydrate onto a solid phase under mild conditions, by using a polymer-bound propargylic ether in the Sonogashira reaction.[10] Furthermore, attaching the galactoside to a solid phase should minimise the risk of galactose hydroxyl groups acting as nucleophiles in the Nicholas reaction.

Methyl β-D-galactoside **1** was converted to the 3,4-*O*-stannylene acetal, which was selectively benzylated with 3-iodobenzyl bromide as the electrophile to afford **2** in 56% yield (Scheme 1). Propargylic alcohol was attached to Merrifield resin in a Williamson reaction,[11] affording **4**, verified by IR analysis. The iodobenzyl derivative **2** was then attached to resin **4** under Sonogashira conditions. IR analysis revealed the disappearance of the terminal alkyne C–H vibration, while a new broad peak in the region of 3500 cm^{-1} showed that the carbohydrate had been successfully attached, affording **5**. Resin **5** could be stored under dry conditions for several months without any sign of deterioration.

Complex formation was effected by treating the substrate with an excess of cobalt octacarbonyl, forming the desired alkyne–cobalt complex **6**, indicated by the deep red colouring of the resin.

In almost all examples of the Nicholas reaction in solution as well as on solid phase, the solvent of choice has been dichloromethane.[12] However, our initial experiments in dichloromethane always resulted in unidentified by-product formation. Hence, we made a comparison of different solvents and found that the Nicholas reaction failed in tetrahydrofuran and acetonitrile, but gave a clean reaction in toluene. Toluene also allowed us to run the reaction at room temperature.

Polymer-bound scaffold **6** was treated with 10 different nucleophiles (Table 1, entries 1–10) under the modified Nicholas conditions. The solutions typically turned red within 2 min indicating initiation of the reaction. After 17 h, the reactions were quenched via the addition of triethylamine, and the cobalt–alkyne complex was then cleaved with iodine.[13] After workup, the crude products **7–16** were



Scheme 1. Preparation of *meta*-substituted 3-*O*-alkynylbenzyl galactosides **7–16**. Reaction conditions: (i) Bu_2SnO, MeOH, reflux, 2 h; (ii) 3-iodobenzyl bromide, *n*-Bu_4NBr, 1,4-dioxane, reflux, 2 h; (iii) NaH, propargyl alcohol, 15-crown-5, THF, 16 h; (iv) $Pd(PPh_3)_4$, CuI, **4**, THF/Et_3N (1:1), rt, 16 h; (v) $Co_2(CO)_8$, dichloromethane, rt, 4 h; (vi) $BF_3 \cdot OEt_2$, nucleophile (see Table 1, entries 1–10), toluene, rt, 16 h or two cycles of 10 min/30 min; (vii) I_2, THF, 0 °C, 1.5 h (yields, see Table 1).

Table 1. Nicholas reaction with polymer-bound substrate **6**

Entry	Nucleophile	Product	Overall yield (%)[b]
1	*N*-Methylindole	**7**	25
2	Benzo[*b*]thiophene	**8**	<5
3[a]	3-Trimethylsilyl-1-cyclohexene	**9**	18
4	1-Trimethylsilyloxy-cyclohexene	**10**	14
5[a]	1,3,5-Trimethoxybenzene	**11**	48
6[a]	1,3-Dimethoxybenzene	**12**	40
7	4-Chloroanisole	**13**	n.r.
8	Fluorene	**14**	n.r.
9[a]	4-(Trimethylsilyoxy)-3-penten-2-one	**15**	<5
10	Allyltrimethylsilane	**16**	50

[a] Two short consecutive reactions.
[b] Isolated yield after chromatography for the four-step sequence calculated from **2**.

purified either by flash chromatography or by HPLC. 1,3-Dimethoxybenzene and 1,3,5-trimethoxybenzene initially gave products, but these decomposed rapidly under the conditions used (entries 5, 6). Products **11** and **12** were formed within 1 min according to TLC analysis and decomposition products could be detected after about 15 min. Fortunately, a shorter reaction time, i.e., 15 min, in combination with a second reaction cycle of 30 min resulted in good yields for 1,3,5-tri- (**11**) and 1,3-di-methoxybenzene (**12**). Also included in the second run was 4-(trimethylsilyloxy)-3-penten-2-one (**15**) but only traces of nucleophile could be isolated. The reaction with 1,3-dimethoxybenzene not surprisingly yielded **12** as a mixture of two regioisomers, inseparable by HPLC (entry 6). Having completed the synthesis of the *meta*-substituted 3-*O*-benzylated galactosides **7**–**16**, our attention turned towards synthesis of the *para*-substituted analogues. Starting again from **1**, the key alkyne-complex **18** was synthesised following the same protocol as for **6** (Scheme 2). With the earlier problems for the methoxy-substituted benzenes in mind, we opted to run the whole set with the methodology used for **11** and **12**.

Unfortunately, this methodology failed to afford any product for 1,3-di- and 1,3,5-trimethoxybenzene (only unidentified decomposition products were observed). For the other nucleophiles products were formed, but the yields were substantially lower. The poor yield may be caused by the *para*-alkenyl in combination with the unprotected hydroxyls, rendering the benzyl ether less stable.

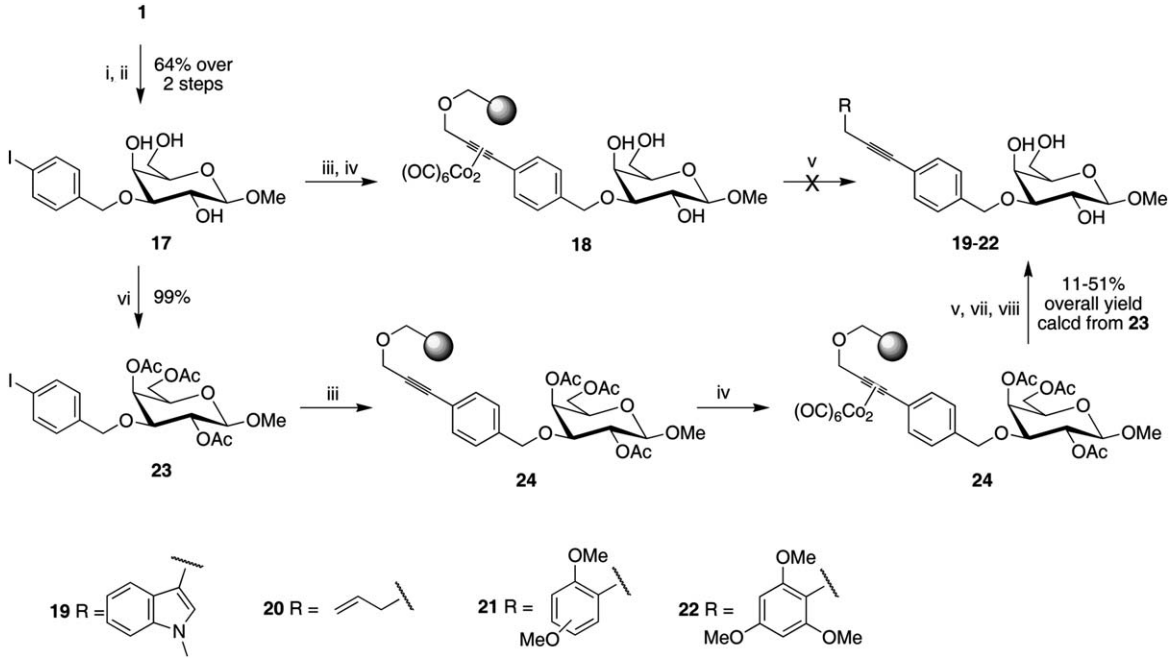
To avoid possible difficulties caused by the unprotected hydroxyls we decided to acetylate **17**, affording **23** in 99% yield, followed by attachment to the solid phase **3** as before, yielding **24** (Scheme 2). After complexation of **24** with dicobalt hexacarbonyl, the nucleophiles that had given the highest yields with substrate **6** earlier were applied in the Nicholas reaction. Oxidative removal of the cobalt complex and deacetylation under Zemplen conditions[14] (catalytic amount of sodium methoxide in methanol) afforded products **19**–**22** without any by-product formation (Table 2, entries 1–4).

Our initial fears that the hydroxyl groups would compete with the *C*-nucleophiles during the Nicholas reaction turned

Table 2. Nicholas reaction with polymer-bound substrate **24**

Entry	Nucleophile	Product	Overall yield (%)[a]
1	*N*-Methylindole	**19**	24
2	Allyltrimethylsilane	**20**	45
3[a]	1,3-Dimethoxybenzene	**21**	51
4	1,3,5-Trimethoxybenzene	**22**	11

[a] Isolated yield after chromatography for the five-step sequence calculated from **23**.



Scheme 2. Preparation of *para*-substituted 3-*O*-alkynylbenzyl galactosides **19**–**22**. Reaction conditions: (i) Bu_2SnO, MeOH, reflux, 2 h; (ii) 4-iodobenzyl bromide, *n*-Bu_4NBr, 1,4-dioxane, reflux, 2 h; (iii) $Pd(PPh_3)_4$, CuI, **4**, THF/Et_3N (1:1), rt, 16 h; (iv) $Co_2(CO)_8$, dichloromethane, rt, 3 h; (v) $BF_3\cdot OEt_2$, nucleophile (see Table 2, entries 1–4), toluene, rt, 2 h; (vi) Ac_2O, pyridine, rt, 16 h; (vii) I_2, THF, 0 °C, 2 h; (viii) NaOMe, MeOH, rt, 16 h (yields, see Table 2).

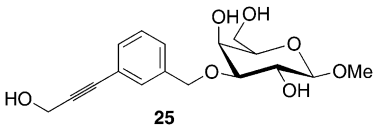


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Figure 3.

out to be unwarranted. We could never detect any other carbohydrate-containing products when using **6** as the substrate. The lower yields for the unprotected carbohydrates are probably caused by boron trifluoride etherate coordinating to the free hydroxyl groups, as well as material loss in the isolation steps.

The final compounds **2**, **7**, **9–12**, **16** and **19–22** as well as compound **25** (Fig. 3) were tested against galectin-1, -3, -7, -8N (N-terminal domain) and -9N (N-terminal domain) using fluorescence polarisation techniques (Table 3).[15,16] While the results for galectin-1, -3 and -8N were less impressive, inhibitors of galectin-7 and -9N with more than an order magnitude improved affinity were discovered (entries **16**, **25**, **21** and **2**, respectively).

Three sub-millimolar inhibitors, compounds **16**, **25** and **21**, were found for galectin-7. The K_d value of 0.39 mM for **16** is more than one order of magnitude improvement over the underivatised reference galactoside **1** and remarkably potent for a monosaccharide derivative. The best inhibitors are the simple straight-chain allyl- and hydroxymethyl-substituted alkynes **16** and **25**, which suggests that the binding pocket of galectin-7 close to galactose O-3 is relatively small and does not allow larger cyclic structures to bind. Several structures of galectin-7 as complexes with ligands have been solved,[17] this allowed computational analysis of galectin-7 in complexation with compounds **16**, **25** and **21**. Although conformational searches of the complexes gave several energy minima for each inhibitor/galectin-7 complex, a coherent picture emerged. The conformations and positions of the galactose ring and the 3-*O*-benzyl group were in all cases similar. The N-terminal of galectin-7, which was close to the alkynyl groups, possessed a high degree of conformational freedom. Hence, conclusive analysis of the conformations of the alkynyl chains proved to be challenging. Nevertheless, some general conclusions could be drawn. The two compounds carrying benzyl ethers substituted in the *meta*-position with straight-chain substituents, **16** and **25**, preferred similar conformations and similar interactions with galectin-7. While the alkynylbenzyl moiety of **16** displayed a better surface complementarity with galectin-7 (Fig. 4a), the hydroxyl group of the alkyne moiety of **25** could form a hydrogen bond with Asn-2 (Fig. 4b).

Table 3. K_d (mM) values against galectin-1, -3, -7, -8N and -9N measured in a competitive fluorescence polarisation assay

		Galectin				
		1	3	7	8N	9N
1	Methyl β-D-galactoside	10	4.4	4.8	5.3	3.4
meta-Substituted benzyl ethers, R=						
2	I	11	2.1	1.3	2.7	*0.26*
7	3-(*N*-Methylindol-3-yl)prop-1-ynyl	n.i.[a]	n.i.	n.i.	n.i.	n.i.
9	(Cyclohexen-3-yl-methyl)prop-1-ynyl	n.i.	n.i.	8.4	1.8	5.7
10	(Cyclohexanon-2-yl-methyl)prop-1-ynyl	11	2.4	4.6	1.5	3.0
11	(1,3,5-Trimethoxyphenyl)prop-1-ynyl	n.i.	4.5	1.5	1.9	4.2
12	(1,3-Dimethoxyphenyl)prop-1-ynyl	n.i.	4.8	1.2	n.i.	1.5
16	Allylprop-1-ynyl	27	2.4	*0.39*	1.0	1.0
25[b]	Hydroxymethylprop-1-ynyl	6.9	2.9	*0.65*	3.8	1.9
para-Substituted benzyl ethers, R=						
19	3-(*N*-Methylindol-3-yl)prop-1-ynyl	n.i.	n.i.	n.i.	n.i.	n.i.
20	Allylprop-1-ynyl	n.i.	4.3	4.9	n.i.	13
21	(1,3-Dimethoxyphenyl)prop-1-ynyl	n.i.	5.4	*0.74*	2.4	2.0
22	(1,3,5-Trimethoxyphenyl)prop-1-ynyl	2.2	1.3	1.5	0.93	1.8

[a] Non-inhibitory.
[b] Prepared via solution phase methods.

The low-energy complexes with **21**, which carries a dimethoxybenzyl-substituted alkyne moiety at the *para*-position, revealed preferred interaction modes significantly different from those of the *meta*-substituted **16** and **25**. The dimethoxybenzyl group of **21** fills a narrow cleft with good surface complementarity (Fig. 4c). The favoured complex calculated between **21** and galectin-7 could also explain why the structurally similar 1,3,5-trimethoxy-substituted **22** has a K_d twice as high as **21**. The third methoxy group of **22** would inevitably sterically interfere with the alkyne moiety if a complex similar to that between **21** and galectin-7 was formed.

The best inhibitor of galectin-9N was **2**, which carries a structurally simple *meta*-iodobenzyl ether at the 3-*O* position. Compound **2** is almost 13 times higher in affinity than methyl β-D-galactoside itself. Disappointingly, additional substitution on the benzyl group lowers the affinity. Although **2** might not be a good structure for further development into galectin-9N inhibitors, it shows significant selectivity over the other galectins investigated. This confirms that the galectin binding sites close to galactose C-3 differ enough to allow for the much desired development of selective inhibitors.

3. Conclusions

We have demonstrated that the Nicholas reaction of alkynyl benzyl ethers can be used to provide a straightforward and flexible route to novel galectin inhibitors. The solid phase approach simplified the purification steps and enabled the use of unprotected carbohydrate in the formation of the *meta*-substituted products. The inhibitors were tested for their affinity towards galectin-1, -3, -7, -8N and -9N using fluorescence polarisation techniques and the majority exhibited affinity for one or more of the tested galectins. In particular, potent monosaccharide inhibitors of galectin-7 were discovered.

4. Experimental

4.1. General methods

All reactions involving moisture or air sensitive compounds were performed under a nitrogen or argon atmosphere using oven-dried glass equipment. Solvents were either used as purchased from commercial sources or purified using standard procedures[18] as appropriate. All reagents were used as purchased. Column chromatography was carried out using Matrix Si 60 Å, 35–70 μm. Thin layer chromatography (TLC) was performed using precoated alumina-backed plates (Merck 25 DC Alufolien Kieselgel 60 F_{254}). Visualisation was effected either by UV fluorescence (υ=254 nm) or by heating the plates after treatment with

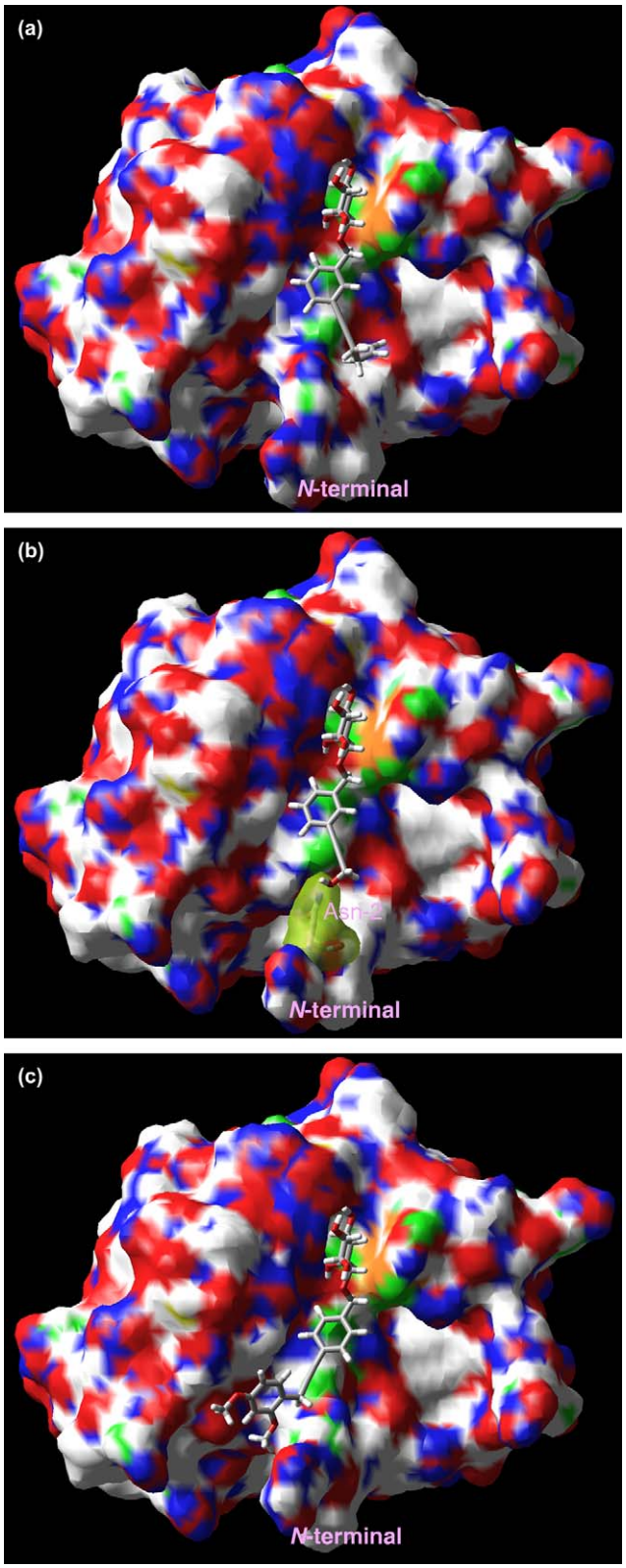

Figure 4. Energy minimised structure of galectin-7 in complex with (a) **16**, (b) **25** and (c) **21**. Molecular modelling was performed with MMFFs force field in water implemented in Macromodel 9.0. Starting conformations were built from the galactose/galectin-7 crystal structure.[12]

10% H_2SO_4. Preparative HPLC was conducted on a Waters 600E system using an XTerra Ms C_{18} 5 μm column and equipped with a Waters 490 Multiwavelength detector, using a stepwise gradient of 0.1 M NH_4OAc (5% acetonitrile) to pure acetonitrile as the mobile phase. Analytical HPLC was conducted on a Waters 600E system using a Chromasil 100 C_8 3.5 mm column with the same mobile phase as the preparative system. NMR spectra were recorded using either a Varian Unity 500 or a JEOL eclipse+ (^{1}H 400 MHz and ^{13}C 100 MHz). All spectra were recorded either in chloroform-d_1 or in methanol-d_4. All chemical shifts (δ_H and δ_C) are quoted in parts per million relative to tetramethylsilane (δ_H 0.00). Optical rotations were measured using a Perkin–Elmer polarimeter 341 C, which has a thermally jacketed 10^{-1} cm cell (path length of 1 dm) and were given in units of 10^{-1} deg cm^2 g^{-1} at 589 nm (sodium D-line). All infrared spectra were obtained using a Perkin–Elmer 1600 FTIR as KBr tablet samples. Only selected absorbancies are reported. Galectin production and fluorescence polarisation experiments with rat galectin-1 and human galectin-3, -7, -8N and -9N were performed as described earlier.[15,16]

4.1.1. Methyl 3-*O*-(3-iodobenzyl)-β-D-galactopyranoside (2). Methyl β-D-galactoside (3.00 g, 15.5 mmol) was dissolved in MeOH (50 mL) and *n*-Bu_2SnO (4.23 g, 17.0 mmol) was added. The mixture was refluxed for 2 h whereafter the solvent was evaporated under reduced pressure. To the crude residue were added 1,4-dioxane (50 mL), 3-iodobenzyl bromide (9.17 g, 31.0 mmol) and *n*-Bu_4NBr (4.98 g, 15.5 mmol). The mixture was sonicated for 10 min and then refluxed overnight. The solvent was evaporated under reduced pressure and the sticky residue was dissolved in MeOH (70 mL) and cooled to 0 °C for 2 h. A white solid was filtered off and the clear solution was concentrated under reduced pressure. The crude residue was diluted with CH_2Cl_2 (100 mL) and cooled to 0 °C, affording methyl [3-*O*-[3-(3-propargyl)-benzyl]]-β-D-galactopyranoside **2** in the form of white crystals (3.56 g, 56%). $[\alpha]_D^{25}$ +22.2 (*c* 0.021, CD_3OD). ^{1}H NMR (400 MHz; CD_3OD) δ 7.83 (s, 1H), 7.58 (s, *J*=9.0 Hz, 1H), 7.40 (d, *J*=7.1 Hz, 1H), 7.06 (t, *J*=7.8 Hz, 1H), 4.69 (d, *J*=12.1 Hz, 1H), 4.56 (d, *J*=12.1 Hz, 1H), 4.11 (d, *J*=7.8 Hz, 1H), 4.02 (d, *J*=3.2 Hz, 1H), 3.71 (m, 2H), 3.62 (dd, *J*=9.6, 7.8 Hz, 1H), 3.49 (s, 3H), 3.43 (t, *J*=6.1 Hz, 1H), 3.32 (dd, *J*=9.7, 3.3 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 142.61, 137.93, 137.71, 131.15, 128.18, 105.10, 94.80, 82.74, 76.55, 71.74, 71.48, 67.03, 62.50, 57.28. Anal. Calcd for $C_{14}H_{19}IO_6$: C, 40.99; H, 4.67; I, 30.94. Found: C, 40.86; H, 4.82; I, 30.78.

4.1.2. Polymer-bound propargyl ether (4). Merrifield resin HL 200–400 mesh (2 g, 2.20 mmol) was mixed with THF (50 mL), 15-crown-5 (874 μL, 4.40 mmol), KI (73 mg, 0.44 mmol) and NaH (60% in mineral oil, 440 mg, 11 mmol) under N_2 for 10 min. Propargyl alcohol (640 μL, 11 mmol) was then added dropwise. The mixture was shaken overnight whereupon the resin was washed with water, DMF, MeOH and CH_2Cl_2 (2×200 mL each) and then dried under N_2. The resin was analysed using IR and exhibited the characteristic alkyne C–H stretch at 3294 cm^{-1}.

4.1.3. Polymer-bound methyl 3-*O*-[3-(3-propargyl)-benzyl]-β-D-galactopyranoside (5). To a solid phase reaction vessel were added polymer-bound propargyl ether **4** (2.20 mmol) and THF/Et_3N (50 mL, 1:1), whereupon the mixture was degassed with N_2 for 10 min. $Pd(PPh_3)_4$

(250 mg, 10 mol %) was then added and the mixture was again degassed for 5 min and then shaken overnight. The resin was washed with water, DMF, MeOH, CH_2Cl_2 and Et_2O (2×100 mL) and dried under N_2. The resin was analysed using IR and the alkyne C–H vibration had been replaced by a broad peak around 3500 cm^{-1}, indicating that reaction had taken place.

4.1.4. Representative procedures for *meta*-substituted products 7–16. *Method A*: To a solid phase vessel containing polymer-bound methyl [3-*O*-[3-(3-propargyl)]-benzyl]-β-D-galactopyranoside (**5**) (2.20 mmol) was added CH_2Cl_2 (50 mL) and the mixture was degassed with N_2 for 10 min. $Co_2(CO)_8$ (2.57 g, 6.6 mmol) was added and the vessel was shaken at ambient temperature for 4 h whereupon the resin was washed with CH_2Cl_2 (6×50 mL) followed by toluene (6×50 mL), affording **6**, which was used directly in the Nicholas reaction. The resin was split into eight parts. To each part were added toluene (5 mL) and one nucleophile (3 equiv). The mixture was shaken for 5 min and then $BF_3 \cdot OEt_2$ (171 μL, 1.35 mmol) was added in one portion. The reactions were shaken overnight whereupon Et_3N was added. The resins were washed with THF and MeOH in alteration until no colourisation of the wash fluid was seen. The solvent was then evaporated under reduced pressure and the crude residues treated with I_2 (10 equiv) in a THF/MeOH mixture (10 mL, 8:2) at ambient temperature until TLC showed complete conversion (normally around 1.5 h). To the reaction mixtures was then added 20 mL of a 1:1 solution of satd $NaHCO_3$ and satd Na_2SO_3. The mixture was extracted with 2×30 mL EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. Reversed phase HPLC afforded products **7**, **10** and **16**. Remaining nucleophiles gave only traces of product.

Method B: To a solid phase vessel containing polymer-bound methyl [3-*O*-[3-(3-propargyl)]-benzyl]-β-D-galactopyranoside (**5**) (1.65 mmol) was added CH_2Cl_2 (50 mL) and the mixture was degassed with N_2 for 10 min. $Co_2(CO)_8$ (1.96 g, 4.95 mmol) was added and the vessel was shaken at ambient temperature for 4 h whereupon the resin was washed with CH_2Cl_2 (6×50 mL) followed by toluene (6×50 mL). The resin was then split into four parts. To each part were added toluene (4 mL) and one nucleophile (1.5 equiv). The mixture was shaken for 5 min and then $BF_3 \cdot OEt_2$ (131 μL, 1.03 mmol) was added. The mixtures were shaken for 10 min and then the resins were washed with toluene (3×2 mL). The combined wash fluids from each mixture were quenched with an excess of triethylamine. The resins were then again treated with nucleophile (1.5 equiv) and $BF_3 \cdot OEt_2$ (131 μL, 1.03 mmol) in toluene (2 mL) for 30 min and then washed with toluene and THF. The solvent was evaporated and the crude residues treated with I_2 (10 equiv) in THF/MeOH (10 mL, 8:2) at ambient temperature until TLC showed complete conversion. To the reaction mixtures was then added 20 mL of a 1:1 solution of satd $NaHCO_3$ and satd Na_2SO_3, and the aqueous phase was extracted with 2×30 mL EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to chromatography on silica with EtOAc as eluent to afford **9**, **11** and **12**. Only traces of **15** were formed.

4.1.5. Methyl [3-*O*-[3-(3-(1-methyl-indol-3-yl)-prop-1-ynyl)]-benzyl]-β-D-galactopyranoside (7). White solid. Yield: 31 mg (25% over four steps, calculated from **2**). $[\alpha]_D^{25}$ +11.9 (*c* 0.007, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.58 (d, *J*=7.9 Hz, 1H), 7.50 (s, 1H), 7.36 (d, *J*=7.4 Hz, 1H), 7.31 (m, 2H), 7.26 (t, *J*=7.6 Hz, 1H), 7.17 (dd, *J*=7.0, 1.1 Hz, 1H), 7.09 (s, 1H), 7.03 (dd, *J*=7.0, 0.9 Hz, 1H), 4.70 (d, *J*=12.0 Hz, 1H), 4.58 (d, *J*=12.0 Hz, 1H), 4.12 (d, *J*=7.4 Hz, 1H), 4.01 (dd, *J*=2.5, 0.7 Hz, 1H), 3.85 (s, 2H), 3.75–3.72 (m, 4H), 3.61 (dd, *J*=7.8, 1.9 Hz, 1H), 4.51–3.49 (m, 4H), 3.42 (dt, *J*=5.6, 0.8 Hz, 1H), 3.32 (dd, *J*=9.6, 3.3 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 140.19, 138.82, 132.04, 131.72, 129.32, 128.60, 128.40, 127.92, 125.35, 122.66, 119.81, 119.65, 110.92, 110.28, 105.94, 89.30, 82.52, 81.87, 76.49, 72.01, 71.73, 67.05, 62.50, 57.26, 32.73, 16.55. HRMS (ES) *m/z* (M+Na) calcd for $C_{26}H_{29}O_6NNa$ *m/e*: 474.1893. Found: 474.1907.

4.1.6. Methyl [3-*O*-[3-(3-(cyclohex-1-enyl)-prop-1-ynyl)]-benzyl]-β-D-galactopyranoside (9). White solid. Yield: 15 mg (18% over four steps, calculated from **2**). $[\alpha]_D^{25}$ +32.8 (*c* 0.005, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.43 (s, 1H), 7.33 (dt, *J*=4.0, 1.3 Hz, 1H), 7.23–7.22 (m, 2H), 5.73–5.74 (m, 2H), 4.69 (d, *J*=11.9 Hz, 1H), 4.57 (d, *J*=12.0 Hz, 1H), 4.11 (d, *J*=7.7 Hz, 1H), 4.00 (d, *J*=2.8 Hz, 1H), 3.75 (m, 2H), 3.62 (dd, *J*=7.8, 1.9 Hz, 1H), 3.48 (s, 3H), 3.42 (t, *J*=6.55 Hz, 1H), 3.32 (dd, *J*=9.7, 3.3 Hz, 1H), 2.34–2.29 (m, 3H), 1.98–1.94 (m, 2H), 1.92–1.85 (m, 1H), 1.78–1.69 (m, 1H), 1.59–1.49 (m, 1H), 1.42–1.34 (m, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 140.15, 132.01, 131.70, 131.48, 129.30, 129.05, 128.35, 125.40, 105.95, 89.63, 82.53, 82.46, 76.522, 72.05, 71.74, 67.05, 62.49, 57.28, 36.57, 30.00, 26.91, 26.23, 22.38. HRMS (FAB) *m/z* (M+Na) calcd for $C_{23}H_{30}O_6$ *m/e*: 425.1940. Found: 425.1937. Anal. Calcd for $C_{23}H_{30}O_6$: C, 68.64; H, 7.51. Found: C, 68.84; H, 7.18.

4.1.7. Methyl [3-*O*-[3-(3-(2-cyclohexanonyl)-prop-1-ynyl)-benzyl]]-β-D-galactopyranoside (10). White solid. Yield: 16 mg (14% over four steps, calculated from **2**). $[\alpha]_D^{25}$ +14.5 (*c* 0.010, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.45 (s, 1H), 7.37 (dt, *J*=4.3, 1.7 Hz, 1H), 7.26–7.24 (m, 2H), 4.72 (d, *J*=12.0 Hz, 1H), 4.60 (d, *J*=12.0 Hz, 1H), 4.14 (d, *J*=7.7 Hz, 1H), 4.04 (dd, *J*=2.4, 0.9 Hz, 1H), 3.78–3.70 (m, 2H), 3.65 (dd, *J*=7.8, 1.9 Hz, 1H), 3.52 (s, 3H), 3.46 (dd, *J*=5.6, 1.1 Hz, 1H), 3.35 (dd, *J*=5.5, 3.3 Hz, 1H), 2.74 (dd, *J*=11.9, 4.9 Hz, 1H), 2.69–2.61 (m, 1H), 2.48–2.40 (m, 1H), 2.40 (d, *J*=6.2 Hz, 1H), 2.36 (d, *J*=7.8 Hz, 1H), 2.15–2.08 (m, 1H), 1.96–1.90 (m, 1H), 1.84–1.73 (m, 1H), 1.71–1.60 (m, 1H), 1.54–1.40 (m, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 212.05, 138.83, 130.64, 130.31, 127.92, 127.05, 123.87, 104.62, 87.60, 81.29, 81.20, 75.18, 70.67, 70.40, 65.70, 61.13, 55.91, 49.50, 41.38, 33.31, 27.70, 24.72, 19.12. Anal. Calcd for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 66.15; H, 7.18.

4.1.8. Methyl [3-*O*-[3-(3-(2,4,6-trimethoxy-phenyl)-prop-1-ynyl)]-benzyl]-β-D-galactopyranoside (11). White solid. Yield: 48 mg (48% over four steps, calculated from **2**). $[\alpha]_D^{25}$ +22.8 (*c* 0.015, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.40 (s, 1H), 7.31 (t, *J*=3.6 Hz, 1H), 7.23–7.16 (m, 2H), 6.20 (s, 2H), 4.67 (d, *J*=11.9 Hz, 1H), 4.56 (d, *J*=11.9 Hz, 1H), 4.12 (d, *J*=7.8 Hz, 1H), 4.00 (d, *J*=2.7 Hz,

1H), 3.82 (s, 6H), 3.77 (s, 3H), 3.73 (t, J=6.6 Hz, 2H), 3.59 (t, J=9.5 Hz, 1H), 3.56 (s, 2H), 3.48 (s, 3H), 3.35 (s, 2H); ^{13}C NMR (100 MHz; CD_3OD) δ 161.84, 159.92, 139.97, 132.05, 131.70, 129.20, 128.02, 125.75, 107.03, 105.90, 91.84, 90.57, 82.44, 78.91, 76.47, 72.06, 71.70, 67.04, 62.48, 57.26, 56.37, 55.81, 13.63. HRMS (FAB) *m/z* (M+Na) calcd for $C_{26}H_{32}O_9$ *m/e*: 511.1944. Found: 511.1942.

4.1.9. Methyl [3-*O*-[3-(3-(2,4-dimethoxy-phenyl)-prop-1-ynyl)]-benzyl]-β-D-galactopyranoside and methyl [3-*O*-[3-(3-(2,6-dimethoxy-phenyl)-prop-1-ynyl)]-benzyl]-β-D-galactopyranoside (12). White solid. Isomeric mixture. Yield: 39 mg (40% over four steps, calculated from **2**). $[\alpha]_D^{25}$ +17.2 (*c* 0.023, CD_3OD). ^{1}H NMR (400 MHz; CD_3OD) δ 7.51 (br s, 2H), 7.40–7.36 (m, 3H), 7.32–7.16 (m, 3H), 6.62 (d, J=8.37 Hz) and 6.48 (d, J=2.4 Hz, 1H), 6.51 (br s, 2H, 0.5H), 4.73 (d, major isomer, J=11.9 Hz, 2H), 4.61 (d, major isomer, J=11.9 Hz, 2H), 4.14 (d, major isomer, J=7.8 Hz, 1H), 4.03 (d, major isomer, J=2.8 Hz, 1H), 3.85 (s, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 3.75–3.71 (m, 4H), 3.70–3.66 (m, 2H), 3.65–3.58 (m, 4H), 3.51 (s, major isomer, 3H), 3.48–3.41 (m, 2H), 3.37–3.32 (m, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 161.50, 159.10, 140.22, 132.09, 131.74, 130.23, 129.34, 129.24, 129.20, 128.45, 128.142, 125.33, 118.44, 105.97, 105.39, 105.10, 99.21, 88.85, 86.00, 82.55, 76.54, 72.04, 71.76, 67.07, 62.50, 61.58, 57.28, 56.45, 55.96, 55.83, 162.68, 20.04. HRMS (FAB) *m/z* (M+Na) calcd for $C_{25}H_{30}O_8$ *m/e*: 481.1838. Found: 481.1837.

4.1.10. Methyl [3-*O*-(3-hex-5-en-1-ynyl)-benzyl]-β-D-galactopyranoside (16). White solid. Yield: 50 mg (50% over four steps, calculated from **2**). $[\alpha]_D^{25}$ +19.5 (*c* 0.030, CD_3OD). ^{1}H NMR (400 MHz; CD_3OD) δ 7.46 (s, 1H), 7.36 (t, J=4.4 Hz, 1H), 7.26 (d, J=4.0 Hz, 2H), 5.95 (m, 1H), 5.12 (d, J=17.6 Hz, 1H), 5.05 (d, J=10.4 Hz, 1H), 4.73 (d, J=12.0 Hz, 1H), 4.61 (d, J=12.0 Hz, 1H), 4.14 (d, J=8.0 Hz, 1H), 3.74 (d, J=3.2 Hz, 1H), 3.67 (m, 2H), 3.66 (dd, J=8.0, 1.8 Hz, 1H), 3.52 (s, 3H), 3.45 (t, J=4.8 Hz, 1H), 3.45 (dd, J=8.0, 3.2 Hz, 1H), 2.48 (t, J=6.4 Hz, 2H), 2.34 (m, 2H); ^{13}C NMR (100 MHz; CD_3OD) δ 138.94, 137.076, 130.82, 130.42, 128.08, 127.173, 124.12, 114.93, 104.75, 88.99, 81.30, 80.74, 75.33, 70.80, 70.53, 65.80, 61.27, 56.10, 33.00, 18.79. HRMS (ES) *m/z* (M+Na) calcd for $C_{20}H_{26}O_6Na$ *m/e*: 385.1627. Found: 385.1611.

4.1.11. Methyl 3-*O*-(4-iodobenzyl)-β-D-galactopyranoside (17). Methyl *O*-β-D-galactoside (3.00 g, 15.5 mmol) was dissolved in MeOH (50 mL) and *n*-Bu_2SnO (4.23 g, 17.0 mmol) was added. The mixture was refluxed for 2 h whereafter the solvent was evaporated. To the crude residue were added 1,4-dioxane (50 mL), 4-iodobenzyl bromide (9.17 g, 31.9 mmol) and *n*-Bu_4NBr (4.98 g, 15.5 mmol). The mixture was sonicated for 10 min and then refluxed overnight. The solvent was evaporated under reduced pressure and the sticky residue was dissolved in MeOH (50 mL) and cooled to 0 °C for 2 h. A white solid was formed and filtered off, and the clear solution was then concentrated under reduced pressure. The crude residue was diluted with CH_2Cl_2 (100 mL) and cooled to 4 °C overnight, affording, after filtration, methyl 3-*O*-(4-iodo)-benzyl-β-D-galactopyranoside **17** as white crystals (4.06 g, 64%). $[\alpha]_D^{25}$ +18.2 (*c* 0.012, CD_3OD). ^{1}H NMR (400 MHz; CD_3OD) δ 7.63 (d, J=8.3 Hz, 2H), 7.20 (d, J=8.3 Hz, 2H), 4.68 (d, J=12.2 Hz, 1H), 4.56 (d, J=12.2 Hz, 1H), 4.10 (d, J=7.8 Hz, 1H), 4.00 (d, J=3.1 Hz, 1H), 3.75–3.66 (m, 2H), 3.62 (dd, J=7.8, 1.8 Hz, 1H), 3.48 (s, 3H), 3.42 (dt, J=6.6, 1.0 Hz, 1H), 3.31 (dd, J=9.6, 3.3 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 139.88, 138.50, 131.01, 105.98, 93.53, 82.63, 76.53, 71.75, 67.05, 62.47, 57.28. Anal. Calcd for $C_{14}H_{19}IO_6$: C, 40.99; H, 4.67. Found: C, 41.12; H, 4.65.

4.1.12. Methyl 2,4,5-*O*-triacetyl-3-*O*-(4-iodobenzyl)-β-D-galactopyranoside (23). Methyl 3-*O*-(4-iodobenzyl)-β-D-galactopyranoside (0.85 g, 2.1 mmol) was dissolved in pyridine (25 mL) and Ac_2O (25 mL) was added. The mixture was refluxed for 2 h whereupon the solvent was evaporated under reduced pressure and the crude residue was subjected to chromatography on silica gel using toluene/EtOAc (5:2) as eluent to afford methyl 2,4,5-*O*-triacetyl-3-*O*-(4-iodobenzyl)-β-D-galactopyranoside **23** as a white solid (1.10 g, 99%). $[\alpha]_D^{25}$ +49.1 (*c* 0.0085, $CDCl_3$). ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.64 (d, J=7.4 Hz, 2H), 7.00 (d, J=7.4 Hz, 2H), 5.49 (s, 1H), 5.10 (t, J=9.0 Hz, 1H), 4.64 (d, J=12.4 Hz, 1H), 4.33 (d, J=12.4 Hz, 1H), 4.30 (d, J=8.0 Hz, 1H), 4.20–4.13 (m, 2H), 3.80 (t, J=6.4 Hz, 1H), 3.53–3.45 (m, 4H), 2.13 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.70, 170.58, 169.58, 137.61, 137.30, 129.65, 102.15, 93.50, 77.23, 70.93, 70.83, 70.45, 65.92, 62.03, 56.98, 21.15, 21.04, 20.96. Anal. Calcd for $C_{20}H_{25}IO_9$: C, 44.79; H, 4.70. Found: C, 44.89; H, 4.88.

4.1.13. Polymer-bound methyl [2,4,5-*O*-triacetyl-3-*O*-(4-iodobenzyl)]-β-D-galactopyranoside (24). To a solid phase reaction vessel were added polymer-bound propargylic ether **4** (1.10 mmol), THF/Et_3N (20 mL, 1:1 mixture), CuI (21 mg, 10 mol %) and methyl *O*-[2,4,5-triacetyl-3-(4-iodobenzyl)]-β-D-galactopyranoside **23** (649 mg, 1.21 mmol). The mixture was thoroughly degassed by bubbling N_2 for 5 min. $Pd(PPh_3)_4$ (127 mg, 10 mol %) was added and the mixture was again degassed for 5 min and then shaken overnight. The resin was washed with THF, CH_2Cl_2 and MeOH repeatedly and thereafter dried under N_2. The resin **24** was analysed using IR and did not exhibit the alkyne C–H stretch at 3294 cm^{-1} seen in **23**, indicating that Sonogashira coupling had taken place.

4.1.14. Representative procedure for *para*-substituted products 19–22. To a solid phase vessel containing polymer-bound methyl *O*-[2,4,5-triacetyl-3-(4-iodobenzyl)]-β-D-galactopyranoside **24** (0.22 mmol) was added CH_2Cl_2 (10 mL) and the mixture was degassed with N_2 for 5 min. $Co_2(CO)_8$ (291 mg, 0.66 mmol) was added and the vessel was shaken at ambient temperature for 3 h whereafter the resin was washed with CH_2Cl_2 (4×10 mL) followed by toluene (4×10 mL), forming polymer-bound complex **25**. To the resin were then added toluene (10 mL) and the nucleophile (0.66 mmol). The mixture was degassed for 5 min and then $BF_3\cdot OEt_2$ (42 μL, 0.66 mmol) was added. The mixture was shaken for 2 h and then quenched with Et_3N. The resin was washed with CH_2Cl_2 and THF. The solvent was then evaporated under reduced pressure and the crude residue treated with I_2 (558 mg) in THF (20 mL) at ambient temperature until TLC showed complete conversion (normally around 2 h). To the reaction mixtures was then added

20 mL of a 1:1 solution of satd $NaHCO_3$ and satd Na_2SO_3. Extraction with 2×30 mL EtOAc, drying over Na_2SO_4 and evaporation of the solvent under reduced pressure was followed by flash chromatography (SiO_2, CH_2Cl_2/MeOH 20:1). Removal of the acetyl moieties was effected by dissolving the product in MeOH (1 mL) and adding 1 mL of a solution of NaOMe in MeOH (20 mg NaOMe/10 mL MeOH). The mixture was stirred overnight at room temperature and neutralised with Amberlyst 120 H+ when TLC indicated complete conversion. The solvent was removed in vacuo and the residue was subjected to flash chromatography to afford products **19**–**22**.

4.1.15. Methyl 3-*O*-[4[(1-methyl-indol-3-yl)-prop-1-ynyl]-benzyl]-β-D-galactopyranoside (19). White solid. Yield: 23.4 mg (24% over five steps, calculated from **23**). $[\alpha]_D^{25}$ +20.0 (*c* 0.01, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.58 (d, *J*=8.0 Hz, 1H), 7.36–7.31 (m, 4H), 7.28 (d, *J*=8.3 Hz, 1H), 7.13 (dt, *J*=7.1, 1.0 Hz, 1H), 7.06 (s, 1H), 7.01 (dt, *J*=7.9, 0.9 Hz, 1H), 4.69 (d, *J*=12.1 Hz, 1H), 4.58 (d, *J*=12.1 Hz, 1H), 4.09 (d, *J*=7.8 Hz, 1H), 3.98 (d, *J*=3.1 Hz, 1H), 3.82 (s, 2H), 3.74–3.66 (m, 5H), 3.61 (dd, *J*=7.8, 1.8 Hz, 1H), 3.48 (s, 3H), 3.40 (t, *J*=5.7 Hz, 1H), 3.31 (dd, *J*=9.7, 3.3 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 139.59, 138.85, 132.45, 128.95, 128.62, 127.88, 124.61, 122.66, 119.79, 119.66, 110.96, 110.28, 105.97, 89.27, 82.52, 81.78, 76.52, 72.11, 71.76, 67.08, 62.49, 57.28, 32.73, 16.56. HRMS (ES) *m/z* (M+Na) calcd for $C_{26}H_{29}O_6NNa$ *m/e*: 474.1893. Found: 474.1891.

4.1.16. Methyl [3-*O*-(4-hex-5-en-1-ynyl)-benzyl]-β-D-galactopyranoside (20). White solid. Yield: 21.3 mg (45% over five steps, calculated from **23**). $[\alpha]_D^{25}$ +21.9 (*c* 0.014, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.34 (d, *J*=8.2 Hz, 2H), 7.27 (d, *J*=8.2 Hz, 2H), 5.95–5.84 (m, 1H), 5.09 (d ab-quart, *J*=17.1, 1.8 Hz, 1H), 5.00 (d ab-quart, *J*=10.2, 1.8 Hz, 1H), 4.71 (d, *J*=12.1 Hz, 1H), 4.60 (d, *J*=12.1 Hz, 1H), 4.10 (d, *J*=7.8 Hz, 1H), 3.99 (d, *J*=3.2 Hz, 1H), 3.75–3.66 (m, 2H), 3.61 (dd, *J*=7.8, 1.9 Hz, 1H), 3.49 (s, 3H), 3.41 (t, *J*=6.5 Hz, 1H), 3.31 (dd, *J*=9.6, 3.3 Hz, 1H), 2.44 (t, *J*=7.2 Hz, 2H), 2.29 (quart, *J*=6.45 Hz, 2H); ^{13}C NMR (100 MHz; CD_3OD) δ 139.54, 138.29, 132.39, 128.92, 124.61, 116.10, 105.98, 90.19, 82.54, 81.86, 76.53, 72.11, 71.78, 67.08, 62.49, 57.27, 34.21, 19.97. HRMS (FAB) *m/z* (M+Na) calcd for $C_{26}H_{34}O_8$ *m/e*: 385.1627. Found: 385.1636.

4.1.17. Methyl 3-*O*-[4-(2,4-dimethoxyphenyl-prop-1-ynyl)]-benzyl-β-D-galactopyranoside and methyl 3-*O*-[4-(2,6-dimethoxyphenyl-prop-1-ynyl)]-benzyl-β-D-galactopyranoside (21). White solid. Yield: 15.1 mg (51% over five steps, calculated from **23**). Isomeric mixture. $[\alpha]_D^{25}$ +17.3 (*c* 0.01, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.41–7.32 (m) and 7.19 (t, *J*=8.3 Hz, 5H), 7.26 (d, *J*=8.3 Hz, 1H), 6.63 (d, *J*=8.4 Hz, 1H), 6.51 (br s, 1H), 6.49 (d, *J*=2.4 Hz, 1H), 4.76–4.70 (m, 1H), 4.62–4.59 (m, 1H), 4.13 (dd, *J*=7.8, 5.7 Hz, 1H), 4.03 (dd, *J*=3.1, 0.9 Hz) and 4.01 (dd, *J*=3.1, 0.9 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 2H), 3.78 (s, 2H), 3.75–3.71 (s, 3H), 3.70–3.67 (m, 1H), 3.65–3.60 (m, 3H), 3.52–3.51 (m, 4H), 3.46–3.42 (m, 2H), 3.36 (dd, *J*=39.6, 3.3 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 161.51, 159.43, 159.11, 139.66, 139.21, 132.46, 132.46, 132.41, 130.19, 129.23, 128.98, 128.97, 128.88, 125.00, 124.57, 118.48, 106.00, 105.99, 105.98, 105.39, 105.12, 99.22, 90.01, 88.80, 83.04, 82.56, 82.51, 76.54, 76.53, 72.16, 72.13, 71.79, 71.77, 67.10, 62.49, 62.48, 57.28, 57.26, 56.44, 55.95, 55.82, 20.04, 13.91. HRMS (FAB) *m/z* (M+Na) calcd for $C_{26}H_{34}O_8$ *m/e*: 481.1838. Found: 481.1841.

4.1.18. Methyl 3-*O*-[4-(2,4,6-trimethoxyphenyl-prop-1-ynyl)]-benzyl-β-D-galactopyranoside (22). White solid. Yield: 12.0 mg (11% over five steps, calculated from **23**). $[\alpha]_D^{25}$ +19.5 (*c* 0.033, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.30 (d, *J*=8.2 Hz, 2H), 7.23 (*J*=8.2 Hz, 2H), 6.18 (s, 2H), 4.68 (d, *J*=12.1 Hz, 1H), 4.58 (d, *J*=12.1 Hz, 1H), 4.09 (d, *J*=7.8 Hz, 1H), 3.98 (d, *J*=3.2 Hz, 1H), 3.80 (s, 6H), 3.74–3.65 (m, 2H), 3.60 (dd, *J*=7.8, 1.8 Hz, 1H), 3.56 (s, 2H), 3.42 (dt, *J*=5.6, 0.9 Hz, 1H), 3.29 (dd, *J*=6.4, 3.3 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) 159.98, 132.40, 128.88, 125.10, 107.17, 105.99, 91.90, 90.50, 82.50, 76.54, 72.18, 71.77, 67.10, 62.48, 57.27, 56.38, 55.82, 13.62. HRMS (FAB) *m/z* (M+Na) calcd for $C_{26}H_{34}O_8$ *m/e*: 511.1944. Found: 511.1942.

4.1.19. Methyl 3-*O*-[3-(3-hydroxypropargyl)-benzyl]-β-D-galactopyranoside (25). Methyl 3-*O*-(3-iodobenzyl)-β-D-galactopyranoside **2** (200 mg, 0.49 mmol) was dissolved in THF/Et_3N (1:1, 10 mL) together with CuI (9 mg, 10 mol %) and propargylic alcohol (34 μL, 0.59 mmol). The mixture was thoroughly degassed with N_2 for 10 min whereafter $Pd(PPh_3)_4$ (28 mg, 5 mol %) was added. The solution was degassed for an additional 5 min and then stirred at ambient temperature until TLC indicated total conversion. The solvent was evaporated under reduced pressure and the residue subjected to multiple chromatographies (SiO_2, EtOAc/MeOH 10:1) to afford methyl 3-*O*-[3-(3-hydroxypropargyl)-benzyl]-β-D-galactopyranoside **32** (137 mg, 87%) as a white solid. $[\alpha]_D^{25}$ +19.8 (*c* 0.016, CD_3OD). ^1H NMR (400 MHz; CD_3OD) δ 7.51 (br s, 1H), 7.40 (d, *J*=6.8 Hz, 1H), 7.32–7.28 (m, 2H), 4.73 (d, *J*=12.0 Hz, 1H), 4.60 (d, *J*=12.0 Hz, 1H), 4.36 (s, 2H), 4.12 (d, *J*=8.0 Hz, 1H), 4.03 (d, *J*=3.2 Hz, 1H), 3.74–3.70 (m, 2H), 3.65–3.61 (m, 1H), 3.50 (s, 3H), 3.44 (tr, *J*=6.0 Hz, 1H), 3.37 (dd, *J*=9.6, 3.2 Hz, 1H); ^{13}C NMR (100 MHz; CD_3OD) δ 140.08, 131.68, 131.40, 128.09, 128.69, 123.97, 105.64, 88.50, 85.14, 82.29, 76.18, 71.58, 71.42, 66.70, 62.17, 57.08, 56.94, 50.90, 49.37. HRMS (FAB) *m/z* (M+Na) calcd for $C_{17}H_{22}NaO_7$ *m/e*: 361.1263. Found: 361.1259.

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Reaction between glutaric anhydride and *N*-benzylidenebenzylamine, and further transformations to new substituted piperidin-2-ones

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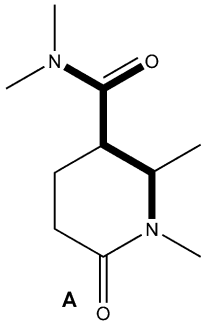
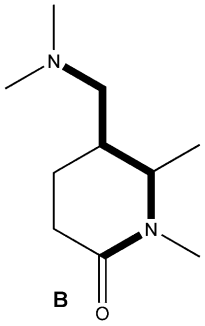
Abstract—The reaction between glutaric anhydride (**1**) and *N*-benzylidenebenzylamine (**3**) was studied in detail by ^{1}H NMR spectroscopy under different reaction conditions. The major product was (±)-*trans*-1-benzyl-6-oxo-2-phenylpiperidine-3-carboxylic acid (**2**), which was converted into new substituted piperidin-2-ones via transformations of the carboxylic group. The final products are expected to possess pharmaceutical activities, and the relevant screenings are in course.

1. Introduction

The piperidine ring is an important core structure in organic chemistry because of its presence in many natural products.[1–4] Substituted piperidines display a wide spectrum of physiological activities such as analgesic and anti-inflammatory,[5] anticonvulsant,[6] aromatase inhibiting,[7] etc. Piperidines possessing an amino substituent show cytotoxicity,[8] and are selective human neurokinin-1 antagonists.[9] Piperidinones are often useful advanced intermediates in the preparation of piperidines[4] and scaffolds in the synthesis of β-turn peptide mimetics.[10] Piperidinones possessing piperazino substituents exhibit antihistaminic and antianaphylactic activities.[11] The diverse biological activities of substituted piperidines have provoked numerous synthetic studies. Several reviews dealing with the recent progress in the synthesis of substituted piperidines and piperidinones have been published.[4,12–14] The reaction of glutaric anhydride (**1**) with *N*-arylidene-*N*-alkylamines provides a straightforward path to the synthesis of the piperidinone ring. It affords many *trans*-1-alkyl-2-aryl-6-oxopiperidine-3-carboxylic acids in one step.[5,7,15,16] However, less attention has been paid to the functional group transformations in the piperidinones obtained. For instance, *trans*-1-methyl-2-(2-methoxyphenyl)-6-oxopiperidine-3-carboxylic acid obtained by cyclocondensation of glutaric anhydride and the appropriate Schiff base was converted to an aza analog of tetrahydrocannabinol by Grignard addition to the carboxylic group in the key step.[16] The aim of the present paper is to investigate other possible transformations of the carboxylic group in order to achieve its replacement by carboxamido or by aminomethyl groups. In this way two types of target structures, **A** and **B**, can be obtained. The carboxamides, represented by structure **A**, contain a β-alanine subunit (the fragment given in bold). The (aminomethyl)piperidinone derivatives are related to structure **B**, which can be considered as inverse amide analogs of γ-aminobutyric acid (GABA).[17–19] The two sets of piperidinone derivatives may be designed to incorporate an amino group in the side chain as a part of another heterocycle such as 4-substituted piperazine, morpholine, piperidine, etc. Such heterocyclic moieties are well known pharmacophore substituents.[20–22] The combination of the piperidinone ring with different heterocyclic moieties in the side chain would result in a series of compounds with potential biological activities. For the purposes of this



A



B

Keywords: Piperidin-2-ones; Glutaric anhydride; Piperazine; Stereochemistry.
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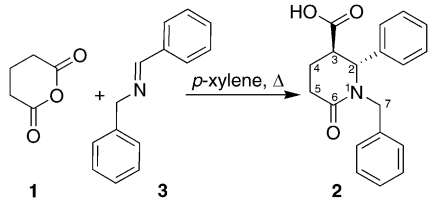
investigation we needed a great amount of *trans*-1-benzyl-2-phenyl-6-oxopiperidine-3-carboxylic acid (**2**). That made us study the reaction of **1** and *N*-benzylidenebenzylamine (**3**) in order to determine the optimal conditions for a fast and easy approach to acid **2**.

2. Results and discussion

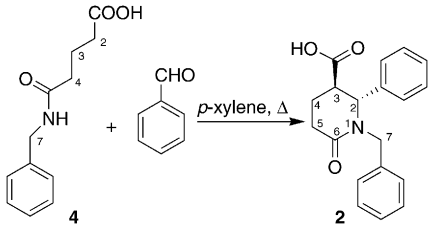
The required starting *trans*-1-benzyl-6-oxo-2-phenylpiperidine-3-carboxylic acid (**2**) was first prepared by Shetty et al. by refluxing a mixture of glutaric anhydride (**1**) and *N*-benzylidenebenzylamine (**3**) in xylene for 10 h in 80% yield[5] (Scheme 1). Chan et al. prepared *trans*-**2** by refluxing **1** and **3** in toluene for 50 h.[15] It was shown that *trans*-**2** formed a non-stoichiometric channel inclusion complex with acetonitrile as evidenced by X-ray analysis.[15] The significant difference in the reaction times[5,15] prompted us to investigate the relationship between the temperature and the time needed for the completion of the reaction. To achieve this we chose to fix the reaction time, in order to estimate the difference in the yields. Mixtures of **1** and **3** were refluxed in aprotic solvents with different boiling points. The solvents used and the yields of *trans*-**2** after 6 h are shown in Table 1.



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

The results indicate that the yield of *trans*-**2** increases with the rise of the temperature. Thus *p*-xylene appears to be the most suitable solvent for this reaction. That was the reason for using *p*-xylene as the solvent in our further research. A detailed investigation of the reaction mixture by TLC showed that glutaric anhydride (**1**) disappeared after 1 h at reflux and the reaction mixture contained along with the acid *trans*-**2**, also glutaric acid mono-*N*-benzylamide **4**. Up to now, there are no literature data about the concomitant formation of acyclic monoamides of glutaric acid during the reaction of the glutaric anhydride (**1**) with imines.[5,15,16] This made us follow the course of the reaction in *p*-xylene by ^{1}H NMR spectroscopy. We prepared four reaction mixtures each containing the same equimolar amounts of the reagents **1** and **3** in *p*-xylene and heated them at different times—0.5, 1, 3 and 6 h. Aliquots were taken and investigated by ^{1}H NMR spectroscopy. The corresponding spectra are presented in Figure 1. The integral intensities are shown below every signal. They are measured relatively to the doublet signal for H-2 (δ 4.81 ppm) in the molecule of the acid **2**. The determination of the relative quantity of the monoamide **4** was done using the doublet signal at 4.25 ppm for the two benzylic CH_2 protons, arbitrarily marked as H-7. Thus, the ^{1}H NMR spectrum after 0.5 h showed the presence of the acid *trans*-**2** and the monoamide **4** in ca. 1:4 ratio. The reaction mixtures also contained benzaldehyde from hydrolysis of the corresponding Schiff base **3** as evidenced by the singlet for C*H*O at 10.0 ppm. A certain amount of benzaldehyde is lost probably during the evaporation of *p*-xylene under reduced pressure. Signals of glutaric anhydride (**1**) could not be detected due to overlap with other signals. After the longest reaction time the ratio of **2** to **4** reached 5:1 and the quantity of the aldehyde was significantly reduced. Formation of other compounds besides **2** and **4** was not detected, which gave rise to the conclusion that compound **4** was converted into **2**. Recently, a similar reactivity of the monoamide of homophthalic acid toward aldehydes to furnish *cis*-2,3-disubstituted 1-oxotetrahydroisoquinoline-4-carboxylic acids has been observed.[23] However, in the latter case the reaction took place in the presence of Al salts and yielded the cis-product.[23] In order to shed more light whether the amide **4** is an intermediate in the reaction, an equimolar mixture of the amide **4** and benzaldehyde was refluxed in *p*-xylene (Scheme 2).

^{1}H NMR analysis of the reaction mixture after 6 h at reflux showed a complex mixture and the presence of the acid *trans*-**2** in very low quantity. In order to increase the yield of *trans*-**2**, we prolonged the reflux to 40 h and isolated **2** in 11% yield. This experiment shows that the acid **2** can be formed by reaction of **4** and benzaldehyde in boiling *p*-xylene. The catalytic effect of other reaction components present in the reaction mixture of **1** and **3** is probably essential and it could explain the low yield of **2** from the reaction of **4** and benzaldehyde. Thus, the acyclic monoamide **4** seems to be an intermediate in the formation of *trans*-**2** in the reaction of **1** and **3**. The question whether the reaction pathway via **4** is the only one or more reaction pathways are possible, remains open and requires further investigations.



Scheme 2. Reaction between **4** and benzaldehyde.

The preparation of carboxamides **5** from *trans*-**2** is shown in Scheme 3. The acid **2** was refluxed with thionyl chloride and the corresponding acid chloride without further purification was treated with an excess of the corresponding amine. The compounds **5a**–**n** thus obtained were purified by recrystallization or column chromatography. Compound **5o** was synthesized by BOC cleavage of **5n** and purified by column chromatography and subsequent recrystallization. The methyl ester **6** was obtained by esterification of the acid **2** with CH_3OH–H_2SO_4. The reduction of the ester **6** with

Table 1. The yield of *trans*-**2** in the cases of different solvents, after 6 h at reflux

Solvent	bp, °C	Yield, %
THF	65	4.4
Benzene	80	9.2
Toluene	110	53.2
p-xylene	138	78.0

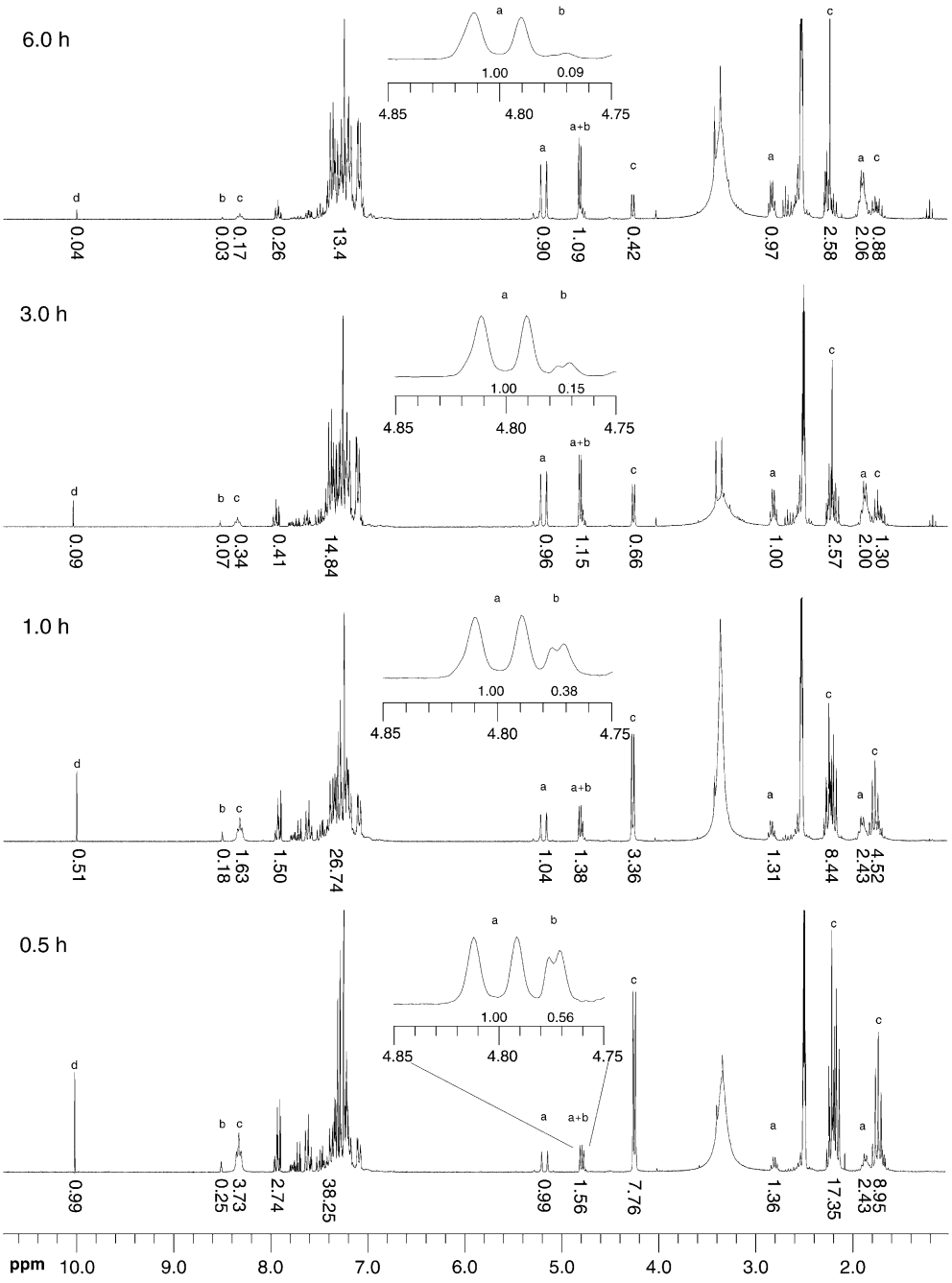
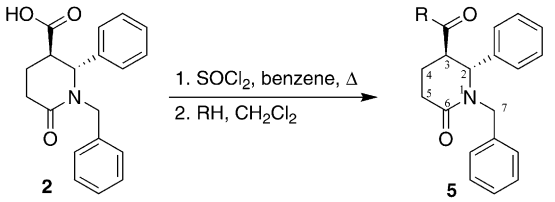


Figure 1. ^{1}H NMR spectra of the reaction mixture, where a—*trans*-**2**, b—imine **3**, c—monoamide **4**, d—benzaldehyde.

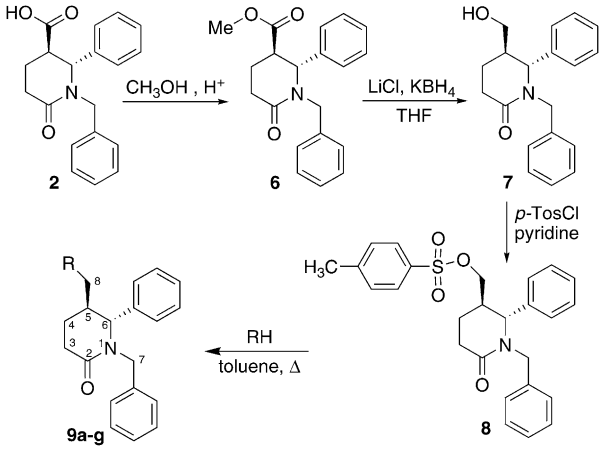
$LiBH_4$ proceeded selectively at the ester group to give *trans*-5-(hydroxymethyl)-6-phenylpiperidin-2-one (**7**). The latter was converted with *p*-toluenesulfonyl chloride (*p*-TosCl) into the tosylate **8**. The reaction of **8** with an excess of the selected secondary heterocyclic amines was carried out in refluxing toluene. The resulting aminomethyl derivatives **9a–g** were purified by means of recrystallization or column chromatography (Scheme 4). The compounds **5** and **9** are new. Their structure and trans relative configuration were established by means of ^{1}H NMR spectral data, which were compared with the data of the previously described structurally similar diastereomeric 1,2-disubstituted 6-oxopiperidine-3-carboxylic acids and their methyl esters[16,24] as well as with the spectral data of the acid **2**.

It is known that the reaction of the anhydride **1** with different *N*-arylidenealkylamines in refluxing xylene affords a mixture of *trans*- and *cis*-1,2-disubstituted 6-oxopiperidine-3-carboxylic acids, with the strong predominance for the *trans*-isomers.[16,24] It has been shown that the *trans*-isomers are thermodynamically more stable and their predominant formation is evidently favored by the reaction conditions (long reflux at ca. 140 °C).[24] The previously reported X-ray analysis of the inclusion complex of the acid *trans*-**2** with acetonitrile, shows that the piperidinone ring is in a highly distorted chair conformation due to the presence of the planar amide fragment and protons H_A and H_B occupy an axial–pseudoaxial position.[15] However, on the basis of ^{1}H NMR spectroscopy it is accepted that the conformation in



Compound	R	Yield, %	Compound	R	Yield, %
5a	H_2N	78	5h	H_3C-N-N	52
5b	N	64	5i	HO-N-N	75
5c	N	58	5j	N-N	84
5d	O-N	73	5k	F-N-N	55
5e	N	44	5l	Cl-N-N	41
5f	N	44	5m	F_3C-N-N	40
5g	HN	61	5n	$(H_3C)_3CO$-N-N	58

Scheme 3. Synthesis of compounds **5a–n**.



Compound	R	Yield, %	Compound	R	Yield, %
9a	N	57	9e	F-N-N	69
9b	O-N	92	9f	Cl-N-N	66
9c	$(H_3C)_3CO$-N-N	90	9g	F_3C-N-N	79
9d	N-N	35			

Scheme 4. Synthesis of compounds **9a–g**.

solution with a pseudoaxial phenyl, respective pseudoequatorial H_A is more favorable because of the smaller $A^{1,2}$ strain between the phenyl group and the *N*-substituent[16] (Fig. 2). In our experiments the acid **2** was isolated always as a single diastereomer. The formation of the *cis*-isomer was not detected, even when the reaction of **1** and **3** was followed by means of ^{1}H NMR spectroscopy. The signal of H_A in the ^{1}H NMR spectrum of **2** appears as a doublet with vicinal

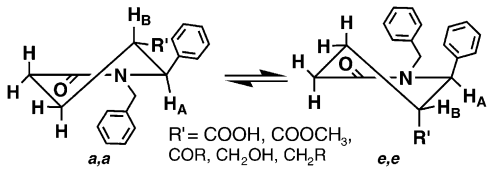


Figure 2.

coupling constant ^3J 5.0 Hz. It is known that six-membered rings in chair conformation are characterized with ^3J of the vicinal axial protons in 8–13 Hz range and ^3J of the vicinal equatorial protons in 1–4 Hz range in their ^{1}H NMR spectra.[25] In analogy to the literature data, we assume that in solution *trans*-**2** spends more time in a conformation with pseudoequatorial H_A and equatorial H_B[16,25] (Fig. 2). In the ^{1}H NMR spectrum of the methyl ester **6**, H_A exhibits a doublet with $^3J_{A,B}$ 3.9 Hz. This value of $^3J_{A,B}$ is in agreement with trans configuration and preferred pseudoequatorial–equatorial solution conformation of H_A and H_B of the ester **6**. Such conformation of the ester **6** is also preferred in solid state, which is evidenced by X-ray analysis.[15] The subsequent reactions of **2** and the intermediate ester **6** do not affect the two stereogenic centers. The trans relative configurations of the alcohol **7**, the tosylate **8** as well as the target amides **5**, and aminomethyl derivatives **9** follow directly from the configuration of the starting compounds, *trans*-**2** and the ester **6**. In the case of the amides **5**, ^3J is in the range of 6.8–9.3 Hz, which can be explained by the conformational equilibrium shifted to a greater extent to the conformer with diaxial protons. This can be due to the planarity of the carboxamido group, which leads to a smaller steric hindrance with the neighboring phenyl moiety. In the ^{1}H NMR spectra of the aminomethyl derivatives **9** the signal of H_A appears as a singlet, which is again in agreement with the trans configuration of compounds **9** and equatorial–pseudoequatorial orientation of H_A and H_B, influenced by the bigger effective volume of the aminomethyl substituent (Fig. 2). It should be noted that the ring numbering of compounds **7–9** is different from that of compounds **2**, **5**, **6** and the H_A and H_B protons have a different number, which is reflected in Section 3.

The pharmacological screening of compounds **5** and **9** is in course.

3. Experimental

3.1. General

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. IR spectra were recorded on a Specord 75 instrument. ^{1}H NMR spectra (250.13 MHz) and ^{13}C NMR spectra (62.90 MHz) were obtained on a Bruker Avance DRX-250 spectrometer. The chemical shifts are given in parts per million (δ) relative to tetramethylsilane as internal standard. Assignments were made using a combination of 1D and 2D spectra (DEPT, COSY). The microanalyses were done at the Faculty of Chemistry, University of Sofia. Thin layer chromatography (TLC) was performed on Merck 1.05554 silica gel 60F254 aluminum plates. Chromatographic filtration and column chromatography were carried out using Acros silica gel (0.060–0.200 mm).

Electrospray ionization (ESI) mass spectra were recorded by flow injection of acetonitrile solution into an ESI source attached to Varian Prostar 240 instrument.

3.2. Preparation of (±)-*trans*-1-benzyl-6-oxo-2-phenylpiperidine-3-carboxylic acid (2)

To a solution of *N*-benzylidenebenzylamine (**3**, 1.71 g, 8.8 mmol) in dried *p*-xylene (18 mL), glutaric anhydride (**1**, 1.0 g, 8.8 mmol) was added. The reaction mixture was refluxed for 6 h. The crystals separated were filtered and recrystallized from ethyl acetate–methanol to yield the acid **2** as white needles (2.12 g, 78%). Mp 168–171 °C. According to lit.,[5] mp of **2** is 171–174 °C. IR (Nujol): 3200–2500 (OH), 1705 (COOH), 1590 (CON) cm^{-1}. ^1H NMR δ (DMSO-d_6): 1.78–1.96 (2H, m, H-4), 2.42–2.65 (2H, m, H-5), 2.76–2.88 (1H, m, H-3), 3.38 (1H, d, H-7, J=15.2 Hz), 4.81 (1H, d, H-2, J=5.0 Hz), 5.19 (1H, d, H-7, J=15.2 Hz), 7.06–7.43 (10H, m, arom. H), 12.40 (1H, br s, COOH). ^{13}C NMR δ (DMSO): 20.2 (1C, C-4), 29.8 (1C, C-5), 46.5 (1C, C-3), 47.5 (1C, C-7), 61.7 (1C, C-2), 127.0 (2C, Ph), 127.7 (2C, Ph), 127.9 (2C, Ph), 128.4 (2C, Ph), 128.9 (2C, Ph), 137.2 (1C, Ph), 140.5 (1C, Ph), 169.2 (1C, C-6), 173.5 (1C, COOH). Anal. Calcd for $C_{19}H_{19}NO_3$: C 73.77%, H 6.19%; found: C 73.79%, H 6.33%.

3.3. Preparation of 5-(benzylamino)-5-oxopentanoic acid (4)

To a solution of **1** (0.228 g, 2 mmol) in CH_2Cl_2 (4 mL), benzylamine (0.22 mL, 2 mmol) was added. The mixture was stirred at room temperature for 15 min and then the solvent was evaporated. The resulting oil was triturated with ethyl acetate, the crystals were collected and recrystallized from water, to give monoamide **4** as colorless platelets (0.333 g, 75%). Mp 105–106 °C. IR (Nujol): 3300 (NH), 3200–2500 (OH), 1690 (COOH), 1635 (CON), 1540 (CONH) cm^{-1}. ^1H NMR δ (DMSO): 1.64–1.81 (2H, m, H-3), 2.12–2.26 (4H, m, H-2, H-4), 4.25 (2H, d, H-7, J=5.8 Hz), 7.17–7.43 (5H, m, arom. H), 8.34 (1H, t, NH, J=5.8 Hz). ^{13}C NMR δ ($CDCl_3$+DMSO): 20.8 (1C, CH_2), 33.2 (1C, CH_2COOH), 34.8 (1C, CH_2CON), 42.6 (1C, CH_2N), 126.8 (1C, Ph), 127.3 (2C, Ph), 128.2 (2C, Ph), 139.0 (1C, Ph), 172.4 (1C, CON), 174.7 (1C, COOH). Anal. Calcd for $C_{12}H_{15}NO_3$: C 65.14%, H 6.83%; found: C 64.86%, H 7.11%.

3.4. Reaction of 4 and benzaldehyde in boiling *p*-xylene

To a solution of **4** (0.222 g, 1 mmol) in *p*-xylene (2 mL), benzaldehyde (0.1 mL, 1 mmol) was added. After 40 h of reflux, the reaction mixture was cooled and diluted with ethyl acetate (20 mL). It was extracted with 5% aq $NaHCO_3$ (3×15 mL) and the combined water layers were acidified with 15% HCl to pH=2. The precipitated solid was filtered and dried, yielding 0.034 g (11%) of colorless crystalline *trans*-**2**, identical in all respects with *trans*-**2**, prepared as above.

3.5. Preparation of amides 5a–o

3.5.1. Preparation of (±)-*trans*-1-benzyl-6-oxo-2-phenylpiperidine-3-carboxylic acid amides 5a–n. A mixture of **2** (0.309 g, 1 mmol) and $SOCl_2$ (0.15 mL, 2 mmol) in benzene (4 mL) was heated at 70 °C for 1 h. The volatile products were evaporated under reduced pressure and the slightly yellow oil was dissolved in CH_2Cl_2 (4 mL). The solution was cooled at −5 °C and 3 mmol of the corresponding amine was added dropwise. In the case of the amide **5a**, dry ammonia was bubbled through the solution at −5 °C. The mixture was stirred at room temperature. After the completion of the reaction (TLC), the reaction mixture was dissolved in ethyl acetate (50 mL) and washed with water (3×20 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The resulting oil was purified by recrystallization or column chromatography and subsequent recrystallization from ethyl acetate–hexane. In this way the following compounds were prepared:

3.5.1.1. (±)-*trans*-1-Benzyl-6-oxo-2-phenylpiperidine-3-carboxamide (5a). White solid; yield: 78%, mp 101–103 °C. IR (Nujol): 3400 (NH), 1680, 1665 (CON) cm^{-1}. ^1H NMR δ (DMSO): 1.73–1.92 (2H, m, H-4), 2.41–2.58 (2H, m, H-5), 2.60–2.73 (1H, m, H-3), 3.33 (1H, d, H-7, J=15.3 Hz), 4.68 (1H, d, H-2, J=6.9 Hz), 5.18 (1H, d, H-7, J=15.3 Hz), 6.87 (1H, s, NH), 7.04–7.41 (11H, m, NH, arom. H). ^{13}C NMR δ (DMSO): 22.5 (1C, C-4), 30.8 (1C, C-5), 46.8 (1C, C-7), 47.8 (1C, C-3), 62.0 (1C, C-2), 126.9 (1C, Ph), 127.2 (2C, Ph), 127.5 (2C, Ph), 127.7 (1C, Ph), 128.4 (2C, Ph), 128.8 (2C, Ph), 137.3 (1C, Ph), 141.0 (1C, Ph), 169.5 (1C, C-6), 173.5 (1C, $CONH_2$). Anal. Calcd for $C_{19}H_{20}N_2O_2$: C 74.00%, H 6.54%; found: C 73.65%, H 6.60%.

3.5.1.2. (±)-*trans*-1-Benzyl-2-phenyl-3-(pyrrolidine-1-carbonyl)-piperidin-6-one (5b). White needles; yield: 64%, mp 137–139 °C. IR ($CHCl_3$): 1620 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.43–1.96 (5H, m, H-4, $4CH_2$), 2.00–2.20 (1H, m, H-4), 2.39–2.69 (2H, m, 2H-5), 2.74–2.93 (2H, m, H-3, CH_2N), 3.07–3.39 (3H, m, CH_2N), 3.44 (1H, d, H-7, J=14.8 Hz), 4.67 (1H, d, H-2, J=8.6 Hz), 5.49 (1H, d, H-7, J=14.8 Hz), 7.02–7.38 (10H, m, arom. H). MS *m/z*: 363 (M^+, 100), 338 (8), 256 (20), 201 (10), 196 (95), 168 (24), 130 (45). Anal. Calcd for $C_{23}H_{26}N_2O_2$: C 76.21%, H 7.23%; found: C 76.05%, H 7.12%.

3.5.1.3. (±)-*trans*-1-Benzyl-2-phenyl-3-(piperidine-1-carbonyl)-piperidin-6-one (5c). Colorless crystals; yield: 58%, mp 129–131 °C. IR ($CHCl_3$): 1625 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 0.61–0.79 (1H, m, CH_2), 1.11–1.33 (2H, m, CH_2), 1.34–1.49 (3H, m, CH_2), 1.81–1.93 (1H, m, H-4), 1.97–2.17 (1H, m, H-4), 2.51–2.68 (1H, m, H-5), 2.73–2.86 (1H, ddd, H-5, J=4.7, 4.7, 17.6 Hz), 2.93–3.12 (3H, m, H-3, $2CH_2N$), 3.13–3.27 (1H, m, CH_2N), 3.42 (1H, d, H-7, J=14.8 Hz), 3.48–3.63 (1H, m, CH_2N), 4.72 (1H, d, H-2, J=8.3 Hz), 5.45 (1H, d, H-7, J=14.8 Hz), 7.02–7.38 (10H, m, arom. H). Anal. Calcd for $C_{24}H_{28}N_2O_2$: C 76.56%, H 7.50%; found: C 76.80%, H 7.69%.

3.5.1.4. (±)-*trans*-1-Benzyl-3-(morpholine-4-carbonyl)-2-phenylpiperidin-6-one (5d). White powder; yield: 73%, mp 143–145 °C. IR ($CHCl_3$): 1630 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.83–1.96 (1H, m, H-4), 2.02–2.22 (1H, m, H-4), 2.53–2.95 (4H, m, 2H-5, $2CH_2N$), 2.94–3.21 (2H, m, H-3, CH_2N), 3.22–3.46 (4H, m, H-7, CH_2N, $2CH_2O$), 3.47–3.73 (2H, m, CH_2O), 4.65 (1H, d, H-2, J=8.5 Hz),

5.48 (1H, d, H-7, J=14.8 Hz), 6.96–7.41 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.2 (1C, C-4), 31.5 (1C, C-5), 42.1 (1C, CH_2N), 44.9 (1C, C-7), 45.9 (1C, CH_2N), 46.7 (1C, C-3), 63.2 (1C, C-2), 66.0 (1C, CH_2O), 66.5 (1C, CH_2O), 127.2 (3C, Ph), 128.2 (2C, Ph), 128.4 (3C, Ph), 129.0 (2C, Ph), 136.5 (1C, Ph), 139.6 (1C, Ph), 170.0 (1C, C-6), 170.3 (1C, CON). Anal. Calcd for $C_{23}H_{26}N_2O_3$: C 72.99%, H 6.92%; found: C 72.73%, H 6.92%.

3.5.1.5. (±)-*trans*-1-Benzyl-*N*,*N*-diisopropyl-6-oxo-2-phenylpiperidine-3-carboxamide (5e). Chromatographic purification (hexane–ethyl acetate=1:1) and recrystallization yielded **5e** as white powder (44%). Mp 75–77 °C. IR ($CHCl_3$): 1620 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 0.54 (3H, d, CH_3, J=6.5 Hz), 1.01 (3H, d, CH_3, J=6.6 Hz), 1.11 (3H, d, CH_3, J=6.8 Hz), 1.27 (3H, d, CH_3, J=6.8 Hz), 1.72–1.89 (1H, m, H-4), 1.99–2.19 (1H, m, H-4), 2.50–2.68 (1H, m, H-5), 2.72–2.84 (1H, m, H-5), 2.90–3.03 (1H, m, H-3), 3.14–3.37 (1H, br s, CHN), 3.45 (1H, d, H-7, J=14.8 Hz), 3.53–3.71 (1H, m, CHN), 4.81 (1H, d, H-2, J=9.3 Hz), 5.43 (1H, d, H-7, J=14.8 Hz), 7.00–7.37 (10H, m, arom. H). Anal. Calcd for $C_{25}H_{32}N_2O_2$: C 76.50%, H 8.22%; found: C 76.45%, H 8.41%.

3.5.1.6. (±)-*trans*-1-Benzyl-*N*,*N*-dicyclohexyl-6-oxo-2-phenylpiperidine-3-carboxamide (5f). Chromatographic purification (hexane–ethyl acetate=3:2) and recrystallization yielded **5f** as white powder (44%). Mp 161–163 °C. IR ($CHCl_3$): 1625 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 0.83–1.88 (20H, m, $10CH_2$), 2.00–2.21 (1H, m, H-4), 2.21–2.48 (1H, br s, CHN), 2.49–2.80 (2H, m, H-4, H-5), 2.71–2.85 (1H, m, H-5), 2.91–3.15 (2H, m, H-3, CHN), 3.44 (1H, d, H-7, J=14.8 Hz), 4.74 (1H, d, H-2, J=8.9 Hz), 5.43 (1H, d, H-7, J=14.8 Hz), 6.97–7.37 (10H, m, arom. H). MS m/z: 472 (M^+, 100), 281 (8). Anal. Calcd for $C_{31}H_{40}N_2O_2$: C 78.77%, H 8.53%; found: C 78.85%, H 8.68%.

3.5.1.7. (±)-*trans*-*N*-(2-(1*H*-indol-3-yl)ethyl)-1-benzyl-6-oxo-2-phenylpiperidine-3-carboxamide (5g). White powder; yield: 61%, mp 178–180 °C. IR (Nujol): 3240 (NH), 1630, 1615 (CON) cm^{-1}. ^{1}H NMR δ (DMSO): 1.69–1.92 (2H, m, H-4), 2.38–2.72 (5H, m, H-3, 2H-5, $2CH_2$), 3.12–3.27 (2H, dt, CH_2N, J=7.1, 13.2 Hz), 3.33 (1H, d, H-7, J=15.3 Hz), 4.70 (1H, d, H-2, J=6.8 Hz), 5.22 (1H, d, H-7, J=15.3 Hz), 6.91–7.47 (15H, m, 1CH, 14 arom. H), 7.94 (1H, t, NHCO, J=5.6 Hz), 10.75 (1H, d, NH, J=1.0 Hz). ^{13}C NMR δ (DMSO): 22.3 (1C, C-4), 25.0 (1C, CH_2), 30.7 (1C, C-5), 39.4 (1C, CH_2N), 46.7 (1C, C-7), 48.0 (1C, C-3), 62.2 (1C, C-2), 111.4 (1C, indole), 111.6 (1C, indole), 118.2 (2C, indole), 120.9 (1C, indole), 122.6 (1C, indole), 126.9 (1C, Ph), 127.0 (2C, Ph), 127.1 (1C, indole), 127.5 (2C, Ph), 127.7 (1C, Ph), 128.3 (2C, Ph), 128.7 (2C, Ph), 136.2 (1C, indole), 137.2 (1C, Ph), 140.7 (1C, Ph), 169.5 (1C, C-6), 171.3 (1C, CON). Anal. Calcd for $C_{29}H_{29}N_3O_2$: C 77.14%, H 6.47%; found: C 77.49%, H 6.81%.

3.5.1.8. (±)-*trans*-1-Benzyl-3-(4-methylpiperazine-1-carbonyl)-2-phenylpiperidin-6-one (5h). Chromatographic purification (ethyl acetate–methanol=4:1 containing 1% aq ammonia) and recrystallization yielded **5h** as white solid (52%). Mp 100–103 °C. IR ($CHCl_3$): 1630 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.81–1.95 (1H, m, H-4), 2.00–2.19 (2H, m, H-4, CH_2N), 2.27 (3H, s, CH_3), 2.33–2.46 (1H, m, CH_2N), 2.51–2.68 (2H, m, H-5, CH_2N), 2.73–2.87 (1H, m, H-5), 2.95–3.22 (3H, m, H-3, CH_2N), 3.30–3.48 (3H, m, H-7, CH_2N), 3.71–3.92 (1H, m, CH_2N), 4.64 (1H, d, H-2, J=8.6 Hz), 5.45 (1H, d, H-7, J=14.8 Hz), 6.99–7.42 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.1 (1C, C-4), 31.5 (1C, C-5), 41.6 (1C, CH_2N), 45.0 (1C, C-7), 45.3 (1C, CH_2N), 45.6 (1C, CH_3), 46.8 (1C, C-3), 54.2 (1C, CH_2N), 54.4 (1C, CH_2N), 63.1 (1C, C-2), 127.2 (3C, Ph), 128.2 (3C, Ph), 128.3 (2C, Ph), 128.9 (2C, Ph), 136.6 (1C, Ph), 139.8 (1C, Ph), 170.1 (2C, C-6, CON). Anal. Calcd for $C_{24}H_{29}N_3O_2$: C 73.63%, H 7.47%; found: C 73.82%, H 7.32%.

3.5.1.9. (±)-*trans*-1-Benzyl-3-(4-(2-hydroxyethyl)piperazine-1-carbonyl)-2-phenylpiperidin-6-one (5i). White needles; yield: 75%, mp 136–138 °C. IR (Nujol): 3355 (OH), 1620 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.80–1.94 (1H, m, H-4), 1.99–2.29 (4H, m, H-4, $3CH_2N$), 2.31–2.45 (3H, m, CH_2N), 2.51–2.69 (1H, m, H-5), 2.73–2.87 (1H, m, H-5), 2.88–3.22 (3H, m, H-3, $2CH_2N$), 3.27–3.37 (1H, m, CH_2N), 3.41 (1H, d, H-7, J=14.8 Hz), 3.55 (2H, t, CH_2O, J=5.3 Hz), 3.58–3.64 (1H, m, CH_2N), 4.67 (1H, d, H-2, J=8.5 Hz), 5.47 (1H, d, H-7, J=14.8 Hz), 7.01–7.40 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.0 (1C, C-4), 31.4 (1C, C-5), 41.7 (1C, CH_2N), 44.9 (1C, C-7), 45.4 (1C, CH_2N), 46.8 (1C, C-3), 52.2 (1C, CH_2N), 52.5 (1C, CH_2N), 57.6 (1C, CH_2N), 59.0 (1C, CH_2O), 63.1 (1C, C-2), 127.2 (3C, Ph), 128.2 (3C, Ph), 128.4 (2C, Ph), 129.0 (2C, Ph), 136.5 (1C, Ph), 139.7 (1C, Ph), 170.1 (2C, C-6, CON). Anal. Calcd for $C_{25}H_{31}N_3O_3$: C 71.23%, H 7.41%; found: C 69.88%, H 7.77%.

3.5.1.10. (±)-*trans*-1-Benzyl-2-phenyl-3-(4-phenylpiperazine-1-carbonyl)piperidin-6-one (5j). White needles; yield: 84%, mp 181–183 °C. IR ($CHCl_3$): 1645 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.85–1.96 (1H, m, H-4), 2.04–2.26 (2H, m, H-4, CH_2N), 2.54–2.82 (3H, m, 2H-5, CH_2N), 2.82–2.94 (1H, m, CH_2N), 3.02–3.15 (3H, m, H-3, $2CH_2N$), 3.23–3.45 (1H, m, CH_2N), 3.38–3.52 (2H, m, H-7, CH_2N), 3.69–3.81 (1H, m, CH_2N), 4.68 (1H, d, H-2, J=8.6 Hz), 5.49 (1H, d, C-7, J=14.8 Hz), 6.74–6.81 (2H, m, arom. H), 6.85–6.92 (1H, m, arom. H), 7.03–7.08 (2H, m, arom. H), 7.13–7.39 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.3 (1C, C-4), 31.6 (1C, C-5), 41.7 (1C, CH_2N), 45.2 (1C, C-7), 45.4 (1C, CH_2N), 46.8 (1C, C-3), 49.0 (1C, CH_2N), 49.2 (1C, CH_2N), 63.2 (1C, C-2), 116.5 (2C, Ph), 120.5 (1C, Ph), 127.2 (3C, Ph), 128.2 (2C, Ph), 128.3 (1C, Ph), 128.4 (2C, Ph), 129.0 (1C, Ph), 129.1 (1C, Ph), 136.6 (1C, Ph), 139.8 (1C, Ph), 150.5 (1C, Ph), 170.0 (1C, C-6), 170.2 (1C, CON). MS m/z: 454 (M^+, 100), 347 (12), 294 (7), 292 (39), 251 (20), 197 (18), 196 (90), 189 (67), 163 (8), 130 (54). Anal. Calcd for $C_{29}H_{31}N_3O_2$: C 76.79%, H 6.89%; found: C 76.74%, H 7.16%.

3.5.1.11. (±)-*trans*-1-Benzyl-3-(4-(4-fluorophenyl)-piperazine-1-carbonyl)-2-phenylpiperidin-6-one (5k). White needles; yield: 55%, mp 153–155 °C. IR ($CHCl_3$): 1635 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.84–1.98 (1H, m, H-4), 2.02–2.22 (2H, m, H-4, CH_2N), 2.53–2.71 (2H, m, H-5, CH_2N), 2.73–2.89 (2H, m, H-5, CH_2N), 2.91–3.03 (1H, m, CH_2N), 3.03–3.17 (2H, m, H-3, CH_2N) 3.23–3.36 (1H, m, CH_2N) 3.37–3.51 (2H, m, H-7, CH_2N), 3.69–3.83

(1H, m, CH_2N), 4.68 (1H, d, H-2, J=8.5 Hz), 5.49 (1H, d, H-7, J=14.8 Hz), 6.67–6.80 (2H, m, arom. H), 6.88–7.00 (2H, m, arom. H), 7.01–7.11 (2H, m, arom. H), 7.13–7.40 (8H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.3 (1C, C-4), 31.5 (1C, C-5), 41.8 (1C, CH_2N), 45.1 (1C, C-7), 45.4 (1C, CH_2N), 46.8 (1C, C-3), 50.0 (1C, CH_2N), 50.3 (1C, CH_2N), 63.2 (1C, C-2), 115.6 (2C, d, Ph, J=22.2 Hz), 118.4 (2C, d, Ph, J=7.8 Hz), 127.3 (3C, Ph), 128.3 (3C, Ph), 128.4 (2C, Ph), 129.0 (2C, Ph), 136.6 (1C, Ph), 139.8 (1C, Ph), 147.2 (1C, d, Ph, J=2.3 Hz), 157.5 (1C, d, CF, J=240.2 Hz), 170.0 (1C, C-6), 170.2 (1C, CON). MS *m/z*: 472 (M^+, 100), 342 (1.75), 279 (3.75), 261 (3.50), 217 (4.25). Anal. Calcd for $C_{29}H_{30}FN_3O_2$: C 73.86%, H 6.41%; found: C 74.18%, H 6.56%.

3.5.1.12. (±)-*trans*-1-Benzyl-3-(4-(3-chlorophenyl)-piperazine-1-carbonyl)-2-phenylpiperidin-6-one (5l). Chromatographic purification (hexane–ethyl acetate=1:1) and recrystallization yielded **5l** as pale pink spheres (41%). Mp 152–154 °C. IR ($CHCl_3$): 1630 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.83–1.97 (1H, m, H-4), 2.03–2.23 (2H, m, H-4, CH_2N), 2.53–2.82 (3H, m, 2H-5, CH_2N), 2.83–2.95 (1H, m, CH_2N), 3.00–3.16 (3H, m, H-3, $2CH_2N$), 3.20–3.34 (1H, m, CH_2N), 3.35–3.48 (2H, m, H-7, CH_2N), 4.67 (1H, d, H-2, J=8.6 Hz), 5.49 (1H, d, H-7, J=14.8 Hz), 6.64 (1H, dd, arom. H, J=2.1, 8.3 Hz), 6.72 (1H, t, arom. H, J=2.1 Hz), 6.83 (1H, dd, arom. H, J=1.7, 7.8 Hz), 7.01–7.40 (11H, m, arom. H). Anal. Calcd for $C_{29}H_{30}ClN_3O_2$: C 71.37%, H 6.20%; found: C 71.71%, H 6.36%.

3.5.1.13. (±)-*trans*-1-Benzyl-2-phenyl-3-(4-(3-(trifluoromethyl)phenyl)piperazine-1-carbonyl)piperidin-6-one (5m). Chromatographic purification (hexane–ethyl acetate=2:3) and recrystallization yielded **5m** as colorless needles (40%). Mp 136–138 °C. IR ($CHCl_3$): 1645 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.85–1.97 (1H, m, H-4), 2.04–2.22 (2H, m, H-4, CH_2N), 2.54–2.83 (3H, m, 2H-5, CH_2N), 2.84–2.98 (1H, m, CH_2N), 3.04–3.18 (3H, m, H-3, $2CH_2N$), 3.24–3.35 (1H, m, CH_2N), 3.36–3.49 (2H, m, H-7, CH_2N), 3.73–3.85 (1H, m, CH_2N), 4.67 (1H, d, H-2, J=8.6 Hz), 5.50 (1H, d, H-7, J=14.8 Hz), 6.89–6.97 (2H, m, arom. H), 7.02–7.09 (2H, m, arom. H), 7.09–7.40 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.3 (1C, C-4), 31.6 (1C, C-5), 41.6 (1C, CH_2N), 45.2 (2C, C-7, CH_2N), 46.8 (1C, C-3), 48.5 (1C, CH_2N), 48.8 (1C, CH_2N), 63.2 (1C, C-2), 112.6 (1C, q, Ph, J=3.8 Hz), 116.7 (1C, q, Ph, J=3.8 Hz), 119.3 (1C, Ph), 124.1 (1C, q, CF_3, J=272.6 Hz), 127.3 (3C, Ph), 128.3 (2C, Ph), 128.4 (1C, Ph), 128.5 (2C, Ph), 129.1 (2C, Ph), 129.6 (1C, Ph), 131.4 (1C, q, CCF_3, J=31.8 Hz), 136.6 (1C, Ph), 139.8 (1C, Ph), 150.7 (1C, Ph), 170.0 (1C, C-6), 170.3 (1C, CON). MS *m/z*: 522 (M^+, 100), 292 (12), 202 (9), 196 (47), 130 (39), 120 (7). Anal. Calcd for $C_{30}H_{30}F_3N_3O_2$: C 69.08%, H 5.80%; found: C 68.79%, H 5.67%.

3.5.1.14. *tert*-Butyl-4-((±)-*trans*-1-benzyl-6-oxo-2-phenylpiperidine-3-carbonyl)piperazine-1-carboxylate (5n). White crystals; yield: 58%, mp 134–136 °C. IR ($CHCl_3$): 1680 ($COOC(CH_3)_3$), 1635 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.42 (9H, s, $3CH_3$), 1.81–2.20 (3H, m, 2H-4, CH_2N), 2.27–2.45 (1H, m, CH_2N), 2.53–2.71 (1H, m, H-5), 2.74–2.98 (2H, m, H-5, CH_2N), 2.98–3.13 (2H, m, H-3, CH_2N), 3.14–3.30 (2H, m, CH_2N), 3.31–3.45 (2H, m, H-7, CH_2N), 3.53–3.66 (1H, m, CH_2N), 4.66 (1H, d, H-2, J=8.5 Hz), 5.47 (1H, d, H-7, J=14.8 Hz), 7.01–7.40 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 23.3 (1C, C-4), 28.3 (3C, $3CH_3$), 31.6 (1C, C-5), 41.7 (2C, CH_2N), 45.3 (3C, C-7, $2CH_2N$), 46.8 (1C, C-3), 63.2 (1C, C-2), 80.3 (1C, $OC(CH_3)_3$), 127.2 (2C, Ph), 127.3 (1C, Ph), 128.3 (2C, Ph), 128.4 (3C, Ph), 129.0 (2C, Ph), 136.6 (1C, Ph), 139.7 (1C, Ph), 154.2 (1C, NCOO), 170.0 (1C, C-6), 170.4 (1C, CON). Anal. Calcd for $C_{28}H_{35}N_3O_4$: C 70.42%, H 7.39%; found: C 70.45%, H 7.32%.

3.5.2. (±)-*trans*-1-Benzyl-2-phenyl-3-(piperazine-1-carbonyl)piperidin-6-one (5o). A mixture of **5n** (0.220 g, 0.5 mmol) and F_3CCOOH (0.53 mL, 7 mmol) was sonicated for 15 min. The mixture was neutralized with 10% aq Na_2CO_3 and extracted with ethyl acetate (30 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The resulting oil was recrystallized from ethyl acetate to yield **5o** as white crystals (0.118 g, 68%). Mp 144–146 °C. IR ($CHCl_3$): 1635 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.58 (1H, s, NH), 1.80–2.19 (3H, m, 2H-4, CH_2N), 2.42–2.81 (5H, m, 2H-5, $3CH_2N$), 2.81–2.98 (1H, m, CH_2N), 2.99–3.17 (2H, m, H-3, CH_2N), 3.21–3.35 (1H, m, CH_2N), 3.41 (1H, d, H-7, J=14.8 Hz), 3.48–3.61 (1H, m, CH_2N), 4.68 (1H, d, H-2, J=8.5 Hz), 5.47 (1H, d, H-7, J=14.8 Hz), 7.01–7.41 (10H, m, arom. H). Anal. Calcd for $C_{23}H_{27}N_3O_2$: C 73.18%, H 7.21%; found: C 73.07%, H 6.83%.

3.6. Preparation of methyl (±)-*trans*-1-benzyl-6-oxo-2-phenylpiperidine-3-carboxylate (6)

A mixture of **2** (5.89 g, 19 mmol), methanol (30 mL), and concd H_2SO_4 (2 mL) was refluxed for 2 h. The cooled reaction mixture was poured into water (100 mL). The suspension was neutralized with 10% aq Na_2CO_3 and extracted with ethyl acetate (3×30 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The crude product was recrystallized from ethyl acetate to give **6** as colorless platelets (4.53 g, 77%). Mp 125–127 °C. IR (Nujol): 1730 ($COOCH_3$), 1630 (CON) cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 1.83–2.10 (2H, m, H-4), 2.52–2.66 (1H, m, H-5), 2.69–2.84 (2H, m, H-3, H-5), 3.34 (1H, d, H-7, J=14.7 Hz), 3.48 (3H, s, CH_3), 4.89 (1H, d, H-2, J=3.9 Hz), 5.60 (1H, d, H-7, J=14.7 Hz), 7.10–7.43 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 19.0 (1C, C-4), 29.3 (1C, C-5), 46.2 (1C, C-7), 47.7 (1C, C-3), 51.8 (1C, OCH_3), 60.6 (1C, C-2), 126.5 (2C, Ph), 127.2 (1C, Ph), 127.9 (1C, Ph), 128.2 (2C, Ph), 128.4 (2C, Ph), 128.9 (2C, Ph), 136.6 (1C, Ph), 139.6 (1C, Ph), 169.7 (1C, C-6), 171.9 (1C, COO). Anal. Calcd for $C_{20}H_{21}NO_3$: C 74.28%, H 6.55%; found: C 74.04%, H 6.82%.

3.7. Preparation of (±)-*trans*-1-benzyl-5-(hydroxymethyl)-6-phenylpiperidin-2-one (7)

To a stirred suspension of LiCl (1.78 g, 42 mmol) and KBH_4 (2.27 g, 42 mmol) in THF (10 mL) was added dropwise a solution of **6** (4.53 g, 14 mmol) in THF (20 mL) for 20 min. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure and the residue was poured in water (100 mL). The suspension

was extracted with ethyl acetate (3×30 mL) and the organic phase was dried (Na_2SO_4). After removal of the solvent, the residue was purified by recrystallization from ethyl acetate, to give **7** as white powder (3.71 g, 90%). Mp 134–136 °C. IR (Nujol): 3300 (OH), 1600 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.52–1.70 (1H, m, H-4), 1.82–2.08 (2H, m, H-4, H-5), 2.58 (2H, t, 2H-3, J=6.9 Hz), 3.31 (1H, d, H-7, J=14.6 Hz), 3.41–3.55 (2H, m, CH_2OH), 4.37 (1H, d, H-6, J=4.9 Hz), 5.59 (1H, d, H-7, J=14.6 Hz), 7.09–7.45 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 19.7 (1C, C-4), 29.7 (1C, C-3), 43.3 (1C, C-5), 47.5 (1C, C-7), 61.0 (1C, C-6), 62.6 (1C, CH_2O), 126.9 (2C, Ph), 127.3 (1C, Ph), 127.7 (1C, Ph), 128.2 (2C, Ph), 128.5 (2C, Ph), 128.7 (2C, Ph), 137.1 (1C, Ph), 140.6 (1C, Ph), 170.7 (1C, C-2). Anal. Calcd for $C_{19}H_{21}NO_2$: C 77.26%, H 7.17%; found: C 77.59%, H 7.00%.

3.8. Preparation of ((±)-*trans*-1-benzyl-2-oxo-6-phenylpiperidin-5-yl)methyl 4-methylbenzenesulfonate (8)

p-Toluenesulfonyl chloride (4.41 g, 23.1 mmol) was added in portions, with stirring, to a solution of **7** (3.41 g, 11.6 mmol) in pyridine (30 mL) maintained at −5 °C. The reaction mixture was allowed to warm to room temperature and the stirring continued for further 4 h. The mixture was poured into ice-water and the product was extracted with ethyl acetate (3×30 mL). The organic layer was dried (Na_2SO_4) and evaporated. The resulting oil was recrystallized from ethyl acetate to yield **8** as colorless needles (4.1 g, 79%). Mp 98–100 °C. IR (Nujol): 1630 (CON), 1180 (OSO_2) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.58–1.74 (1H, m, H-4), 1.79–1.95 (1H, m, H-4), 2.08–2.22 (1H, m, H-5), 2.46 (3H, s, CH_3), 2.54 (2H, t, H-3, J=6.8 Hz), 3.28 (1H, d, H-7, J=14.7 Hz), 3.76–3.90 (2H, m, CH_2O), 4.18 (1H, d, H-6, J=6.1 Hz), 5.49 (1H, d, H-7, J=14.7 Hz), 6.93–7.08 (4H, m, arom. H), 7.22–7.35 (8H, m, arom. H), 7.59–7.65 (2H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 20.1 (1C, C-4), 21.6 (1C, CH_3), 29.8 (1C, C-3), 41.0 (1C, C-5), 47.3 (1C, C-7), 60.8 (1C, C-6), 69.5 (1C, CH_2O), 126.9 (2C, Ph), 127.4 (1C, Ph), 127.8 (2C, Ph), 128.1 (1C, Ph), 128.2 (2C, Ph), 128.5 (2C, Ph), 128.9 (2C, Ph), 129.8 (2C, Ph), 132.4 (1C, CS), 136.6 (1C, Ph), 139.4 (1C, Ph), 145.0 (1C, CCH_3), 169.8 (1C, C-2). Anal. Calcd for $C_{26}H_{27}NO_4S$: C 69.44%, H 6.05%; found: C 69.74%, H 6.22%.

3.9. Preparation of (±)-*trans*-5-aminomethylpiperidin-2-ones 9a–g

To a solution of **8** (0.90 g, 2 mmol) in toluene (5 mL) was added the corresponding amine (6 mmol). The reaction mixture was refluxed until the completion of the reaction determined by TLC. The mixture was allowed to cool down to room temperature and ethyl acetate (50 mL) was added. The organic layer was washed with water (4×20 mL) and then dried (Na_2SO_4). The solvent was removed under reduced pressure. The resulting oil was recrystallized or purified by column chromatography and subsequent recrystallization from ethyl acetate–hexane, if not stated otherwise. In this way the following compounds were prepared:

3.9.1. (±)-*trans*-1-Benzyl-6-phenyl-5-((piperidin-1-yl)methyl)piperidin-2-one (9a). White powder; yield: 57%, mp 95–97 °C. IR ($CHCl_3$): 1635 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.24–1.55 (7H, m, H-4, $6CH_2$), 1.81–2.37 (8H, m, 2H-3, H-4, H-5, 2H-8, $2CH_2N$), 2.41–2.63 (2H, m, CH_2N), 3.28 (1H, d, H-7, J=14.4 Hz), 4.64 (1H, s, H-6), 5.61 (1H, d, H-7, J=14.4 Hz), 7.10–7.43 (10H, m, arom. H). Anal. Calcd for $C_{24}H_{30}N_2O$: C 79.52%, H 8.34%; found: C 79.18%, H 8.20%.

3.9.2. (±)-*trans*-1-Benzyl-5-(morpholinomethyl)-6-phenylpiperidin-2-one (9b). White powder; yield: 92%, mp 115–117 °C. IR (Nujol): 1640 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.39–1.54 (1H, m, H-4), 1.86–2.03 (4H, m, H-4, H-5, $2CH_2N$), 2.08–2.26 (2H, m, H-8, CH_2N), 2.26–2.41 (2H, m, H-8, CH_2N), 2.41–2.65 (2H, m, 2H-3), 3.24 (1H, d, H-7, J=14.3 Hz), 3.43–3.60 (4H, m, CH_2O), 4.66 (1H, s, H-6), 5.67 (1H, d, H-7, J=14.3 Hz), 7.10–7.44 (10H, m, arom. H). Anal. Calcd for $C_{23}H_{28}N_2O_2$: C 75.79%, H 7.74%; found: 75.69%, H 7.59%.

3.9.3. *tert*-Butyl-4-(((±)-*trans*-1-benzyl-2-oxo-6-phenylpiperidin-5-yl)methyl)piperazine-1-carboxylate (9c). White powder; yield: 90%, mp 162–163 °C. IR (Nujol): 1680 ($COOC(CH_3)_3$), 1625 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.45 (10H, s, H-4, $9CH_3$), 1.83–2.02 (4H, m, H-4, H-5, $2CH_2N$), 2.05–2.15 (1H, m, H-8), 2.18–2.33 (3H, m, H-8, $2CH_2N$), 2.38–2.63 (2H, m, 2H-3), 3.13–3.31 (5H, m, H-7, $4CH_2N$), 4.65 (1H, s, H-6), 5.67 (1H, d, H-7, J=14.3 Hz), 7.11–7.45 (10H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 19.4 (1C, C-4), 28.3 (4C, C-3, $3CH_3$), 37.6 (1C, C-5), 47.8 (1C, C-7), 52.9 (3C, CH_2N), 58.8 (2C, CH_2N), 61.0 (1C, C-6), 79.4 (1C, $OC(CH_3)_3$), 126.5 (2C, Ph), 127.3 (1C, Ph), 127.4 (1C, Ph), 128.3 (2C, Ph), 128.7 (2C, Ph) 128.8 (2C, Ph), 137.5 (1C, Ph), 141.5 (1C, Ph), 154.6 (1C, NCOO), 170.0 (1C, C-2). Anal. Calcd for $C_{28}H_{37}N_3O_3$: C 72.54%, H 8.04%; found: C 72.32%, H 7.95%.

3.9.4. (±)-*trans*-1-Benzyl-6-phenyl-5-((4-phenylpiperazin-1-yl)methyl)piperidin-2-one (9d). Chromatographic purification (hexane–ethyl acetate=3:2) and recrystallization yielded **9d** as white powder (35%). Mp 117–119 °C. IR (Nujol): 1635 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.40–1.55 (1H, m, H-4), 1.86–2.06 (2H, m, H-4, H-5), 2.08–2.22 (3H, m, H-8, $2CH_2N$), 2.30 (1H, dd, H-8, J=9.8, 12.4 Hz), 2.40–2.65 (4H, m, 2H-3, $2CH_2N$), 2.93–3.09 (4H, m, CH_2N), 3.25 (1H, d, H-7, J=14.3 Hz), 4.68 (1H, s, H-6), 5.66 (1H, d, H-7, J=14.3 Hz), 6.80–6.93 (3H, m, arom. H), 7.12–7.44 (12H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 19.5 (1C, C-4), 28.4 (1C, C-3), 37.7 (1C, C-5), 48.0 (1C, C-7), 49.1 (2C, CH_2N), 53.2 (2C, CH_2N), 58.8 (1C, CH_2N), 61.2 (1C, C-6), 115.9 (2C, Ph), 119.6 (1C, Ph), 126.6 (2C, Ph), 127.3 (1C, Ph), 127.4 (1C, Ph), 128.4 (2C, Ph), 128.7 (2C, Ph) 128.9 (2C, Ph), 129.0 (2C, Ph), 137.6 (1C, Ph), 141.7 (1C, Ph), 151.2 (1C, Ph), 170.1 (1C, C-2). Anal. Calcd for $C_{29}H_{33}N_3O$: C 79.23%, H 7.57%; found: C 79.49%, H 7.83%.

3.9.5. (±)-*trans*-1-Benzyl-5-((4-(4-fluorophenyl)piperazin-1-yl)methyl)-6-phenylpiperidin-2-one (9e). Colorless crystals; yield: 69%, mp 149–151 °C. IR ($CHCl_3$): 1615 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.40–1.55 (1H, m, H-4), 1.86–2.06 (2H, m, H-4, H-5), 2.08–2.23 (3H, m, H-8, $2CH_2N$), 2.31 (1H, dd, H-8, J=9.8, 12.4 Hz), 2.39–2.65 (4H, m, 2H-3, $2CH_2N$), 2.85–3.01 (4H, m, CH_2N), 3.25 (1H, d, H-7, J=14.3 Hz), 4.68 (1H, s, H-6), 5.66 (1H, d,

H-7, J=14.3 Hz), 6.78–7.01 (4H, m, arom. H), 7.12–7.44 (10H, m, arom. H). Anal. Calcd for $C_{29}H_{32}FN_3O$: C 76.12%, H 7.05%; found: C 76.44%, H 7.14%.

3.9.6. (±)-*trans*-1-Benzyl-5-((4-(3-chlorophenyl)piperazin-1-yl)methyl)-6-phenylpiperidin-2-one (9f). Chromatographic purification (hexane–ethyl acetate=3:2) and recrystallization yielded **9f** as white crystals (66%). Mp 155–157 °C. IR ($CHCl_3$): 1615 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.39–1.54 (1H, m, H-4), 1.86–2.05 (2H, m, H-4, H-5), 2.06–2.21 (3H, m, H-8, $2CH_2N$), 2.30 (1H, dd, H-8, J=9.8, 12.4 Hz), 2.39–2.66 (4H, m, 2H-3, $2CH_2N$), 2.91–3.09 (4H, m, CH_2N), 3.25 (1H, d, H-7, J=14.3 Hz), 4.67 (1H, s, H-6), 5.67 (1H, d, H-7, J=14.3 Hz), 6.70–6.84 (3H, m, arom. H), 7.11–7.44 (11H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 19.5 (1C, C-4), 28.4 (1C, C-3), 37.6 (1C, C-5), 47.9 (1C, C-7), 48.6 (2C, CH_2N), 52.9 (2C, CH_2N), 58.7 (1C, CH_2N), 61.2 (1C, C-6), 113.7 (1C, Ph), 115.5 (1C, Ph), 119.1 (1C, Ph), 126.6 (2C, Ph), 127.3 (1C, Ph), 127.4 (1C, Ph), 128.4 (2C, Ph) 128.8 (2C, Ph), 128.9 (2C, Ph), 129.9 (1C, Ph), 134.8 (1C, CCl), 137.6 (1C, Ph), 141.6 (1C, Ph), 152.2 (1C, Ph), 170.1 (1C, C-2). Anal. Calcd for $C_{29}H_{32}ClN_3O$: C 73.48%, H 6.80%; found: C 73.21%, H 6.71%.

3.9.7. (±)-*trans*-1-Benzyl-6-phenyl-5-((4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)methyl)piperidin-2-one (9g). White powder; yield: 79%, mp 151–153 °C (methanol). IR ($CHCl_3$): 1615 (CON) cm^{-1}. ^1H NMR δ ($CDCl_3$): 1.40–1.55 (1H, m, H-4), 1.87–2.06 (2H, m, H-4, H-5), 2.07–2.23 (3H, m, H-8, $2CH_2N$), 2.32 (1H, dd, H-8, J=9.9, 12.3 Hz), 2.40–2.66 (4H, m, 2H-3, $2CH_2N$), 2.96–3.14 (4H, m, CH_2N), 3.25 (1H, d, H-7, J=14.3 Hz), 4.68 (1H, s, H-6), 5.67 (1H, d, H-7, J=14.3 Hz), 6.98–7.44 (14H, m, arom. H). ^{13}C NMR δ ($CDCl_3$): 19.5 (1C, C-4), 28.4 (1C, C-3), 37.7 (1C, C-5), 48.0 (1C, C-7), 48.7 (2C, CH_2N), 53.0 (2C, CH_2N), 58.7 (1C, CH_2N), 61.2 (1C, C-6), 112.0 (1C, q, Ph, J=3.8 Hz), 115.7 (1C, q, Ph, J=3.8 Hz), 118.6 (1C, Ph), 124.3 (1C, q, CF_3, J=272.3 Hz), 126.6 (2C, Ph), 127.4 (1C, Ph), 127.5 (1C, Ph), 128.4 (2C, Ph) 128.8 (2C, Ph), 128.9 (2C, Ph), 129.5 (1C, Ph), 131.3 (1C, q, CCF_3, J=31.7 Hz), 137.6 (1C, Ph), 141.6 (1C, Ph), 151.3 (1C, Ph), 170.1 (1C, C-2). Anal. Calcd for $C_{30}H_{32}F_3N_3O$: C 70.99%, H 6.35%; found: C 71.21%, H 6.63%.

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Synthesis of peptidomimetic-spirostane hybrids via Ugi reaction: a versatile approach for the formation of peptide–steroid conjugates

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Abstract—A general approach towards the preparation of peptide–steroid conjugates has been addressed. Utilizing the Ugi reaction, five peptidomimetic-steroid hybrids were achieved in good to excellent yields from carboxy- and amino-spirostanes and mono-protected α-amino acids. Diverse synthetic routes, specially focused on the formation of secosteroids, were implemented to introduce Ugi-type functionalities at different positions of the steroidal nucleus. This versatile approach is suitable for the formation of stable conjugates of steroids with other biologically relevant molecules.

1. Introduction

The conjugation of steroids to other chemically or biologically relevant molecules represents a valuable strategy to generate new properties in the resulting molecular hybrid. Similarly to the naturally occurring saponins,[1] many synthetic biomolecule-steroid conjugates have shown to possess physico–chemical and biological features arising from the junction of the two molecular entities. E.g., synthetic sugar–steroid conjugates have been synthesized to provide novel amphiphilic molecules capable to interact with phospholipid membranes.[2] Likewise, peptide–steroid conjugates have been employed as synthetic receptors of oligopeptide sequences,[3] as protease-like artificial enzymes,[4] and as mimics of the natural cationic peptide antibiotics.[5] Additionally, the attachment of detectable labels (e.g., fluorescent) to currently used steroidal hormones is an important approach in the development of clinical immunoassays. Indeed, this objective requires the production of novel reagents and methodologies toward improvements in the conjugation process.[6]

The formation of a chemically and metabolically stable linkage between a steroid and a biomolecule, a bioactive compound or a detectable tag is a crucial step for the potential applicability of the conjugate. By far the most commonly used chemical linkages in steroidal conjugates (i.e., glycosidic, amide, and ester) present an undeniable drawback: the sensitivity towards chemical or enzymatic hydrolysis. Another more stable and thereby widely exploited linkage, such as the oxime bond, is generally fixed to the presence of oxo-functions at the steroid.[6,7]

Herein we report on the use of the Ugi four-component reaction (Ugi-4CR) as a versatile approach towards the formation of peptide–steroid conjugates. The Ugi-4CR is the one-pot condensation of a primary amine, an oxo-component, a carboxylic acid, and an isocyanide to afford an *N*-substituted dipeptide backbone[8] (Scheme 1). This and other related scaffolds arising from the Ugi-4CR, or its variants, have found relevance in medicinal chemistry in the last decades.[9] Particularly interesting are oligomeric peptidomimetics containing *N*-substituted amide bonds (i.e., peptoids), which have shown a wide variety of biological applications[10] and a high resistance towards proteolytic degradation.[11]

The Ugi-4CR has been recently utilized to assemble very large cholane-based macrocycles tethered by highly diverse peptoid moieties.[12] However, a general conjugation process of biomolecules to steroids utilizing this approach has not been previously addressed. Apart from the accessible structural diversity arising from the possibility of utilizing carboxy, amino, isocyano, or oxo-steroids as building blocks, this strategy provides a very straightforward method to access stable peptide–steroid conjugates with potential biological activities.

Keywords: Steroids; Spirostanes; Steroid conjugates; Multicomponent reactions; Ugi reaction.
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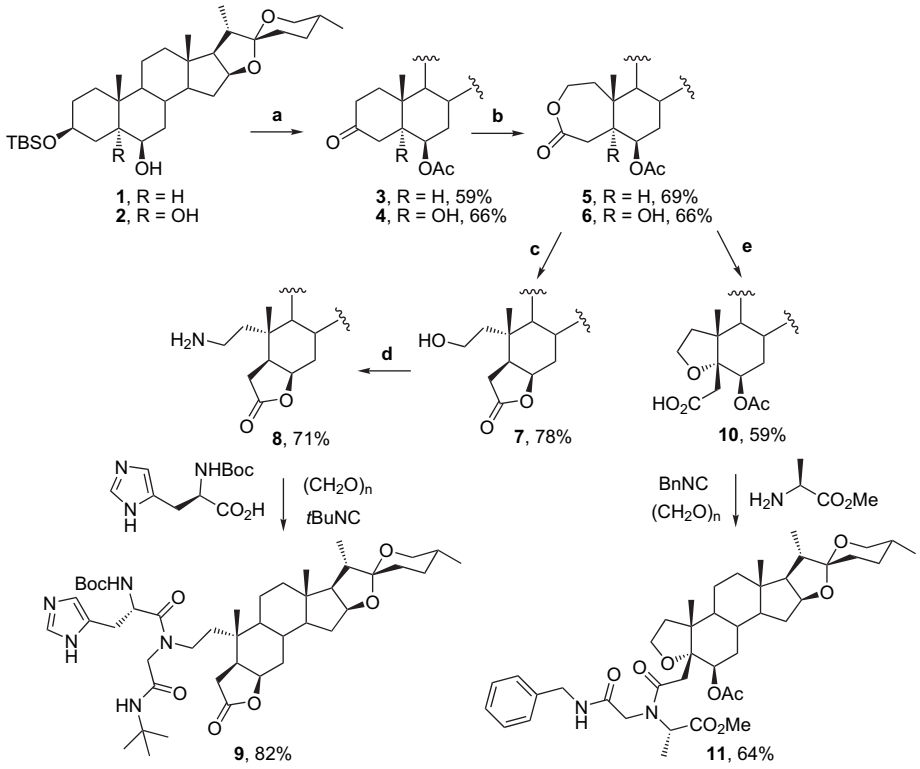


Scheme 1. Reagents and conditions: (a) i: Ac_2O, Py; ii: TBAF, THF; iii: PCC, CH_2Cl_2; (b) *m*CPBA/CH_2Cl_2; (c) from **5**, i: 5% KOH, MeOH, reflux; ii: Dowex 50W, set pH 3; (d) i: MsCl, CH_2Cl_2, Et_3N; ii: NaN_3, DMPU; iii: H_2, Lindlar catalyst; (e) from **6**; i: H_2SO_4, MeOH; ii: LiOH, THF/H_2O. TBAF=Tetrabutylammonium fluoride; DMPU=1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidonone.

2. Results and discussion

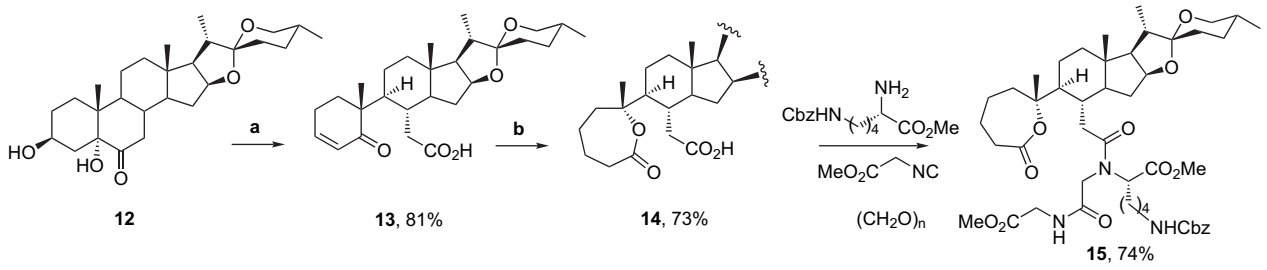
This article focuses on the conjugation process of interesting α-amino acids at different positions of the spirostane skeleton. However, we also aim to illustrate that this approach is suitable for introducing oligopeptides either by their C- or N-terminus at varied positions of the steroidal nucleus. Therefore, we concentrated on functionalizing steroids with carboxy and amino functions and taking advantage of the wide set of commercially available isocyanides (isonitriles) that can be employed to incorporate aromatic, aliphatic or other amino acid into the final hybrid compound. A key feature of these synthetic routes is the avoidance of the established succinate moiety as a source of carboxy groups. Instead, we concentrated on developing highly practical pathways that easily allow incorporating the above mentioned functional groups for the Ugi-4CR. These approaches are specially focused on secosteroid syntheses via lactone-ring opening and further activation of the Ugi-type functionality.

Spirostanes were chosen because of various features that make them amenable starting material to produce bioactive compounds. Derivatives of these sapogenins have exhibited a variety of biological activities depending on the functionalization pattern incorporated in their structures, e.g., ecdysteroid[13] and brassinosteroid[14] type activities have been reported upon introduction on rings A and B of functionalities typical of these natural products. Likewise, the general interest on these widely available steroids has been increased due to their unquestionable potential to access analogues[15] of cephalostatins[16] and ritterazines.[17]

Scheme 1 summarizes the synthesis of peptidomimetic-spirostane hybrids functionalized on ring A. Baeyer–Villiger oxidation of 3-oxosteroids **3** and **4** gave access to the corresponding lactones, which upon ring opening processes afforded varied secosteroids properly functionalized for the conjugation process. Thus, treatment of lactone **5** with basic conditions followed by acidification to pH 3 rendered the γ-lactone **7** in 78% yield after chromatography purification. The experimental condition were established to favor the kinetic product (5-exo-trig ring closing), thereby avoiding the regeneration of the ε-lactone. Further replacement of the primary hydroxyl group by an amino function allowed accomplishing the conjugation of the *N*-Boc-protected α-amino acid L-histidine in 82% yield by using *tert*-butylisocyanide. It must be mentioned that paraformaldehyde was always employed as the oxo-component to avoid the stereoisomers formation during the conjugation process.

The 5α-hydroxylated lactone **6** allowed an alternative pathway, in which an acid-catalyzed methanolysis led to dehydration at C-5 and subsequent nucleophilic attack of the primary hydroxyl at C-2 to yield a cyclic ether. Cleavage of the methyl ester function on the later intermediate furnished the final steroidal acid **10**. The *C*-protected α-amino acid L-alanine and benzylisocyanide were employed to achieve the formation of conjugate **11** in 64% yield. The lower isolated yield of this compound compared to **9** may be due to the less accessible character of the β-face directed carboxy function at C-3 because of typical steric effect.

As depicted in Scheme 2, the introduction on ring B of Ugi-type functionalities is accomplished by treatment of

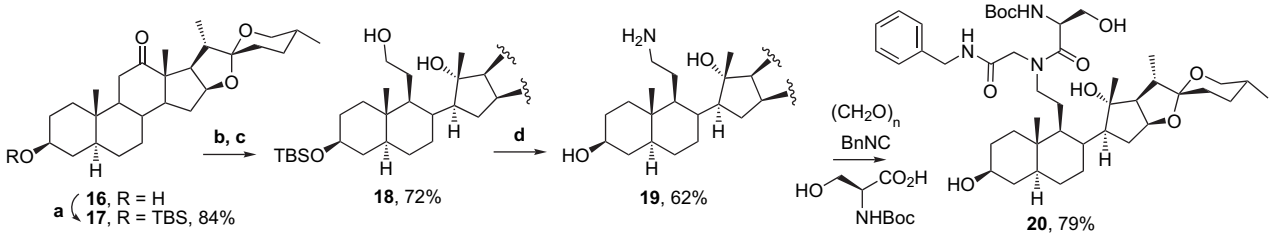


Scheme 2. Reagents and conditions: (a) i: Ac_2O, Py; ii: Jones, 60 °C; (b) i: H_2, Pd/C 10%; ii: *m*CPBA; CH_2Cl_2.

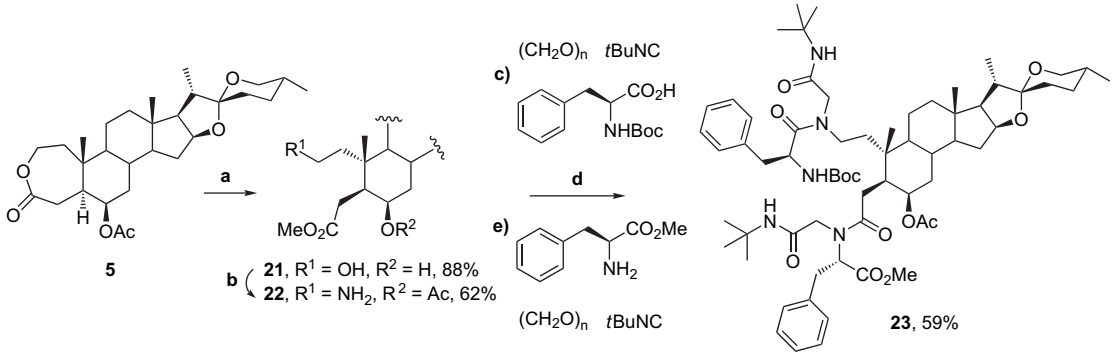
ketol **12** with strong oxidative conditions to allow C–C bond cleavage. Secospirostane **13** was then submitted to palladium-catalyzed hydrogenation and subsequent Baeyer–Villiger oxidation of the oxo-function at C-5 to afford the A-ring lactone **14** properly functionalized for the further Ugi-4CR. Thus, the carboxy functionality at C-6 was employed to achieve the conjugate **15** in 74% yield by using ε-*N*-Cbz-L-lysine methyl ester as the amino component and methyl isocyanoacetate. The use of the polyfunctional amino acid L-lysine and a functionalized isocyanide makes of the dipeptide–secospirostane hybrid **15** an amenable scaffold for further peptide coupling as well as for introducing other biologically relevant moieties. Indeed, this was possible due to the multicomponent nature of the Ugi-4CR, which easily allows the incorporation of multiple functionalized building blocks in a one-pot procedure.

To accomplish the formation of steroid conjugates functionalized on ring C, we turned to the use of hecogenin as starting material of the synthetic planning shown in Scheme 3. Protection of the hydroxyl at C-3 with *tert*-butyldimethylsilane (TBS) enabled further functionalizations on ring C without affecting the ring A. Once more, the Baeyer–Villiger oxidation of the oxo-function at C-12 allowed accessing Ugi-type functionalities via reductive ring opening with $LiAlH_4$ and subsequent replacement of the primary hydroxyl at C-12 by an amino group. Benzylisocyanide was then employed in the conjugation process of *N*-Boc-protected L-serine to the aminosteroid **19**, to afford the dipeptide–secospirostane hybrid **20** in 79% yield.

Having established the value of this strategy, we focused on extending it towards a double conjugation procedure of natural α-amino acids by either its amino or carboxy groups. In this sense, the *C*-protected secosteroidal amino acid **22** was produced by acid-catalyzed lactone-ring opening of intermediate **5** followed by incorporation of the required amino group at C-2 using a standard protocol. A sequential Ugi-4CR/deprotection/Ugi-4CR approach enabled the easy incorporation of two L-phenylalanine units to obtain the conjugate **23** in 61% yield (Scheme 4). Indeed, the use of both *C*- and *N*-protected α-amino acids as the carboxylic and amino components of the reaction sequence, respectively, confirms the versatile character of this methodology to produce highly functionalized conjugates with very low synthetic cost.



Scheme 3. Reagents and conditions: (a) TBSCl, imidazole, DMF; (b) *m*CPBA, CH_2Cl_2; (c) $LiAlH_4$, THF; (d) i: MsCl, CH_2Cl_2, Et_3N; ii: NaN_3, DMPU; iii: H_2, Lindlar catalyst; iv: TBAF, THF, TBS=*tert*-Butyldimethylsilane.



Scheme 4. Reagents and conditions: (a) i: H_2SO_4, MeOH, reflux; ii: NaOMe, MeOH; (b) i: MsCl, CH_2Cl_2, Et_3N; ii: NaN_3, DMPU; iii: Ac_2O, Py; iv: H_2, Lindlar catalyst; (d) LiOH, THF/H_2O.

3. Conclusions

As it has been demonstrated, various proteogenic amino acids could be conjugated to spirostanes via the Ugi-4CR to form peptidomimetic–steroid hybrids in yields ranging from 60% to 85%. Indeed, the high value of this synthetic approach lies on its potential applicability towards a general conjugation strategy of varied types of molecules with steroids. The multicomponent character of the reaction allowed utilizing the steroid and the α-amino acids either as the amino or the carboxylic component.

4. Experimental

4.1. General

Melting points were determined on a Stuart Scientific apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker ACF-250 spectrometer at 250.13 MHz and 62.9 MHz for ^{1}H and ^{13}C, respectively, using TMS as an internal standard. The high resolution ESI mass spectra were obtained from a Bruker Apex 70e Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an Infinity™ cell, a 7.0 tesla superconducting magnet. Reactions were monitored by thin-layer chromatography on precoated plates with silica gel (Merck) and spots were visualized with a 1% w/v spray of vanillin in perchloric acid and subsequent heating. 'Usual work-up' refers to dilution with an organic solvent, washing the extract consecutively with 5% HCl and/or 5% $NaHCO_3$, and brine, drying over anhydrous Na_2SO_4 and removal of the solvent under reduced pressure. The solid compounds were recrystallized from selected solvents for the melting point measurements. Flash column chromatography was performed on silica gel 60 (Merck, >230 mesh). Solvents were purified and dried according to standard procedures. Compounds **1**, **2**, and **12** were obtained from the widely available diosgenin as described in Ref. 13.

4.1.1. (25*R*)-6β-Acetoxy-5α-spirostan-3-one (3). Ac_2O (6 mL, 64 mmol) was added to a solution of compound **1** (5.8 g, 10.2 mmol) in anhydrous pyridine (60 mL) and the reaction mixture was stirred at room temperature for 18 h. The mixture was poured into 400 mL of cold water and the solid was filtered under reduced pressure and washed several times with water. The resulting crude product was dried and dissolved in anhydrous THF (60 mL). Tetrabutylammonium fluoride (TBAF) (4 mL, 1 M in THF) was added to the solution and the reaction mixture was stirred under nitrogen atmosphere for 6 h. The usual work-up (Et_2O) yielded a crude product, which was dissolved in CH_2Cl_2 (150 mL) and added to a suspension of pyridinium chlorochromate (PCC) (3.8 g, 17.6 mmol) in CH_2Cl_2 (200 mL) at 0 °C. The reaction mixture was stirred for 1 h at room temperature and then filtered through a pad of alumina. The solution was evaporated under reduced pressure and the crude product was purified by flash column chromatography (hexane/AcOEt, 4:1) to afford the ketone **3** (2.72 g, 59%). Mp (MeOH): 204–206 °C. ^{1}H NMR ($CDCl_3$): δ=0.79 (d, 3H, J=6.4 Hz, H-21); 0.81 (s, 3H, H-18); 0.96 (d, 3H, J=6.3 Hz, H-27); 1.35 (s, 3H, H-19); 2.05 (s, 3H, CH_3CO); 3.36 (t, 1H, J=10.8 Hz, H-26ax); 3.45 (dd, 1H, J=4.1/10.8 Hz, H-26eq); 4.40 (m, 1H, H-16α); 4.96 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=37.0 (C-1); 37.9 (C-2); 211.3 (C-3); 44.2 (C-4); 49.1 (C-5); 72.6 (C-6); 36.3 (C-7); 30.4 (C-8); 53.8 (C-9); 35.4 (C-10); 20.9 (C-11); 39.6 (C-12); 40.5 (C-13); 55.5 (C-14); 31.4 (C-15); 80.6 (C-16); 62.0 (C-17); 16.5 (C-18); 15.2 (C-19); 41.6 (C-20); 14.5 (C-21); 109.3 (C-22); 31.3 (C-23); 28.7 (C-24); 30.2 (C-25); 66.8 (C-26); 17.1 (C-27); 21.4 (*C*H$_3$CO); 170.2 (CH$_3$*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 495.3084 [M+Na]$^+$ (Calculated for $C_{29}H_{44}NaO_5$: 495.3087).

4.1.2. (25*R*)-6β-Acetoxy-5-hydroxy-5α-spirostan-3-one (4). Diol **2** (3.0 g, 7.16 mmol) was treated in a similar way as described in Section 4.1.1 to give the ketol **4** (1.53 g, 66 %). Mp (MeOH): 212–213 °C. ^{1}H NMR ($CDCl_3$): δ=0.79 (d, 3H, J=6.2 Hz, H-21); 0.82 (s, 3H, H-18); 0.97 (d, 3H, J=6.2 Hz, H-27); 1.39 (s, 3H, H-19); 2.09 (s, 3H, CH_3CO); 3.37 (t, 1H, J=10.6 Hz, H-26ax); 3.45 (dd, 1H, J=4.1/10.8 Hz, H-26eq); 4.41 (m, 1H, H-16α); 4.73 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=31.6 (C-1); 37.7 (C-2); 211 (C-3); 48.4 (C-4); 76.9 (C-5); 75.6 (C-6); 33.6 (C-7); 30.1 (C-8); 45.2 (C-9); 38.9 (C-10); 20.9 (C-11); 39.8 (C-12); 40.5 (C-13); 55.4 (C-14); 31.4 (C-15); 80.6 (C-16); 62.0 (C-17); 16.5 (C-18); 15.9 (C-19); 41.6 (C-20); 14.5 (C-21); 109.2 (C-22); 31.3 (C-23); 28.7 (C-24); 30.2 (C-25); 66.8 (C-26); 17.1 (C-27); 21.4 (*C*H$_3$CO); 170.0 (CH$_3$*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 511.3038 [M+Na]$^+$ (Calculated for $C_{29}H_{44}NaO_6$: 511.3036).

4.1.3. (25*R*)-A-Homo-2a-oxa-6β-acetoxy-5α-spirostan-3-one (5). A solution of *m*CPBA (2.0 g, 11.6 mmol) in CH_2Cl_2 (100 mL) was added to a stirred solution of ketone **3** (2.2 g, 4.6 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The reaction was allowed to reach room temperature and stirred for 4 h in the dark. The mixture was diluted with $CHCl_3$ (150 mL) and washed sequentially with solutions of 10% Na_2SO_3 (2×200 mL) and 10% $NaHCO_3$ (2×200 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting crude product was purified by flash chromatography (hexane/EtOAc, 5:1) to yield the lactone **5** (1.57 g, 69%). Mp (EtOH): 202–205 °C. ^{1}H NMR ($CDCl_3$): δ=0.77 (d, 3H, J=6.3 Hz, H-27); 0.79 (s, 3H, H-18); 0.97 (d, 3H, J=6.6 Hz, H-21); 1.26 (s, 3H, H-19); 2.03 (s, 3H, CH_3CO); 3.36 (t, 1H, J=10.8 Hz, H-26ax); 3.47 (dd, 1H, J=4.2/10.9 Hz, H-26eq); 4.21 (m, 1H, H-2α); 4.39 (m, 1H, H-2β); 4.41 (m, 1H, H-16α); 4.94 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=41.0 (C-1); 63.9 (C-2); 175.0 (C-3); 29.0 (C-4); 52.1 (C-5); 72.1 (C-6); 36.5 (C-7); 30.0 (C-8); 53.3 (C-9); 35.7 (C-10); 21.0 (C-11); 39.6 (C-12); 40.3 (C-13); 55.4 (C-14); 31.5 (C-15); 80.7 (C-16); 62.1 (C-17); 16.5 (C-18); 15.1 (C-19); 41.6 (C-20); 14.5 (C-21); 109.3 (C-22); 31.3 (C-23); 28.7 (C-24); 30.2 (C-25); 66.8 (C-26); 17.2 (C-27); 170.1 (CH$_3$*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 511.3039 [M+Na]$^+$ (Calculated for $C_{29}H_{44}NaO_6$: 511.3036).

4.1.4. (25*R*)-A-Homo-2a-oxa-6β-acetoxy-5-hydroxy-5α-spirostan-3-one (6). Ketol **4** (1.5 g, 3.1 mmol) was treated in a similar way as described in Section 4.1.3 to give the lactone **6** (1.02 g, 66%). Mp (MeOH): 218–220 °C. ^{1}H NMR ($CDCl_3$): δ=0.78 (d, 3H, J=6.3 Hz, H-27); 0.79 (s, 3H, H-18); 0.97 (d, 3H, J=6.4 Hz, H-21); 1.25 (s, 3H, H-19); 2.08 (s, 3H, CH_3CO); 3.37 (t, 1H, J=10.9 Hz, H-26ax);

3.47 (dd, 1H, *J*=4.2/10.9 Hz, H-26eq); 4.19 (m, 1H, H-2α); 4.38 (m, 1H, H-2β); 4.40 (m, 1H, H-16α); 4.93 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=36.2 (C-1); 65.1 (C-2); 169.8 (C-3); 42.9 (C-4); 76.6 (C-5); 72.1 (C-6); 31.7 (C-7); 29.7 (C-8); 44.7 (C-9); 41.5 (C-10); 20.9 (C-11); 39.7 (C-12); 40.3 (C-13); 55.4 (C-14); 31.5 (C-15); 80.7 (C-16); 61.9 (C-17); 16.5 (C-18); 15.8 (C-19); 41.6 (C-20); 14.5 (C-21); 109.3 (C-22); 31.3 (C-23); 28.7 (C-24); 30.2 (C-25); 66.8 (C-26); 17.1(C-27); 170.0 (CH_3CO). HRMS (ESI-FT-ICR) *m/z*: 527.2987 $[M+Na]^+$ (Calculated for $C_{29}H_{44}NaO_7$: 527.2985).

4.1.5. (25*R*)-2,3-Seco-2-hydroxy-3-carboxy-5α-spirostan-6β-yl (7). Lactone **5** (1.5 g, 3.1 mmol) was dissolved in a 2% solution of KOH in MeOH (70 mL). The reaction mixture was refluxed for 3 h, then acidified with resin Dowex 50W (H^+ form) until pH 3 and stirred at 0 °C for 2 h. The resin was then filtered off and the filtrate was evaporated under reduced pressure to give a white crude product. Recrystallization from AcOEt afforded the pure γ-lactone **7** (1.17 g, 78%). Mp (AcOEt): 202–203 °C. ^{1}H NMR ($CDCl_3$): δ=0.75 (s, 3H, H-18); 0.77 (d, 3H, *J*=6.3 Hz, H-27); 0.91 (s, 3H, H-19); 0.94 (d, 3H, *J*=6.8 Hz, H-21); 3.35 (t, 1H, *J*=11.0 Hz, H-26ax); 3.46 (dd, 1H, *J*=4.1/10.9 Hz, H-26eq); 3.65 (m, 2H, H-2); 4.39 (m, 1H, H-16); 4.53 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=35.0 (C-1); 58.4 (C-2); 178.1 (C-3); 42.2 (C-4); 46.2 (C-5); 79.9 (C-6); 33.1 (C-7); 30.3 (C-8); 41.3 (C-9); 36.8 (C-10); 20.9 (C-11); 39.6 (C-12); 40.2 (C-13); 56.5(C-14); 31.6 (C-15); 80.6 (C-16); 62.0 (C-17); 16.3 (C-18); 17.4 (C-19); 41.7 (C-20); 14.5 (C-21); 109.4 (C-22); 31.4 (C-23); 28.8 (C-24); 29.0 (C-25); 66.9 (C-26); 17.1 (C-27). HRMS (ESI-FT-ICR) *m/z*: 469.2932 $[M+Na]^+$ (Calculated for $C_{27}H_{42}NaO_5$: 469.2930).

4.1.6. (25*R*)-2,3-Seco-2-amino-3-carboxy-5α-spirostan-6β-yl (8). Mesyl chloride (0.44 mL, 3.7 mmol) was added dropwise at 0 °C to a solution of lactone **7** (1.1 g, 2.4 mmol) in anhydrous CH_2Cl_2 (80 mL) and Et_3N (3.8 mL, 30 mmol). The reaction mixture was stirred at 0 °C for 1 h and then washed with brine (2×100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduce pressure. The resulting crude product was dissolved in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidonone (DMPU, 30 mL) and the solution was treated with NaN_3 (403 mg, 7.2 mmol). The reaction mixture was stirred vigorously under an argon atmosphere at 50 °C for 48 h and then, the usual work-up (EtOAc) yielded a crude product, which was dissolved in 150 mL of absolute EtOH. Lindlar catalyst (350 mg) was added to the solution and the mixture was treated successively with hydrogen and vacuum, and finally stirred under hydrogen atmosphere for 36 h. The catalyst was removed by filtration and the resulting solution was evaporated under reduced pressure to give a crude product. Flash column chromatography purification ($CHCl_3$/MeOH/Et_3N, 10:1:0.5) furnished the amine **8** (758 mg, 71%). Mp (MeOH): 195–197 °C. ^{1}H NMR ($CDCl_3$): δ=0.76 (s, 3H, H-18); 0.77 (d, 3H, *J*=6.4 Hz, H-27); 0.90 (s, 3H, H-19); 0.94 (d, 3H, *J*=6.8 Hz, H-21); 3.36 (t, 1H, *J*=11.1 Hz, H-26ax); 3.46 (dd, 1H, *J*=4.2/11.0 Hz, H-26eq); 3.54 (m, 2H, H-2); 4.41 (m, 1H, H-16); 4.53 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=35.1 (C-1); 57.9 (C-2); 178.0 (C-3); 42.3 (C-4); 46.2 (C-5); 79.8 (C-6); 33.2 (C-7); 30.3 (C-8); 41.4 (C-9); 36.8 (C-10); 20.9 (C-11); 39.8 (C-12); 40.2 (C-13); 56.6 (C-14); 31.6 (C-15); 80.6 (C-16); 62.0 (C-17); 16.3 (C-18); 17.2 (C-19); 41.7 (C-20); 14.5 (C-21); 109.4 (C-22); 31.4 (C-23); 28.8 (C-24); 29.1 (C-25); 66.9 (C-26); 17.1 (C-27). HRMS (ESI-FT-ICR) *m/z*: 446.3272 $[M+H]^+$ (Calculated for $C_{27}H_{44}NO_4$: 446.3270).

4.1.7. Peptide-steroid conjugate 9. A solution of amine **8** (700 mg, 1.6 mmol) and paraformaldehyde (48 mg, 1.6 mmol) in MeOH (60 mL) were stirred at room temperature for 1 h to accomplish the formation of the corresponding imine. *N*-Boc-L-histidine (402 mg, 1.6 mmol) and *tert*-butylisocyanide (0.18 mL, 1.6 mmol) were then added and the reaction mixture was stirred for 6 h at room temperature. The solution was concentrated under reduced pressure and then, the usual work-up ($CHCl_3$) yielded a crude product. Flash column chromatography purification ($CHCl_3$/MeOH, 20:1) furnished the conjugate **9** (1.01 g, 82%). Mp (AcOEt): 226–227 °C. ^{1}H NMR ($CDCl_3$): δ=0.77 (s, 3H, H-18); 0.77 (d, 3H, *J*=6.5 Hz, H-27); 0.94 (s, 3H, H-19); 0.93 (d, 3H, *J*=6.8 Hz, H-21); 1.32 (s, 9H, $(CH_3)_3CNH$); 1.43 (s, 9H, $(CH_3)_3C$); 3.36 (t, 1H, *J*=11.0 Hz, H-26ax); 3.46 (dd, 1H, *J*=4.1/11.0 Hz, H-26eq); 3.57 (m, 2H, H-2); 4.40 (m, 1H, H-16); 3.72 (s, 3H, OCH_3); 4.02–3.94 (m, 6H, CH_2); 4.25–4.18 (m, 2H, CH_2); 4.45 (m, 1H, NC*H*); 4.54 (m, 1H, H-6α); 6.74 (m, 1H, C*H*-imidazole); 7.32 (m, 1H, C*H*-imidazole). ^{13}C NMR ($CDCl_3$): δ=14.5 (CH_3); 16.3 (CH_3); 17.1 (CH_3); 17.2 (CH_3); 20.9 (CH_2); 28.8 (CH_2); 28.5 (CH_3); 28.3 (CH_3); 29.1 (*C*H); 30.3 (*C*H); 31.4 (CH_2); 31.6 (CH_2); 33.2 (CH_2); 35.1 (CH_2); 36.8 (*C*); 39.8 (CH_2); 40.2 (*C*); 41.4 (*C*H); 41.7 (*C*H); 42.3 (CH_2); 45.6 (CH_2); 46.2 (*C*H); 49.6 (CH_2); 50.6 (CH_2); 51.8 (CH_3); 56.6 (*C*H); 57.9 (CH_2); 62.0 (*C*H); 66.9 (CH_2); 79.6 (*C*); 79.8 (*C*H); 80.6 (*C*H); 109.4 (*C*); 155.7 (*C*O); 168.5 (*C*O); 169.6 (*C*O); 178.0 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 818.5044 $[M+Na]^+$ (Calculated for $C_{44}H_{69}NaN_5O_8$: 818.5045).

4.1.8. (25*R*)-2,3-Seco-6β-acetoxy-2(5)-oxa-5α-spirostan-3-oic acid (10). Fuming H_2SO_4 (2 mL) was added to a solution of lactone **6** (1.0 g, 1.9 mmol) in MeOH (50 mL) and the reaction mixture was stirred at reflux with the appearance of a precipitate after 10 min. The solid was then filtered under reduced pressure, washed with cold MeOH (2×20 mL) and dissolved in a mixture of THF/H_2O (2:1, 300 mL). LiOH (210 mg, 5.0 mmol) was added and the reaction mixture was stirred at 0 °C for 2 h and then acidified with aqueous 10% $NaHSO_4$ to pH 3. The usual work-up (AcOEt) gave a product, which was purified by flash column chromatography ($CHCl_3$/MeOH/AcOH, 15:1:0.1) to afford the acid **10** (583 mg, 59%). Mp (heptane/AcOEt): 255–256 °C. ^{1}H NMR ($CDCl_3$): δ=0.77 (d, 3H, *J*=6.8 Hz, H-21); 0.77 (s, 3H, H-18); 0.95 (d, 3H, *J*=6.9 Hz, H-27); 0.97 (s, 3H, H-19); 2.05 (s, 3H, CH_3CO); 2.41 (d, 1H, *J*=13.1 Hz, H-4); 2.63 (d, 1H, *J*=13.1 Hz, H-4); 3.35 (t, 1H, *J*=10.9 Hz, H-26ax); 3.44 (dd, 1H, *J*=4.1/10.8 Hz, H-26eq); 3.90 (m, 2H, H-2); 4.36 (m, 1H, H-16α); 5.21 (m, 1H, H-6α). ^{13}C NMR ($CDCl_3$): δ=37.9 (C-1); 63.9 (C-2); 169.3 (C-3); 36.4 (C-4); 83.8 (C-5); 70.5 (C-6); 30.8 (C-7); 29.3 (C-8); 45.7 (C-9); 46.6 (C-10); 22.7 (C-11); 40.6 (C-12); 39.8 (C-13); 56.7 (C-14); 31.6 (C-15); 80.7 (C-16); 62.2 (C-17); 16.4 (C-18); 15.1 (C-19); 41.6 (C-20); 14.5 (C-21); 109.4 (C-22); 31.5 (C-23); 28.6 (C-24); 30.4

(C-25); 66.7 (C-26); 17.2 (C-27); 169.7 (CH_3CO). HRMS (ESI-FT-ICR) *m/z*: 503.3009 $[M-H]^-$ (Calculated for $C_{29}H_{43}O_7$: 503.3008).

4.1.9. Peptide-steroid conjugate 11. A solution of L-alanine methyl ester hydrochloride (139 mg, 1.0 mmol), paraformaldehyde (30 mg, 1.0 mmol), and triethylamine (0.14 mL, 1.0 mmol) in MeOH (60 mL) were stirred at room temperature for 1 h to accomplish the formation of the corresponding imine. Acid **10** (500 mg, 1.0 mmol) and benzylisocyanide (0.12 mL, 1.0 mmol) were then added and the reaction mixture was stirred for 10 h at room temperature. The solution was concentrated under reduced pressure and then, the usual work-up ($CHCl_3$) yielded a crude product. Flash column chromatography purification ($CHCl_3$/MeOH, 20:1) furnished the pure conjugate **11** (471 mg, 64%). Mp (MeOH): 211–214 °C. ^{1}H NMR ($CDCl_3$): δ=0.77 (d, 3H, *J*=6.8 Hz, H-21); 0.77 (s, 3H, H-18); 0.94 (d, 3H, *J*=6.7 Hz, H-27); 1.14 (s, 3H, H-19); 1.53 (d, 3H, *J*=7.2 Hz, (CH_3)CHN); 2.03 (s, 3H, CH_3CO); 3.35 (t, 1H, *J*=10.8 Hz, H-26ax); 3.44 (dd, 1H, *J*=4.1/10.9 Hz, H-26eq); 3.83 (s, 3H, OCH_3); 3.92 (m, 2H, H-2); 4.15–4.08 (m, 2H, NC*H*); 4.29–4.26 (m, 2H, CH_2); 4.39 (m, 1H, H-16α); 4.50–4.56 (m, 2H, CH_2); 5.23 (m, 1H, H-6α); 7.13 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ=14.5 (*C*H_3); 15.1 (*C*H_3); 16.4 (*C*H_3); 17.2 (*C*H_3); 22.7 (*C*H_2); 28.6 (*C*H_2); 29.3 (*C*H); 30.4 (*C*H); 30.6 (*C*H_3); 30.8 (*C*H_2); 31.5 (*C*H_2); 31.6 (*C*H_2); 36.4 (*C*H_2); 37.9 (*C*H_2); 39.8 (*C*); 40.6 (*C*H_2); 41.6 (*C*H); 44.8 (*C*H_2); 45.7 (*C*H); 46.6 (*C*); 53.7 (*C*H_3); 55.7 (*C*H_2); 56.7 (*C*H); 62.2 (*C*H); 63.9 (*C*H_2); 66.7 (*C*H_2); 70.5 (*C*H); 80.7 (*C*H); 83.8 (*C*); 109.4 (*C*); 126.8 (*C*H); 127.3 (*C*H); 128.1 (*C*H); 131.2 (*C*H); 168.9 (*C*O); 169.6 (*C*O); 169.9 (*C*O); 174.2 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 759.4199 $[M+Na]^+$ (Calculated for $C_{42}H_{60}NaN_2O_9$: 759.4197).

4.1.10. (25*R*)-5,6-Seco-5-oxo-3-spirostan-6-oic acid (13). Ketol **12** (2.5 g, 5.6 mmol) was dissolved in anhydrous pyridine (50 mL) and treated with Ac_2O (3 mL, 32 mmol) in a similar way as described in Section 4.1.1. The resulting crude product was dissolved in acetone (100 mL) and treated with 5 mL of Jones reagent. The reaction mixture was stirred at reflux for 2 h, concentrated until half volume and poured into 400 mL of cold water. The solid was filtered under reduced pressure, washed several times with water and then recrystallized from MeOH/H_2O (60 mL, 2:1) to afford the pure ketoacid **13** (2.03 g, 81%). Mp (MeOH/H_2O): 182–185 °C. ^{1}H NMR ($CDCl_3$): δ=0.77 (d, 3H, *J*=6.4 Hz, H-27); 0.80 (s, 3H, H-18); 0.95 (d, 3H, *J*=6.7 Hz, H-21); 1.11 (s, 3H, H-19); 3.33 (t, 1H, *J*=10.8 Hz, H-26ax); 3.46 (dd, 1H, *J*=10.8/4.0 Hz, H-26eq); 4.34 (m, 1H, H-16α); 5.84 (m, 1H, *J*=10.0/1.4 Hz, H-4); 6.75 (m, 1H, *J*=10.0 Hz, H-3). ^{13}C NMR ($CDCl_3$): δ=24.7 (C-1); 39.9 (C-2); 146.6 (C-3); 128.5 (C-4); 207.9 (C-5); 172.4 (C-6); 40.4 (C-7); 41.8 (C-8); 51.3 (C-9); 35.6 (C-10); 35.5 (C-11); 36.1 (C-12); 48.0 (C-13); 55.1 (C-14); 31.4 (C-15); 80.2 (C-16); 61.4 (C-17); 16.2 (C-18); 18.2 (C-19); 42.2 (C-20); 14.6 (C-21); 109.0 (C-22); 31.6 (C-23); 28.8 (C-24); 30.3 (C-25); 66.8 (C-26); 17.2 (C-27). HRMS (ESI-FT-ICR) *m/z*: 443.2797 $[M-H]^-$ (Calculated for $C_{27}H_{39}O_5$: 443.2796).

4.1.11. (25*R*)-5,6-Seco-A-homo-5(10)-oxa-5-oxo-spirostan-6-oic acid (14). Pd/C 10% (800 mg) was added to a solution of ketoacid **13** (2.0 g, 4.5 mmol) in absolute EtOH (100 mL). The reaction mixture was treated successively with hydrogen and vacuum and finally stirred under hydrogen atmosphere for 24 h. The catalyst was removed by filtration and the resulting solution was evaporated under reduced pressure to give a crude product. This product was dissolved in CH_2Cl_2 (80 mL) and treated with *m*CPBA (1.55 g, 9.0 mmol) in a similar way as described in Section 4.1.3 to give the lactone **14** (1.54 g, 73%). Mp (MeOH): 199–200 °C. ^{1}H NMR ($CDCl_3$): δ=0.78 (d, 3H, *J*=6.2 Hz, H-27); 0.81 (s, 3H, H-18); 0.96 (d, 3H, *J*=6.9 Hz, H-21); 1.28 (s, 3H, H-19); 3.34 (t, 1H, *J*=10.8 Hz, H-26ax); 3.46 (dd, 1H, *J*=10.8/4.0 Hz, H-26eq); 4.35 (m, 1H, H-16). ^{13}C NMR ($CDCl_3$): δ=24.7 (C-1); 39.9 (C-2); 29.2 (C-3); 41.2 (C-4); 176.1 (C-5); 172.5 (C-6); 40.8 (C-7); 41.6 (C-8); 50.9 (C-9); 79.1 (C-10); 35.6 (C-11); 36.7 (C-12); 48.2 (C-13); 55.1 (C-14); 31.4 (C-15); 80.4 (C-16); 61.8 (C-17); 16.2 (C-18); 22.3 (C-19); 42.2 (C-20); 14.6 (C-21); 109.0 (C-22); 31.6 (C-23); 28.8 (C-24); 30.3 (C-25); 66.8 (C-26); 17.2 (C-27). HRMS (ESI-FT-ICR) *m/z*: 461.2906 $[M-H]^-$ (Calculated for $C_{27}H_{41}O_6$: 461.2903).

4.1.12. Peptide–steroid conjugate 15. ε-*N*-Cbz-L-lysine methyl ester hydrochloride (596 mg, 1.7 mmol), paraformaldehyde (51 mg, 1.7 mmol), triethylamine (023 mL, 1.7 mmol), steroidal acid **14** (800 mg, 1.7 mmol), and methyl isocyanoacetate (0.2 mL, 1.7 mmol) were reacted in MeOH (80 mL) in a similar way as described in Section 4.1.9. Flash column chromatography purification ($CHCl_3$/MeOH, 15:1) furnished the pure conjugate **15** (1.1 g, 74%). Mp (heptane/AcOEt): 219–223 °C. ^{1}H NMR ($CDCl_3$): δ=0.77 (d, 3H, *J*=6.2 Hz, H-27); 0.84 (s, 3H, H-18); 0.95 (d, 3H, *J*=6.9 Hz, H-21); 1.21 (s, 3H, H-19); 3.34 (t, 1H, *J*=10.8 Hz, H-26ax); 3.46 (dd, 1H, *J*=10.8/4.0 Hz, H-26eq); 3.68 (s, 3H, OCH_3); 3.72 (s, 3H, OCH_3); 4.17 (m, 2H, CH_2); 4.26 (m, 1H, NC*H*); 4.39 (m, 1H, H-16); 5.10 (s, 2H, OCH_2); 7.32 (m, 5H, Ph–Cbz). ^{13}C NMR ($CDCl_3$): δ=14.7 (*C*H_3); 16.4 (*C*H_3); 17.2 (*C*H_3); 22.5 (*C*H_3); 24.9 (*C*H_2); 28.6 (*C*H_2); 28.8 (*C*H_2); 29.2 (*C*H_2); 29.6 (*C*H_2); 29.8 (*C*H_2); 30.4 (*C*H); 30.7 (*C*H_2); 31.4 (*C*H_2); 31.6 (*C*H_2); 35.6 (*C*H_2); 36.8 (*C*H_2); 39.5 (*C*H_2); 40.2 (*C*H_2); 41.3 (*C*H); 41.4 (*C*H_2); 42.2 (*C*H); 44.4 (*C*H_2); 44.8 (*C*H); 45.2 (*C*H_2); 48.5 (*C*); 50.5 (*C*H); 54.9 (*C*H); 61.8 (*C*H); 66.7 (*C*H_2); 66.9 (*C*H_2); 79.1 (*C*); 80.6 (*C*H); 109.0 (*C*); 127.7 (*C*H); 127.9 (*C*H); 128.4 (*C*H); 136.5 (*C*); 157.0 (*C*O); 169.4 (*C*O); 171.1 (*C*O); 174.9 (*C*O); 175.2 (*C*O); 176.1 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 890.4780 $[M+H]^+$ (Calculated for $C_{47}H_{69}N_3NaO_{12}$: 890.4779).

4.1.13. (25*R*)-3β-(*tert*-Butyldimethylsilyloxy)-5α-spirostan-12-one (17). TBSCl (2.23 g, 17.5 mmol) and imidazole (1.23 g, 18.1 mmol) were added to a solution of hecogenin **16** (5.2 g, 12.1 mmol) in anhydrous DMF (80 mL). The reaction mixture was stirred under nitrogen atmosphere for 3 h and then, the usual work-up ($CHCl_3$) yielded a crude product, which was purified by flash column chromatography (hexane/AcOEt, 3:1) to give the ketone **17** (5.79 g, 84%). Mp (EtOH): 187–189 °C. ^{1}H NMR ($CDCl_3$): δ=0.07 (s, 3H, (CH_3)$_2$Si); 0.09 (s, 3H, (CH_3)$_2$Si); 0.76 (s, 3H, H-18); 1.17 (s, 3H, H-19); 0.78 (d, 3H, *J*=6.4 Hz, H-27); 0.92 (s, 9H, (CH_3)$_3$CSi); 0.94 (d, 3H, *J*=6.5 Hz, H-21); 3.36 (t, 1H, *J*=10.9 Hz, H-26ax); 3.49 (dd, 1H, *J*=10.8/3.9 Hz, H-26eq); 3.54 (br m, 1H, H-3α); 4.40 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=36.2 (C-1); 27.2 (C-2); 71.1 (C-3); 33.8

(C-4); 44.4 (C-5); 28.1 (C-6); 31.4 (C-7); 34.3 (C-8); 55.4 (C-9); 36.1 (C-10); 37.7 (C-11); 213.2 (C-12); 55.0 (C-13); 55.6 (C-14); 31.2 (C-15); 79.2 (C-16); 53.5 (C-17); 16.1 (C-18); 11.9 (C-19); 42.2 (C-20); 13.2 (C-21); 109.2 (C-22); 31.4 (C-23); 28.8 (C-24); 30.2 (C-25); 66.8 (C-26); 17.1 (C-27); 26.1 ((CH_3)$_3$CSi); 18.3 ((CH_3)$_3$*C*Si); −2.9 ((CH_3)$_2$Si). HRMS (ESI-FT-ICR) *m*/*z*: 567.3848 [M+Na]$^+$ (Calculated for $C_{33}H_{56}NaSiO_4$: 567.3846).

4.1.14. (25*R*)-12,13-Seco-3β-(*tert*-butyldimethylsilyloxy)-5α-spirostane-12,13-diol (18). Ketone **17** (4.0 g, 7.35 mmol) was treated with *m*CPBA (2.0 g, 11.6 mmol) for 36 h in a similar way as described for the synthesis of **5** to give the corresponding lactone. This crude product was dissolved in dry THF (100 mL) and added dropwise at 0 °C to a solution of $LiAlH_4$ (570 mg, 15 mmol) in dry THF (100 mL). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 20 h. An aqueous 5% NaOH solution (100 mL) was added slowly to the reaction mixture and the stirring was continued for 30 min. The resulting white powder was then filtered off and washed with AcOEt (3×100 mL). The collected organic phase was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give a crude product. Flash column chromatography purification ($CHCl_3$/MeOH, 10:1) afforded the compound **18** (2.97 g, 72%). Mp (AcOEt): 231–233 °C. ^{1}H NMR ($CDCl_3$): δ=0.08 (s, 3H, (CH_3)$_2$Si); 0.09 (s, 3H, (CH_3)$_2$Si); 0.80 (d, 3H, *J*=6.7 Hz, H-27); 0.82 (s, 3H, H-19); 0.92 (s, 9H, (CH_3)$_3$CSi); 1.04 (d, 3H, *J*=6.2 Hz, H-21); 1.10 (s, 3H, H-18); 3.36 (t, 1H, *J*=10.9 Hz, H-26ax); 3.41 (2H, m, H-12); 3.46 (dd, 1H, *J*=11.0/4.0 Hz, H-26eq); 3.53 (br m, 1H, H-3α); 4.41 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=37.9 (C-1); 29.0 (C-2); 70.8 (C-3); 35.8 (C-4); 44.8 (C-5); 30.6 (C-6); 32.8 (C-7); 34.3 (C-8); 52.1 (C-9); 37.8 (C-10); 38.0 (C-11); 64.5 (C-12); 78.5 (C-13); 48.6 (C-14); 31.2 (C-15); 79.8 (C-16); 51.7 (C-17); 20.3 (C-18); 12.4 (C-19); 43.7 (C-20); 14.1 (C-21); 109.2 (C-22); 31.6 (C-23); 28.8 (C-24); 31.2 (C-25); 67.5 (C-26); 17.4 (C-27); 26.1 ((CH_3)$_3$CSi); 18.3 ((CH_3)$_3$*C*Si); −2.9 ((CH_3)$_2$Si). HRMS (ESI-FT-ICR) *m*/*z*: 587.4107 [M+Na]$^+$ (Calculated for $C_{33}H_{60}NaSiO_5$: 587.4108).

4.1.15. (25*R*)-12,13-Seco-12-amino-5α-spirostane-3β,13-diol (19). Diol **18** (2.5 g, 4.4 mmol) was dissolved in CH_2Cl_2 (100 mL) and treated in a similar way as described in Section 4.1.6 to give a crude product that was identified by ^{1}H NMR analysis as the expected amine. This product was dissolved in THF (150 mL) and tetrabutylammonium fluoride (TBAF) (4 mL, 1 M in THF) was added. The reaction mixture was stirred under nitrogen atmosphere for 6 h and then the usual work-up (Et_2O) yielded a crude product. Flash column chromatography purification ($CHCl_3$/MeOH/Et_3N, 10:1:0.5) afforded the amine **19** (1.24 g, 62%). Mp (heptane/AcOEt): 218–220 °C. ^{1}H NMR ($CDCl_3$/CD_3OD, 95:5): δ=0.79 (s, 3H, H-19); 0.81 (d, 3H, *J*=6.5 Hz, H-27); 1.01 (d, 3H, *J*=6.4 Hz, H-21); 1.08 (s, 3H, H-18); 3.36 (t, 1H, *J*=10.9 Hz, H-26ax); 3.37 (m, 2H, H-12); 3.44 (dd, 1H, *J*=10.8/4.0 Hz, H-26eq); 3.54 (br m, 1H, H-3α); 4.41 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$/CD_3OD, 95:5): δ=37.2 (C-1); 32.1 (C-2); 70.2 (C-3); 36.9 (C-4); 44.8 (C-5); 31.2 (C-6); 32.7 (C-7); 34.5 (C-8); 52.4 (C-9); 37.8 (C-10); 38.0 (C-11); 62.8 (C-12); 78.3 (C-13); 50.4 (C-14); 31.2 (C-15); 79.8 (C-16); 51.7 (C-17); 20.3 (C-18); 12.4 (C-19); 43.7 (C-20); 14.1 (C-21); 109.2 (C-22); 31.6 (C-23); 28.8 (C-24); 31.2 (C-25); 67.5 (C-26); 17.4 (C-27). HRMS (ESI-FT-ICR) *m*/*z*: 450.3586 [M+H]$^+$ (Calculated for $C_{27}H_{48}NO_4$: 450.3583).

4.1.16. Peptide–steroid conjugate 20. Steroidal amine **19** (800 mg, 1.8 mmol), paraformaldehyde (53 mg, 1.8 mmol), *N*-Boc-L-serine (365 mg, 1.8 mmol), and benzylisocyanide (0.22 mL, 1.8 mmol) were reacted in MeOH (100 mL) in a similar way as described in Section 4.1.7. Flash column chromatography purification ($CHCl_3$/MeOH, 15:1) furnished the conjugate **20** (1.10 g, 79%). Mp (AcOEt): 231–233 °C. ^{1}H NMR ($CDCl_3$): δ=0.78 (s, 3H, H-19); 0.81 (d, 3H, *J*=6.7 Hz, H-27); 1.04 (d, 3H, *J*=6.6 Hz, H-21); 1.12 (s, 3H, H-18); 1.43 (s, 9H, (CH_3)$_3$C); 3.36 (t, 1H, *J*=10.9 Hz, H-26ax); 3.39 (m, 2H, H-12); 3.44 (dd, 1H, *J*=10.8/4.1 Hz, H-26eq); 3.55 (br m, 1H, H-3α); 3.67–3.62 (m, 2H, CH_2OH); 4.25–4.23 (m, 1H, NC*H*); 4.28–4.32 (m, 4H, CH_2); 4.40 (m, 1H, H-16α); 7.13 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ=17.4 (*C*H$_3$); 12.7 (*C*H$_3$); 14.2 (*C*H$_3$); 20.2 (*C*H$_3$); 28.5 (*C*H$_3$); 28.8 (*C*H$_2$); 31.2 (*C*H$_2$); 31.3 (*C*H+*C*H$_2$); 31.6 (*C*H$_2$); 32.3 (*C*H$_2$); 32.6 (*C*H$_2$); 34.5 (*C*H); 36.7 (*C*H$_2$); 37.0 (*C*H$_2$); 37.9 (*C*); 38.2 (*C*H$_2$); 43.6 (*C*H); 44.9 (*C*H); 45.3 (*C*H$_2$); 46.8 (*C*H); 50.5 (*C*H); 51.6 (*C*H); 52.5 (*C*H); 62.9 (*C*H$_2$); 63.3, (*C*H$_2$); 66.8 (*C*H$_2$); 67.5 (*C*H$_2$); 70.3 (*C*H); 78.6 (*C*); 79.5 (*C*); 80.0 (*C*H); 109.3 (*C*); 126.8 (*C*H); 127.3 (*C*H); 128.2 (*C*H); 131.1 (*C*H); 155.8 (*C*O); 168.7 (*C*O); 170.1 (*C*O). HRMS (ESI-FT-ICR) *m*/*z*: 806.4933 [M+Na]$^+$ (Calculated for $C_{44}H_{69}NaN_3O_9$: 806.4932).

4.1.17. Methyl (25*R*)-2,3-seco-2,6β-dihydroxy-5α-spirostan-3-oate (21). H_2SO_4 (30%, 4 mL) was added dropwise to a solution of lactone **5** (2.0 g, 4.1 mmol) in MeOH (100 mL) and the reaction mixture was stirred at reflux for 8 h. The usual work-up (AcOEt) yielded a crude product, which dissolved in a 1 M solution of NaOMe in MeOH (200 mL). The reaction mixture was stirred for 2 h and then concentrated under reduced pressure. Flash column chromatography purification ($CHCl_3$/MeOH, 20:1) afforded the ester **21** (1.74 g, 88%). Mp (heptane/AcOEt): 242–243 °C. ^{1}H NMR ($CDCl_3$): δ=0.76 (s, 3H, H-18); 0.77 (d, 3H, *J*=6.5 Hz, H-27); 0.81 (s, 3H, H-19); 0.94 (d, 3H, *J*=6.6 Hz, H-21); 3.36 (t, 1H, *J*=11.0 Hz, H-26ax); 3.46 (dd, 1H, *J*=4.1/10.9 Hz, H-26eq); 3.72 (m, 2H, H-2); 3.85 (m, 1H, H-6α); 4.41 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=38.8 (C-1); 57.7 (C-2); 173.9 (C-3); 28.3 (C-4); 46.1 (C-5); 71.2 (C-6); 33.8 (C-7); 30.5 (C-8); 41.1 (C-9); 36.8 (C-10); 20.8 (C-11); 39.7 (C-12); 40.2 (C-13); 56.5 (C-14); 31.6 (C-15); 80.5 (C-16); 62.1 (C-17); 16.3 (C-18); 17.3 (C-19); 41.5 (C-20); 14.5 (C-21); 109.3 (C-22); 31.4 (C-23); 28.8 (C-24); 29.0 (C-25); 66.9 (C-26); 17.1 (C-27). HRMS (ESI-FT-ICR) *m*/*z*: 501.3192 [M+Na]$^+$ (Calculated for $C_{28}H_{46}NaO_6$: 501.3194).

4.1.18. Methyl (25*R*)-2,3-seco-6β-acetoxy-2-amino-5α-spirostan-3-oate (22). Compound **21** (1.6 g, 3.3 mmol) was dissolved in dry CH_2Cl_2 (100 mL) and submitted to mesylation (0.6 mL of MsCl and 6.2 mL of Et_3N) and subsequent nucleophilic replacement with NaN_3 (650 mg, 10 mmol) in a similar way as described in Section 4.1.6 to give the expected azide (identified by ESIMS analysis). This

intermediate was dissolved in pyridine (60 mL) and treated with Ac_2O (3 mL) exactly as described in Section 4.1.1. The resulting acetate was dissolved in absolute ethanol (100 mL) and submitted to catalytic hydrogenation (400 mg of Lindlar catalyst) as described in Section 4.1.6 to afford the crude amine **22**. Flash column chromatography purification (CH_2Cl_2/MeOH/Et_3N, 20:1:0.1) furnished the pure amine **22** (1.06 g, 62%). Mp (MeOH): 217–218 °C. ^1H NMR ($CDCl_3$): δ=0.77 (s, 3H, H-18); 0.77 (d, 3H, J=6.6 Hz, H-27); 0.82 (s, 3H, H-19); 0.96 (d, 3H, J=6.6 Hz, H-21); 2.02 (s, 3H, CH_3CO); 3.36 (t, 1H, J=11.0 Hz, H-26ax); 3.45 (dd, 1H, J=4.0/10.9 Hz, H-26eq); 3.60 (m, 2H, H-2); 4.38 (m, 1H, H-6α); 4.41 (m, 1H, H-16α). ^{13}C NMR ($CDCl_3$): δ=38.2 (C-1); 53.6 (C-2); 173.8 (C-3); 28.4 (C-4); 46.5 (C-5); 72.6 (C-6); 36.3 (C-7); 30.4 (C-8); 53.8 (C-9); 35.4 (C-10); 20.9 (C-11); 39.6 (C-12); 40.5 (C-13); 55.5 (C-14); 31.4 (C-15); 80.6 (C-16); 62.0 (C-17); 16.5 (C-18); 15.2 (C-19); 41.6 (C-20); 14.5 (C-21); 109.3 (C-22); 31.3 (C-23); 28.7 (C-24); 30.2 (C-25); 66.8 (C-26); 17.1 (C-27); 21.3 (*C*H_3CO); 170.0 (CH_3*C*O). HRMS (ESI-FT-ICR) *m/z*: 542.3458 $[M+Na]^+$ (Calculated for $C_{30}H_{49}NO_6Na$: 542.3456).

4.1.19. Peptide–steroid conjugate 23. Steroidal amine **22** (700 mg, 1.3 mmol), paraformaldehyde (41 mg, 1.3 mmol), *N*-Boc-L-phenylalanine (357 mg, 1.3 mmol), and *tert*-butylisocyanide (0.15 mL, 1.3 mmol) were reacted in MeOH (80 mL) in a similar way as described in Section 4.1.7. The resulting crude product was dissolved in a mixture of THF/H_2O (2:1, 200 mL), treated with LiOH (210 mg, 5.0 mmol) and stirred at 0 °C for 2 h. The reaction mixture was then acidified with aqueous 10% $NaHSO_4$ to pH 3 and extracted with AcOEt (2×100 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The resulting acid reacted with L-phenylalanine methyl ester hydrochloride (280 mg, 1.3 mmol), paraformaldehyde (41 mg, 1.3 mmol), triethylamine (0.18 mL, 1.3 mmol), and *tert*-butylisocyanide (0.15 mL, 1.3 mmol) in MeOH (60 mL) in a similar way as described in Section 4.1.9. Flash column chromatography purification ($CHCl_3$/MeOH, 15:1) furnished the pure conjugate **23** (874 mg, 59%). Mp (AcOEt): 233–234 °C. ^1H NMR ($CDCl_3$): δ=0.78 (s, 3H, H-18); 0.77 (d, 3H, J=6.4 Hz, H-27); 0.85 (s, 3H, H-19); 0.95 (d, 3H, J=6.6 Hz, H-21); 1.32–1.35 (s, 18H, (CH_3)$_3$CNH); 1.44 (s, 9H, (CH_3)$_3$C); 2.03 (s, 3H, CH_3CO); 3.36 (t, 1H, J=10.9 Hz, H-26ax); 3.45 (dd, 1H, J=4.1/10.9 Hz, H-26eq); 3.62 (m, 2H, H-2); 3.71 (s, 3H, CH_3O); 4.12–4.27 (m, 4H, CH_2); 4.33–4.39 (m, 3H, NC*H*); 4.41 (m, 1H, H-16α); 7.21–7.25 (m, 10H, Ph). ^{13}C NMR ($CDCl_3$): δ=14.4 (*C*H_3); 15.4 (*C*H_3); 16.6 (*C*H_3); 17.1 (*C*H_3); 21.1 (*C*H_2); 21.2 (*C*H_3); 28.1 (*C*H_2); 28.3 (*C*H_3); 28.5 (*C*H_3); 28.8 (*C*H_2); 30.2 (*C*H); 30.7 (*C*H_2); 31.3 (*C*H_2); 31.4 (*C*H_2); 35.7 (*C*); 36.7 (*C*H_2); 38.0 (*C*H_2); 39.8 (*C*H_2); 40.7 (*C*); 41.5 (*C*H); 44.0 (*C*H_2); 44.2 (*C*H_2); 44.5 (*C*H_2); 45.8 (*C*H); 45.9 (*C*H); 46.1 (*C*H); 53.2 (*C*H_3); 53.3 (*C*H); 53.9 (*C*H_2); 55.2 (*C*H); 62.1 (*C*H); 66.8 (*C*H_2); 72.8 (*C*H); 79.5 (*C*); 80.4 (*C*H); 109.3 (*C*); 120.5 (*C*H); 121.1 (*C*H); 121.4 (*C*H); 127.1 (*C*H); 128.8 (*C*H); 129.4 (*C*); 155.8 (*C*O); 168.6 (*C*O); 168.8 (*C*O); 169.2 (*C*O); 169.5 (*C*O); 170.1 (*C*O); 170.3 (*C*O); 174.8 (*C*O). HRMS (ESI-FT-ICR) *m/z*: 1162.7036 $[M+Na]^+$ (Calculated for $C_{65}H_{97}N_5O_{12}Na$: 1162.7033).

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An efficient and versatile synthesis of all structural types of acylpolyamine spider toxins

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Dedicated to Professor T. Okuno on the occasion of his 65th birthday

Abstract—An efficient and versatile synthesis of acylpolyamine spider toxins of all structural types classified by extensive MS analysis has been achieved. By using 2-nitrobenzenesulfonamide as an effective activating and/or protecting group (the Nosyl strategy), the naturally occurring toxins **1**–**8** corresponding to Types A–F were concisely synthesized in high overall yield.

1. Introduction

Acylpolyamine toxins, found in spider and wasp venoms,[1,2] have attracted much interest in the field of neurobiology because of its unique activity as open channel blocker of glutamate receptors.[3,4] In order to investigate biological properties of these toxins in detail, synthesis of a number of congeners and derivatives is necessary because only limited quantity is available from natural sources. Therefore, the acylpolyamine toxins have been an interesting target for organic synthesis.[5–8]

Recent developments of mass spectrometric techniques accelerated and facilitated structural elucidation of acylpolyamine spider toxins even at low picomolar levels.[9,10] Itagaki et al. revealed that, using highly sensitive analytical method with LC-MS and MS/MS, the *Nephila* and *Nephilengys* spider venom glands contain a complex mixture of closely related toxins, a majority of which have not been previously detected by the classical analytical method.[11–13] Furthermore, these new results led to a classification of the toxin structures. These spider toxins consist of three structural elements: a lipophilic head; a polyamine backbone; and a polyamine chain terminal, which are linearly connected in this order. There are a variety of each element and the combination of each element results in a complex mixture of the venom gland constituents as classified into the generalized structures Types A–F based on the distinct polyamine backbone structure (Fig. 1).[11–13] Figure 2 shows the representative toxins for each type. However, the relationships between these structural types and its biological activities are still not documented well,[14] and in the case of novel toxins, the structures should be confirmed by synthesis.[12,15,16] This situation prompted us to establish an efficient and versatile synthetic method for acylpolyamine toxins. We report herein the successful results along this line, which enabled us to synthesize eight naturally occurring acylpolyamine spider toxins, JSTX-3 (**1**), NPTX-8 (**2**), NPTX-1 (**3**), NPTX-473 (**4**), NPTX-501 (**5**), NSTX-3 (**6**), joramine (**7**), and Arg-636 (**8**) (Fig. 2), covering all structural types.[17]

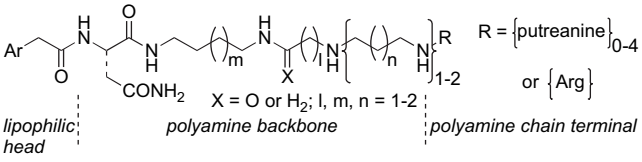

Figure 1. Generalized structure of acylpolyamine spider toxins.

2. Results and discussion

2.1. Basic methodology

Our synthetic strategy is based on the structural classification as mentioned above and the use of 2-nitrobenzenesulfonamide

Keywords: Acylpolyamine; Spider toxins; Ns strategy; Structural classification; Versatile synthesis.
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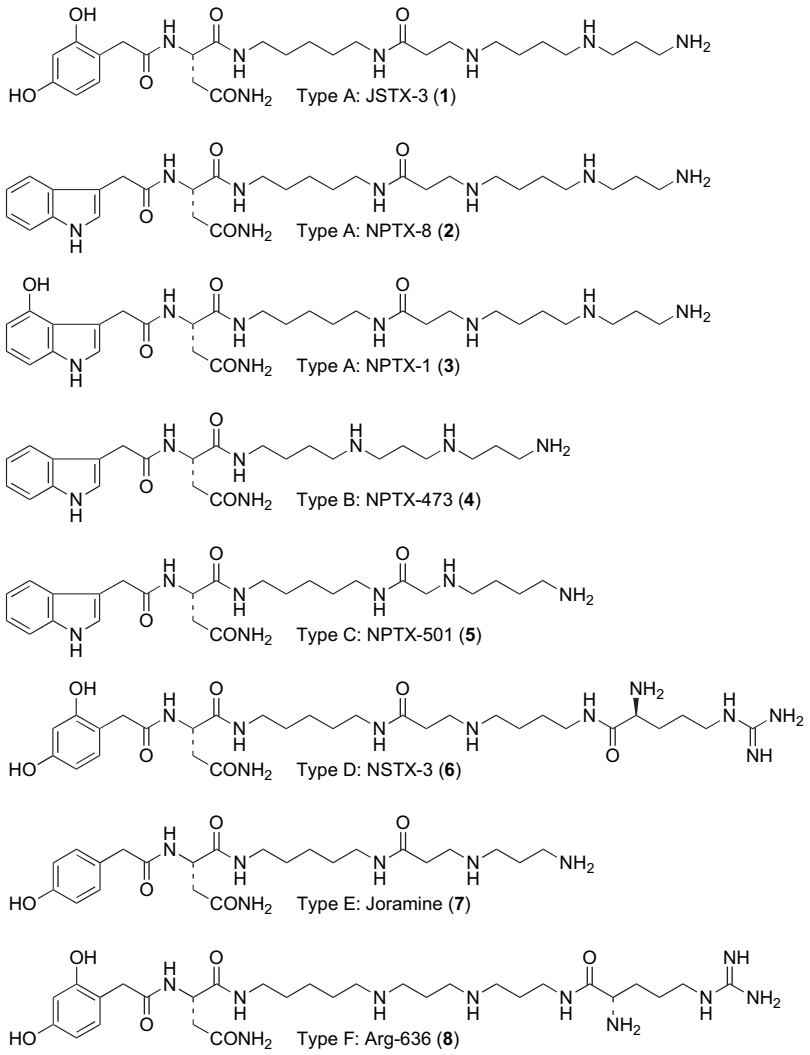
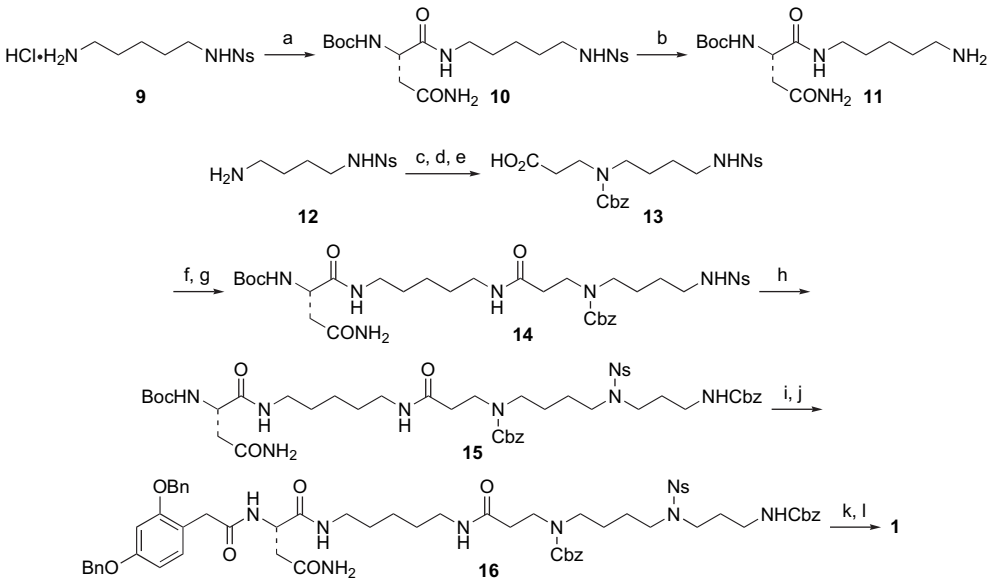


Figure 2. Structures of acylpolyamine spider toxins.

(Ns or Nosyl) as an activating and/or protecting group (the Ns strategy).[18–20] The three structural elements can be considered as building blocks, that is, construction of the polyamine backbone, followed by successive connection of the lipophilic head and polyamine chain terminal would afford a variety of natural toxins as well as their analogs. Accordingly, effective preparation of each polyamine backbone should pave the way for convenient and versatile synthesis of these toxins because this element has the richest variation among those three elements (Fig. 2). In order to efficiently construct the polyamine backbones, selective protection and/or activation of amino groups are needed. Fukuyama et al. reported that using the Ns group as both protecting and activating group is exceptionally versatile for the preparation of a variety of secondary amines (Ns strategies),[18–20] and demonstrated its utility for polyamine synthesis.[21–23] Therefore, their protocol is applied to the construction of the polyamine backbones.

2.2. Synthesis of Type A toxins

JSTX-3 (**1**), isolated from the venom of *Nephila clavata* in 1986, is one of the first found acylpolyamine spider toxins.[24] This polyamine backbone is classified into Type A, containing cadaverine as a diamine constituent. Thus, the synthesis of **1** started from mono-Ns-cadaverine hydrochloride **9**,[21,22,25] which was readily coupled with commercially available *N*-^{t}Boc-L-asparagine-*p*-nitrophenyl ester for 0.5 h to give the asparagyl cadaverine unit **10** (Scheme 1). Initial attempts to remove the Ns group in **10** by the original procedures of the Ns strategy led to poor results; for example, treatment of **10** with thioacetic acid gave the corresponding primary amine **11** only in 20% yield. These results prompted us to improve the procedures for deprotection of 2-nitrobenzenesulfonamide to corresponding primary amine, and as a consequence, we were able to establish the modified procedures using 2-mercaptoethanol/DBU or thiophenol/Cs_2CO_3 in DMF.[26] With these modified procedures, the primary amine **11** was obtained in high yield (>85%). On the other hand, the carboxylic acid **13** was prepared from mono-Ns-putrescine **12**[20,21] in excellent yield through sequential reactions by the Michael addition to methyl acrylate,[27] protection by CbzCl, and hydrolysis of the methyl ester. Coupling of the amine **11** with the succinimide ester prepared from **13** by DCC afforded the polyamine backbone precursor **14** in 90% yield. When acid anhydride method with pivaloyl chloride or the coupling agent EDC for this coupling reaction was used, the yields were less than 50%.



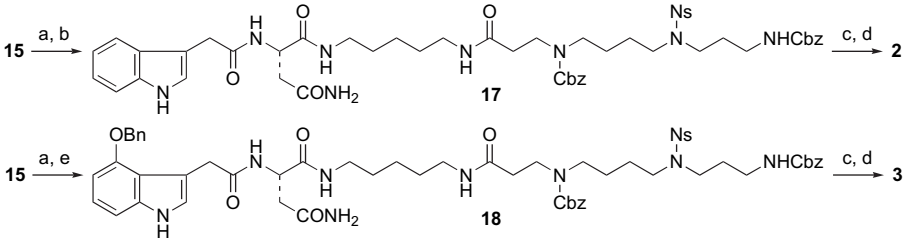
Scheme 1. Synthesis of JSTX-3 (**1**). Reagents and conditions: (a) N^{α}-Boc-L-Asn-ONp, Et_3N/DMF, 0 °C to rt, 0.5 h, 97%; (b) 2-mercaptoethanol, DBU/DMF, rt, 2 h, 85%; (c) methyl acrylate/EtOH, rt, 5 h; (d) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 2 h, 86% (two steps); (e) NaOH/H_2O–MeOH, 0 °C to rt, 1 h, 96%; (f) HOSu, DCC/CH_2Cl_2, 0 °C, 5 h; (g) **11**, DMF, rt, 0.5 h, 90% (two steps); (h) *N*-Cbz-3-bromopropylamine, Cs_2CO_3/DMF, 50 °C, 1 h, 92%; (i) TFA/$CHCl_3$, 0 °C to rt, 1 h; (j) 2,4-dibenzyloxyphenylacetic acid-OSu, Et_3N/DMF, rt, 2 h, 87% (two steps); (k) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (l) H_2-Pd(OH)$_2$/AcOH, rt, 3 h, 66% (two steps).

Following the Ns strategy, the polyamine chain was elongated by alkylation of **14** with *N*-Cbz-3-bromopropylamine in the presence of Cs_2CO_3 to furnish the fully protected polyamine backbone **15** in 92% yield. After removal of the Boc group in **15** by TFA, the resultant amine was condensed with dibenzyl-2,4-dihydroxyphenylacetic acid *N*-hydroxysuccinimide ester[28] to give the fully protected JSTX-3 **16** in 87% yield. Successive removal of the Ns and Cbz protective groups by 2-mercaptoethanol and hydrogenation, respectively, afforded **1** in high yield. Thus, JSTX-3 (**1**) was synthesized from mono-Ns-cadaverine **9** in a 39% overall yield via nine steps. The synthetic compound was identical with authentic specimen in HPLC co-elution and comparison of the ESI-MS/MS spectra, and other spectroscopic data (IR, ^{1}H and ^{13}C NMR, FABHRMS) were consistent with those previously reported.[28]

The synthesis of NPTX-8 (**2**) and -1 (**3**), originally isolated from the Japanese spider *N. clavata*,[29–31] and later found in the Madagascarian spider *Nephilengys borbonica*[11] and Brazilian garden spider *Nephilengys cruentata*[32] as well, demonstrated the convenience of this synthetic method. Both these toxins are also classified into Type A, containing indoleacetate chromophore as the lipophilic head. Therefore, coupling of the polyamine backbone **15** with appropriate indoleacetate moieties instead of 2,4-dihydroxyphenyl moiety would readily lead to these toxins. It was indeed accomplished as shown in Scheme 2. The succinimide ester of indoleacetic acid or 4-benzyloxyindoleacetic acid[33] was coupled with the amine derived from **15** by TFA gave the fully protected toxins **17** or **18**, respectively, from which the natural toxins **2** and **3** were obtained by the same deprotection procedures as above. The synthetic compounds were fully characterized by the spectroscopic data (IR, ^{1}H and ^{13}C NMR, FABHRMS, ESI-MS/MS), and identified with the natural toxins.[34,35] Thus, NPTX-8 and -1 were synthesized from mono-Ns-cadaverine **9** in 35 and 36% overall yields, respectively. In this way, a variety of Type A toxins and its analogs can be synthesized.

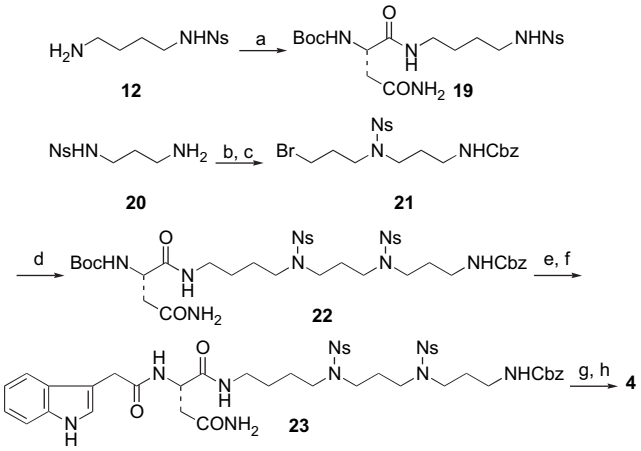
2.3. Synthesis of Type B toxin

The polyamine backbone of Type B has no amide bond in its polyamine chain and the putrescine (C_4) unit instead of the cadaverine (C_5) unit connects to the asparagine moiety. Accordingly, the alkylation procedures of the Ns strategy are



Scheme 2. Syntheses of NPTX-8 (**2**) and NPTX-1 (**3**). Reagents and conditions: (a) TFA/$CHCl_3$, 0 °C to rt, 1 h; (b) indoleacetic acid-OSu, Et_3N/DMF, rt, 2 h, 84% (two steps); (c) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (d) H_2-Pd(OH)$_2$/AcOH, rt, 2 h, 61% (two steps); (e) 4-benzyloxyindoleacetic acid-OSu, Et_3N/DMF, rt, 2 h, 86% (two steps).

useful for construction of this polyamine chain, and the synthesis should start with mono-Ns-putrescine **12**.[21,22] Scheme 3 shows the synthesis of Type B toxin NSTX-473 (**4**), found in the venom gland of the Madagascarian spider *N. borbonica*.[11] Condensation of the putrescine unit **12** with *N*-^{t}Boc-L-asparagine-*p*-nitrophenyl ester furnished the acylputrescine **19** in 95% yield. On the other hand, mono-Ns-diaminopropane **20**[21,22] was protected by CbzCl, and subsequently alkylated with 1,3-dibromopropane to give the bromide **21** in high yield. Thus, the obtained two segments **19** and **21** were connected by the alkylation procedure of the Ns strategy to afford the Type B polyamine backbone **22** in 94% yield. Removal of the Boc protective group by TFA, followed by coupling with indoleacetic acid succinimide ester furnished the fully protected toxin **23** in 84% yield. Finally, deprotection of the Ns and Cbz groups from **23** in the same manner as for the Type A toxins afforded NPTX-473 (**4**) in a 45% overall yield from **12** via six steps. The synthetic compound was fully characterized by the spectroscopic data (IR, ^{1}H and ^{13}C NMR, FABHRMS, ESI-MS/MS), and consistent with the proposed structure.[11]

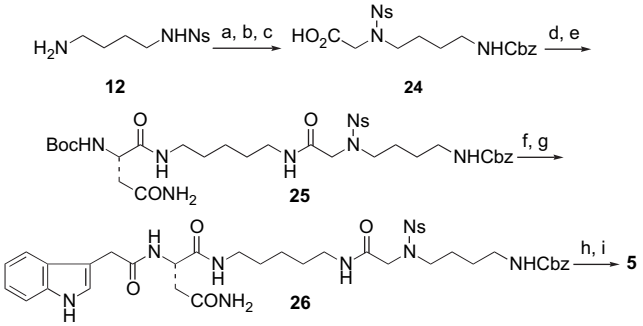


Scheme 3. Synthesis of NPTX-473 (**4**). Reagents and conditions: (a) N^{α}-Boc-L-Asn-ONp, DMF, 0 °C to rt, 2 h, 95%; (b) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 2 h, 88%; (c) 1,3-dibromopropane, Cs_2CO_3/DMF, 50 °C, 0.5 h, 91%; (d) **19**, TBAI, Cs_2CO_3/DMF, 70 °C, 1 h, 94%; (e) TFA/$CHCl_3$, 0 °C to rt, 1 h; (f) indoleacetic acid-OSu, Et_3N/DMF, rt, 2 h, 84% (two steps); (g) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (h) H_2-Pd(OH)$_2$/AcOH, rt, 2 h, 60% (two steps).

It is noteworthy that the structure of this polyamine chain is the same as that of PhTX-433, a glutamate receptor antagonist from the venom of the solitary wasp *Philanthus triangulum*.[36,37] Therefore, the method established here is applicable to the synthesis of PhTX-433 and its derivatives.[38]

2.4. Synthesis of Type C toxin

The Type C structure is close to but simpler than that of Type A; that is, the β-alanine moiety is replaced by the glycine moiety and the C_3 unit of the right terminal is missing. Scheme 4 shows the synthesis of NPTX-501 (**5**), a Type C toxin found in Madagascarian spiders *Nephilengys madagascariensis* and *N. borbonica*.[10,11] The primary amine of mono-Ns-putrescine **12** was protected by CbzCl, then alkylated with methyl bromoacetate by the Ns strategy at 50 °C for 0.5 h and the resultant methyl ester was hydrolyzed to yield the carboxylic acid **24** in high yield. Coupling the asparagine unit **11** with the succinimide ester prepared from **24** furnished the Type C acylpolyamine backbone **25** in excellent yield. Removal of the Boc group in **25** by TFA, followed by coupling of the resultant amine with indoleacetic acid succinimide ester afforded the fully protected toxin **26**. Deprotection of the Ns and Cbz by the same procedures as above produced NPTX-501 (**5**) in a 33% overall yield from **12** via nine steps. The synthetic compound was fully characterized by the spectroscopic data (IR, ^{1}H and ^{13}C NMR, FABHRMS, ESI-MS/MS), and consistent with the proposed structure.[10,11]



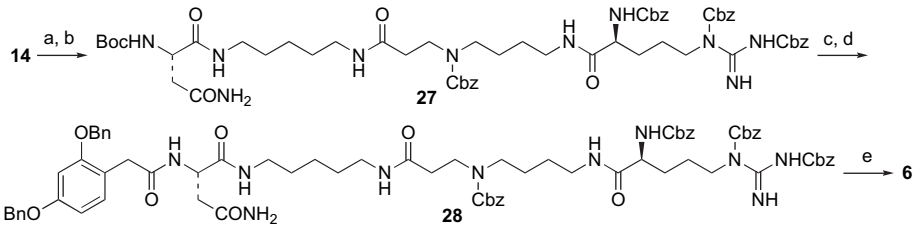
Scheme 4. Synthesis of NPTX-501 (**5**). Reagents and conditions: (a) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 1 h, 95%; (b) methyl bromoacetate, Cs_2CO_3/DMF, 50 °C, 0.5 h, 86%; (c) NaOH/H_2O–MeOH, 0 °C to rt, 2 h, 86%; (d) HOSu, DCC/CH_2Cl_2, 0 °C, 4 h; (e) **11**, DMF, rt, 0.5 h; (f) TFA/$CHCl_3$, 0 °C to rt, 1 h; (g) indoleacetic acid-OSu, Et_3N/DMF, rt, 2 h, 73% (four steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) H_2-Pd(OH)$_2$/AcOH, rt, 2 h, 65% (two steps).

2.5. Synthesis of Type D toxin

The polyamine backbone of Type D is quite similar to that of Type A and missing only the C_3 unit at the right terminal. Therefore, the toxins of these two types can be synthesized from the common polyamine precursor **14**. Thus, the Type D toxin NSTX-3 (**6**), isolated from the Papua New Guinean spider *Nephila maculata*,[24] was synthesized in a manner similar to that of Type A toxins (Schemes 1 and 2) as shown in Scheme 5. Removal of the Ns protective group of **14**,[26] followed by condensation with tri-Cbz-arginine succinimide ester furnished **27** in 76% yield. Deprotection of the Boc protective group of **28** by TFA, and subsequent condensation with 2,4-dihydroxyphenylacetic acid *N*-hydroxysuccinimide ester afforded the fully protected toxin **14** in 87% yield. Finally, hydrogenation of **28** afforded NSTX-3 (**6**) in a 36% overall yield from **9** via nine steps. The synthetic compound was fully characterized by the spectroscopic data (IR, ^{1}H and ^{13}C NMR, FABHRMS, ESI-MS/MS), and identified with the natural[24] and synthetic toxins.[39–41]

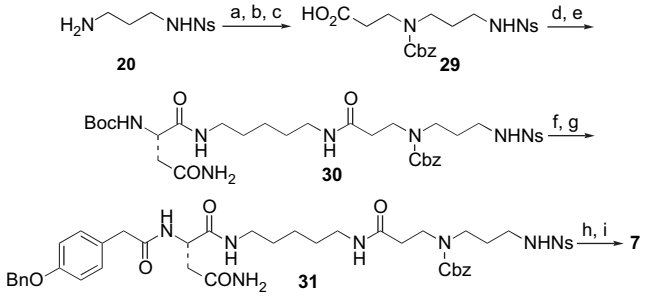
2.6. Synthesis of Type E toxin

The Type E polyamine backbone is only slightly different from Type D, that is, the C_3 unit is on the right terminal instead of the C_4 unit. Accordingly, starting with mono-Ns-1,3-diaminopropane **20** and using the same procedures as that for the Type D would give Type E toxin. Scheme 6 shows the synthesis of joramine (**7**), a minor component of the venom of *N. clavata*.[42] The carboxylic acid **29** was obtained from a series of reactions with Michael addition,



Scheme 5. Synthesis of NSTX-3 (**6**). Reagents and conditions: (a) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (b) tri-Cbz-arginine-OSu, DMF, rt, 2 h; 76% (two steps); (c) TFA/$CHCl_3$, 0 °C to rt, 1 h; (d) 2,4-dibenzyloxyphenylacetic acid-OSu, Et_3N/DMF, rt, 2 h, 87% (two steps); (e) H_2-$Pd(OH)_2$/AcOH, rt, 4 h, 74%.

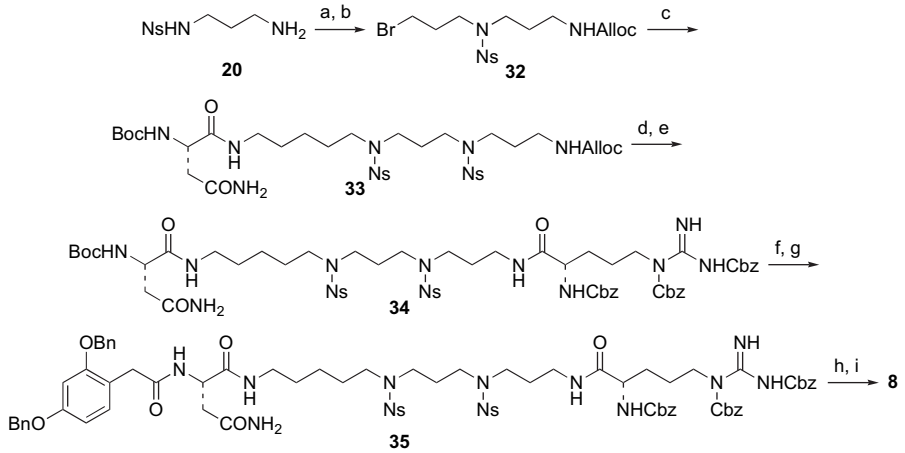
Cbz protection, and ester hydrolysis. The acid **29** was coupled with the amine unit **11** to afford the polyamine backbone **30** in 82% yield. After removal of the Boc group by TFA, the resultant amine was coupled with 4-benzyloxyphenylacetic acid succinimide ester to furnish the fully protected toxin **31**, which was converted to joramine (**7**) by the usual manner. Thus, the Type E toxin joramine (**7**) was synthesized in a 36% overall yield from **20** via nine steps. The synthetic compound was fully characterized by the spectroscopic data (IR, ^1H and ^{13}C NMR, FABHRMS, ESI-MS/MS), and identified with the natural toxin.[42]



Scheme 6. Synthesis of joramine (**7**). Reagents and conditions: (a) methyl acrylate/EtOH, rt, 5 h; (b) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 2 h, 86% (two steps); (c) NaOH/H_2O–MeOH, 0 °C to rt, 1 h, 85%; (d) HOSu, DCC/CH_2Cl_2, 0 °C, 5 h; (e) **11**, DMF, rt, 0.5 h, 82% (two steps); (f) TFA/$CHCl_3$, 0 °C to rt, 1 h; (g) 4-benzyloxyphenylacetic acid-OSu, Et_3N/DMF, rt, 2 h, 88% (two steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) H_2-$Pd(OH)_2$/AcOH, rt, 3 h, 69% (two steps).

2.7. Synthesis of Type F toxin

The Type F polyamine structure is quite similar to that of Type B; the only difference is the cadaverine (C_5) unit instead of the putrescine (C_4) unit connected to asparagine. Accordingly, this polyamine backbone can be constructed by basically the same way as used for Type B. Argiotoxin-636 (Argiopine, **8**), isolated from the Argiope spiders[43,44] in 1986 as well as JSTX-3,[45] can be classified into Type F and its synthesis is shown in Scheme 7. In this case, however, in order to attach arginine moiety at the polyamine terminal, selective protection of the terminal primary amine of the polyamine backbone is necessary. Thus, allyloxycarbonyl (Alloc) group was used for the third protective group for construction of polyamine chain. Mono-Ns-diaminopropane **20** was converted to the corresponding allyl carbamate, which was further alkylated by 1,3-dibromopropane in the presence of Cs_2CO_3 at 50 °C. By the same way as used for Type B toxin, the resultant bromide **32** was coupled with the asparagine/cadaverne conjugate **10** to afford the Type F polyamine backbone **33** in excellent yield. The Alloc moiety in **33** was selectively removed using tetrakis-(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) as a catalyst and pyrrolidine as a nucleophile at room temperature in high yield.[46] Instead of $Pd(PPh_3)_4$, tris(dibenzylideneacetone)-dipalladium(0) was also effective for this deprotection. Thus, the obtained primary amine was coupled with the arginine derivative by its *N*-hydroxysuccinimide ester to give **34**. Boc removal and subsequent acylation of **34**, followed by deprotection in the usual manner afforded



Scheme 7. Synthesis of argiotoxin-636 (**8**). Reagents and conditions: (a) AllocCl, Et_3N/CH_2Cl_2, 0 °C, 0.5 h, 93%; (b) 1,3-dibromopropane, Cs_2CO_3/DMF, 50 °C, 0.5 h, 90%; (c) **10**, TBAI, Cs_2CO_3/DMF, 70 °C, 1 h; (d) $Pd(PPh_3)_4$, PPh_3, pyrrolidine/CH_2Cl_2, rt, 0.5 h; (e) tri-Cbz-arginine-OSu, DMF, rt, 1 h, 66% (three steps); (f) TFA/$CHCl_3$, 0 °C to rt, 1 h; (g) 2,4-dibenzyloxyphenylacetic acid-OSu, Et_3N/DMF, rt, 2 h, 80% (two steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) H_2-$Pd(OH)_2$/AcOH, rt, 2 h, 51% (two steps).

argiotoxin-636 (**8**) in a 26% overall yield from mono-Ns cadaverine **9** via eight steps. The synthetic compound was fully characterized by the spectroscopic data (IR, ^{1}H and ^{13}C NMR, FABHRMS, ESI-MS/MS), and identified with the natural[43,44] and synthetic toxins.[47–50]

3. Conclusion

We have established an efficient and versatile synthesis of acylpolyamine spider toxins based on the structural classification of the *Nephila* and *Nephilengys* spider toxins using the 2-nitrobenzenesulfonamide group (the Ns strategy). The naturally occurring toxins **1**–**8** corresponding to each structural type have been efficiently synthesized by this method in a high overall yield with few steps. This method is so versatile that it would enable us to synthesize a variety of analogues as well as naturally occurring toxins. Therefore, it would be highly useful for structure–activity relationship and mode of action studies of the acylpolyamine toxins in more detail. Studies along this line are currently underway.[51,52]

4. Experimental

4.1. General

Optical rotation was measured on a Jasco DIP-370 polarimeter. IR spectra were recorded on a Perkin–Elmer infrared spectrometer model 1750. NMR spectra were measured on a Varian DPX-300 spectrometer. Low and high resolution mass spectra were recorded on a JEOL JMS D-300 mass spectrometer. ESI-MS/MS spectra were measured on a Micromass Q-TOF Ultima API fitted with an electrospray ion source in positive ionization mode. Preparative HPLC was performed on a Shimadzu LC-10 instrument equipped with a CAPCELL PAK C-18 (5 μm, 10×250 mm) with a flow rate of 5 mL min^{-1} and detection at 210 nm. Column chromatography was carried out on silica gel 60 (70–230 mesh), and preparative TLC was run on silica gel 60F$_{254}$. All dried solvents were purchased from Aldrich Chemical Co. The authentic specimen of JSTX-3 was purchased from Wako Chemical Co.

4.1.1. *N*-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-*N*′-(2-nitrobenzenesulfonyl)-1,5-diaminopentane (10). To a stirred solution of *N*-(5-amino-pentyl)-2,4-dinitro-benzenesulfonamide HCl salt[22] (**9**) (500 mg, 1.54 mmol) in DMF (5 mL) were added *N*$^{\alpha}$-Boc-L-asparagine-*p*-nitrophenyl ester (544 mg, 1.54 mmol) and Et$_3$N (156 mg, 1.54 mmol) at 0 °C. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated aqueous NaHCO$_3$ solution (5×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×30 mL) and combined organic layers were dried over Na$_2$SO$_4$. Filtration and concentration followed by chromatography on silica gel (1–4% MeOH/CH$_2$Cl$_2$) gave the title compound **10** (745 mg, 97%) as a white powder. $[\alpha]_D^{24}$ −3.85 (*c* 2.83, MeOH). IR (Nujol) 1683, 1657, 1542, 1461, 1162, 856 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ 1.26 (m, 2H), 1.36 (s, 9H), 1.41 (m, 4H), 2.52 (dd, 1H, *J*=6.3, 15.3 Hz), 2.58 (dd, 1H, *J*=6.0, 15.3 Hz), 2.96 (t, 2H, *J*=6.9 Hz), 3.07 (t, 1H, *J*=6.6 Hz), 4.29 (dd, 1H, *J*=6.0, 6.3 Hz), 7.75 (m, 3H), 8.00 (m, 1H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 24.7, 28.7, 29.7, 30.4, 38.3, 40.2, 44.2, 53.0, 80.9, 125.8, 131.5, 133.5, 134.9, 149.6, 157.5, 173.8, 175.1. HRMS calcd for $C_{14}H_{27}N_4O_4$ (M$^+$−Ns) 315.2032, found 315.2036.

4.1.2. *N*-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-1,5-diaminopentane (11). To a stirred solution of sulfonamide **10** (500 mg, 997 μmol) in DMF (2 mL) were added 2-mercaptoethanol (234 mg, 3.00 mmol) and DBU (456 mg, 3.00 mmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (1–8% MeOH/CH$_2$Cl$_2$, then, 7.5% MeOH/CHCl$_3$ containing 2.5% *i*-PrNH$_2$) to afford the title compound **11** (269 mg, 85%) as a white powder. Spectral data (^{1}H and ^{13}C NMR, IR, MS) obtained were completely in agreement with those reported previously.[53]

4.1.3. Methyl 8-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaoctanoate. A solution of methyl acrylate (158 mg, 1.84 mmol) in EtOH (10 mL) was added at room temperature over 5 h to a stirring solution of *N*-(5-aminobutyl)-2-nitrobenzenesulfonamide[22] (**12**) (500 mg, 1.83 mmol) in EtOH (20 mL). The solvent was evaporated in vacuo, and the residue was subjected to silica gel chromatography (5–20% MeOH/CH$_2$Cl$_2$) to give the secondary amine as a colorless oil, which was used in the next step without further purification.

To a cold (0 °C) and stirred solution of the secondary amine in CH$_2$Cl$_2$ (20 mL) were added CbzCl (30% in toluene, 1.04 g, 1.83 mmol) and Et$_3$N (185 mg, 1.83 mmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated NH$_4$Cl solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over Na$_2$SO$_4$. Filtration and concentration followed by chromatography on silica gel (30–50% EtOAc/hexane) gave methyl ester (775 mg, 86%, two steps) as a colorless oil. IR (neat) 1735, 1696, 1542, 1422, 1365, 1166, 853 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ 1.54 (m, 4H), 2.57 (m, 2H), 3.09 (m, 2H), 3.25 (t, 2H, *J*=6.3 Hz), 3.49 (t, 2H, *J*=7.2 Hz), 3.65 (s, 3H), 5.11 (s, 2H), 5.48 (br s, 1H), 7.34 (m, 5H), 7.73 (m, 2H), 7.84 (m, 1H), 8.11 (m, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 25.0, 26.7, 33.7, 43.0, 43.3, 47.2, 51.8, 67.2, 125.4, 127.9, 128.1, 128.5, 131.0, 132.7, 133.5, 133.7, 136.5, 148.0, 155.9, 172.0. HRMS calcd for $C_{21}H_{24}N_3O_7S$ (M$^+$−OMe) 462.1335, found 462.1353.

4.1.4. 8-(2-Nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaoctanoic acid (13). To a cold (0 °C) and stirred solution of the above methyl ester (700 mg, 1.42 mmol) in MeOH (5 mL) was added 3.0 M aqueous NaOH solution (1.42 mL, 4.26 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was adjusted to pH 2.0 by concentrated HCl. The resultant mixture was diluted with EtOAc (100 mL) and washed with H$_2$O (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na$_2$SO$_4$. Filtration and concentration followed by chromatography on silica gel (50% EtOAc/hexane, then, EtOAc) gave the title compound **13** (652 mg, 96%) as a colorless oil.

IR (film) 1686, 1541, 1424, 1365, 1165, 854 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.54 (m, 4H), 2.61 (m, 2H), 3.10 (m, 2H), 3.26 (t, 2H, J=6.0 Hz), 3.50 (t, 2H, J=7.2 Hz), 5.11 (s, 2H), 5.54 (br s, 1H), 7.36 (m, 5H), 7.71 (m, 2H), 7.82 (m, 1H), 8.10 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.0, 26.7, 33.0, 42.9, 43.3, 47.5, 67.3, 125.3, 127.9, 128.1, 128.6, 131.0, 132.8, 133.6, 133.7, 136.4, 148.0, 156.1, 176.3. HRMS calcd for $C_{13}H_{18}N_3O_6S$ (M^+−Cbz) 344.0917, found 344.0917.

4.1.5. *N*-[N^{α}-(*tert*-Butoxycarbonyl)-L-asparaginyl]-N'-[8-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaoctanoyl]-1,5-diaminopentane (14). To a cold (0 °C) and stirred suspension of carboxylic acid **13** (363 mg, 757 μmol) and HOSu (175 mg, 1.52 mmol) in CH_2Cl_2 (5 mL) was added DCC (235 mg, 1.14 mmol) slowly. After being stirred for 5 h at 0 °C, the reaction mixture was filtrated through a pad of silica gel with EtOAc. The filtrate was concentrated in vacuo to give the crude succinimide ester as a colorless oil.

To a stirred solution of obtained ester in DMF (6 mL) was added amine **11** (241 mg, 762 μmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–8% EtOH/CH_2Cl_2) gave the title compound **14** (532 mg, 90%, two steps) as a colorless solid. $[\alpha]_D^{24}$ −2.61 (*c* 2.30, MeOH). IR (Nujol) 1674, 1542, 1449, 1369, 1165, 854 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 1.21 (m, 2H), 1.32 (s, 9H), 1.36 (m, 8H), 2.30 (t, 2H, J=6.6 Hz), 2.47 (dd, 1H, J=6.6, 12.3 Hz), 2.53 (dd, 1H, J=5.7, 12.3 Hz), 2.92 (m, 2H), 3.02 (t, 2H, J=7.2 Hz), 3.06 (t, 2H, J=6.9 Hz), 3.13 (t, 2H, J=6.9 Hz), 3.37 (m, 2H), 4.26 (dd, 1H, J=5.7, 6.6 Hz), 4.98 (s, 2H), 7.23 (m, 5H), 7.68 (m, 3H), 7.93 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.1, 26.6, 28.0, 28.7, 29.8, 29.9, 36.5, 38.4, 40.3, 40.5, 44.0, 45.6, 53.1, 68.3, 80.9, 125.8, 128.9, 129.1, 129.6, 131.5, 133.5, 134.9, 135.0, 138.1, 149.6, 157.5, 157.8, 173.6, 173.8, 175.1. FABHRMS calcd for $C_{35}H_{51}N_7O_{11}SNa$ $(M+Na)^+$ 800.3265, found 800.3276.

4.1.6. *N*-[N^{α}-(*tert*-Butoxycarbonyl)-L-asparaginyl]-N'-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (15). To a solution of sulfonamide **14** (200 mg, 257 μmol) and *N*-Cbz-3-bromopropylamine[53] (106 mg, 390 μmol) in DMF (2 mL) was added Cs_2CO_3 (169 mg, 519 μmol). After being stirred for 1 h at 50 °C, the reaction mixture was diluted with EtOAc (100 mL) and washed with H_2O (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–8% MeOH/CH_2Cl_2) gave the title compound **15** (230 mg, 92%) as a colorless oil. $[\alpha]_D^{22}$ −2.28 (*c* 2.46, MeOH). IR (Nujol) 1688, 1667, 1636, 1546, 1462, 1163, 852 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 1.30 (m, 2H), 1.43 (s, 9H), 1.48 (m, 8H), 1.69 (m, 2H), 2.41 (t, 2H, J=6.0 Hz), 2.56 (dd, 1H, J=6.0, 16.5 Hz), 2.62 (dd, 1H, J=5.7, 16.5 Hz), 3.10 (m, 4H), 3.16 (t, 2H, J=6.3 Hz), 3.27 (m, 6H), 3.48 (m, 2H), 4.36 (dd, 1H, J=5.7, 6.0 Hz), 5.05 (s, 2H), 5.09 (s, 2H), 7.34 (m, 10H), 7.73 (m, 3H), 7.96 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.1, 26.4, 28.7, 29.8, 29.9, 30.0, 36.6, 38.4, 39.1, 40.3, 45.6, 46.4, 53.1, 67.4, 68.3, 80.9, 125.4, 128.8, 129.0, 129.2, 129.5, 129.6, 131.4, 133.1, 134.0, 135.2, 138.1, 138.4, 149.6, 157.8, 158.8, 173.6, 173.8, 175.1. FABHRMS calcd for $C_{46}H_{64}N_8O_{13}SNa$ $(M+Na)^+$ 991.4211, found 991.4217.

4.1.7. *N*-[N^{α}-(2,4-Dibenzyloxyphenylacetyl)-L-asparaginyl]-N'-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (16). To a cold (0 °C) and stirred suspension of polyamine **15** (165 mg, 170 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless oil.

To a stirred solution of the amine TFA salt in DMF (1 mL) were added Et_3N (18 mg, 178 μmol) and 2,4-dibenzyloxyphenylacetic acid succinimidyl ester[28] (116 mg, 260 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (1–10% EtOH/CH_2Cl_2) to afford the title compound **16** (177 mg, 87%, two steps) as a white powder. $[\alpha]_D^{22}$ −0.75 (*c* 2.01, DMF). IR (Nujol) 1695, 1664, 1642, 1544, 1463, 1173, 852 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.16 (quin, 2H, J=6.9 Hz), 1.29 (m, 4H), 1.38 (m, 4H), 1.59 (m, 2H), 2.28 (t, 2H, J=6.6 Hz), 2.35 (dd, 1H, J=7.5, 15.3 Hz), 2.46 (dd, 1H, J=6.3, 15.3 Hz), 2.95 (m, 6H), 3.21 (m, 8H), 3.41 (s, 2H), 4.50 (dd, 1H, J=6.6, 7.5 Hz), 4.99 (s, 2H), 5.04 (s, 4H), 5.08 (s, 2H), 6.52 (d, 1H, J=8.4 Hz), 6.68 (s, 1H), 6.85 (s, 1H), 7.07 (d, 1H, J=8.1 Hz), 7.32 (m, 22H), 7.60 (t, 1H, J=5.4 Hz), 7.82 (m, 3H), 7.95 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 23.8, 25.3, 28.6, 28.8, 28.9, 34.4, 35.1, 36.4, 37.5, 38.0, 38.5, 38.7, 44.0, 45.5, 46.5, 47.4, 50.0, 65.4, 66.2, 69.4, 69.5, 100.7, 105.9, 117.3, 124.4, 127.3, 127.7, 127.9, 128.0, 128.5, 128.6, 129.8, 131.1, 132.0, 132.6, 134.6, 137.3, 137.4, 147.8, 155.3, 156.3, 157.1, 158.5, 170.1, 170.5, 170.9, 171.8. FABHRMS calcd for $C_{63}H_{74}N_8O_{14}SNa$ $(M+Na)^+$ 1221.4943, found 1221.4943.

4.1.8. JSTX-3 (1). To a stirred solution of polyamine **16** (40 mg, 33.4 μmol) in DMF (1 mL) were added 2-mercaptoethanol (8.0 mg, 102 μmol) and DBU (15 mg, 98.5 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 3 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. JSTX-3 (**1**) was eluted at 9.54 min and was obtained

as a colorless TFA salt (20 mg, 66%, two steps). $[\alpha]_D^{22}$ −4.74 (*c* 0.97, H_2O). IR (Nujol) 3518, 2924, 1695, 1547 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 1.04 (quin, 2H, *J*=7.8 Hz), 1.29 (m, 4H), 1.63 (m, 4H), 1.94 (quin, 2H, *J*=7.5 Hz), 2.50 (t, 2H, *J*=6.9 Hz), 2.60 (dd, 1H, *J*=7.5, 15.3 Hz), 2.64 (dd, 1H, *J*=5.7, 15.3 Hz), 2.96 (m, 12H), 3.14 (t, 2H, *J*=6.9 Hz), 3.34 (d, 1H, *J*=15.3 Hz), 3.44 (d, 1H, *J*=15.3 Hz), 4.46 (dd, 1H, *J*=5.7, 7.5 Hz), 6.31 (m, 2H), 6.95 (d, 1H, *J*=9.0 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.5, 22.6, 23.1, 23.7, 27.6, 27.7, 30.8, 35.9, 36.4, 36.8, 39.1, 39.2, 43.4, 44.4, 46.7, 46.9, 50.8, 102.8, 107.4, 113.7, 132.4, 155.1, 156.1, 171.5, 172.3, 174.6, 174.9. FABHRMS calcd for $C_{27}H_{48}N_7O_6$ $(M+H)^+$ 566.3670, found 566.3660. ESI-MS/MS: Supplementary data 1 and 2.

4.1.9. *N*-[N^{α}-(Indoleacetyl)-L-asparaginyl]-*N*′-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (17). To a cold (0 °C) and stirred suspension of polyamine **15** (115 mg, 119 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (13 mg, 129 μmol) and indoleacetic acid succinimide ester[45] (49 mg, 180 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/CH_2Cl_2) gave the title compound **17** (102 mg, 84%, two steps) as a white powder. $[\alpha]_D^{24}$ +2.55 (*c* 0.51, MeOH). IR (Nujol) 1668, 1543, 1461, 1250, 1161, 852 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.17 (m, 2H), 1.28 (m, 4H), 1.38 (m, 4H), 1.60 (m, 2H), 2.29 (t, 2H, *J*=6.6 Hz), 2.36 (dd, 1H, *J*=6.9, 15.3 Hz), 2.48 (dd, 1H, *J*=6.0, 15.3 Hz), 2.95 (m, 6H), 3.22 (m, 8H), 3.54 (s, 2H), 4.50 (dd, 1H, *J*=6.0, 6.9 Hz), 4.99 (s, 2H), 5.03 (s, 2H), 6.84 (s, 1H), 6.94 (t, 1H, *J*=7.8 Hz), 7.04 (t, 1H, *J*=7.8 Hz), 7.18 (s, 1H), 7.32 (m, 12H), 7.52 (d, 1H, *J*=7.5 Hz), 7.58 (d, 1H, *J*=5.1 Hz), 7.82 (m, 3H), 7.95 (m, 2H), 8.09 (d, 1H, *J*=8.1 Hz), 10.85 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 23.8, 25.3, 28.7, 28.8, 28.9, 32.7, 35.1, 37.5, 38.0, 38.6, 38.7, 43.2, 44.1, 45.5, 46.5, 47.4, 50.0, 65.4, 66.2, 108.9, 111.5, 118.5, 118.8, 121.1, 124.0, 124.4, 127.4, 127.9, 128.0, 128.5, 128.6, 129.8, 132.0, 132.6, 134.6, 136.3, 137.2, 137.4, 147.7, 155.4, 156.3, 170.2, 170.8, 170.9, 171.7. FABHRMS calcd for $C_{51}H_{63}N_9O_{12}SNa$ $(M+Na)^+$ 1048.4214, found 1048.4216.

4.1.10. NPTX-8 (2). To a stirred solution of polyamine **17** (40 mg, 39.0 μmol) in DMF (1 mL) were added 2-mercaptoethanol (10 mg, 128 μmol) and DBU (20 mg, 131 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. NPTX-8 (**2**) was eluted at 12.26 min and was obtained as a colorless TFA salt (22 mg, 61%, two steps). $[\alpha]_D^{22}$ −2.86 (*c* 0.77, H_2O). IR (Nujol) 3481, 2924, 1697, 1539 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 1.00 (quin, 2H, *J*=7.2 Hz), 1.23 (quin, 4H, *J*=7.2 Hz), 1.60 (m, 4H), 1.94 (quin, 2H, *J*=8.4 Hz), 2.46 (t, 2H, *J*=6.6 Hz), 2.52 (dd, 1H, *J*=7.5, 15.3 Hz), 2.61 (dd, 1H, *J*=5.7, 15.3 Hz), 2.94 (m, 12H), 3.09 (t, 2H, *J*=6.6 Hz), 3.67 (s, 2H), 4.49 (dd, 1H, *J*=5.7, 7.5 Hz), 7.04 (t, 1H, *J*=7.8 Hz), 7.13 (t, 1H, *J*=7.8 Hz), 7.20 (s, 1H), 7.39 (d, 1H, *J*=7.8 Hz), 7.46 (d, 1H, *J*=7.8 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.5, 22.6, 23.0, 23.6, 27.5, 27.6, 30.7, 32.2, 36.1, 36.4, 39.0, 39.1, 43.4, 44.4, 46.6, 46.9, 50.8, 107.4, 111.9, 118.2, 119.5, 122.1, 124.9, 126.5, 136.2, 171.4, 172.1, 174.4, 175.0. FABHRMS calcd for $C_{29}H_{49}N_8O_4$ $(M+H)^+$ 573.3895, found 573.3853. ESI-MS/MS: Supplementary data 3.

4.1.11. *N*-[N^{α}-(4-Benzyloxyindoleacetyl)-L-asparaginyl]-*N*′-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (18). To a cold (0 °C) and stirred suspension of polyamine **15** (200 mg, 206 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (21 mg, 208 μmol) and 4-benzyloxyindoleacetic acid succinimide ester[33] (117 mg, 309 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/CH_2Cl_2) gave the title compound **18** (201 mg, 86%, two steps) as a white powder. $[\alpha]_D^{22}$ +8.07 (*c* 1.50, DMF). IR (Nujol) 1698, 1665, 1639, 1542, 1376, 1248 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.16 (m, 2H), 1.28 (m, 4H), 1.39 (m, 4H), 1.61 (m, 2H), 2.29 (t, 2H, *J*=6.6 Hz), 2.38 (m, 2H), 2.95 (m, 6H), 3.22 (m, 8H), 3.74 (s, 2H), 4.51 (m, 1H), 4.99 (s, 2H), 5.03 (s, 2H), 5.18 (s, 2H), 6.49 (d, 1H, *J*=4.5 Hz), 6.83 (br s, 1H), 6.92 (m, 2H), 7.05 (br s, 1H), 7.32 (m, 15H), 7.47 (m, 2H), 7.56 (m, 1H), 7.80 (m, 4H), 7.94 (m, 2H), 10.88 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 23.8, 24.8, 25.4, 28.7, 28.8, 28.9, 34.5, 35.1, 37.5, 38.0, 38.6, 38.7, 44.1, 45.5, 46.5, 47.4, 49.9, 65.4, 66.3, 69.2, 100.7, 105.3, 108.5, 117.4, 122.0, 122.9, 124.5, 127.4, 127.7, 127.9, 128.0, 128.5, 128.6, 129.8, 132.0, 132.6, 134.6, 137.2, 137.4, 137.9, 138.1, 147.8, 153.0, 155.3, 156.3, 170.2, 170.9, 171.3, 171.9. FABHRMS calcd for $C_{58}H_{70}N_9O_{13}S$ $(M+H)^+$ 1132.4814, found 1132.4833.

4.1.12. NPTX-1 (3). To a stirred solution of polyamine **18** (40 mg, 35.3 μmol) in DMF (1 mL) were added 2-mercaptoethanol (9 mg, 115 μmol) and DBU (18 mg, 118 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-PrNH_2) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-PrNH_2) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. NPTX-1 (**3**) was eluted at 11.31 min and was obtained as a colorless TFA salt (20 mg, 61%, two steps). $[\alpha]_D^{22}$ +1.05 (*c* 1.14, H_2O). IR (Nujol) 3513, 2924, 1670 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 0.90 (quin, 2H, *J*=7.2 Hz), 1.16 (m, 4H), 1.61 (m, 4H), 1.93 (quin, 2H, *J*=7.5 Hz), 2.46 (t, 2H, *J*=6.6 Hz), 2.54 (dd, 1H, *J*=6.0, 16.5 Hz), 2.60 (dd, 1H, *J*=6.0, 16.5 Hz), 2.97 (m, 12H), 3.11 (t, 2H, *J*=6.6 Hz), 3.68 (d, 1H, *J*=15.9 Hz), 3.76 (d, 1H, *J*=15.9 Hz), 4.46 (t, 1H, *J*=6.0 Hz), 6.41 (t, 1H, *J*=4.2 Hz), 6.93 (d, 2H, *J*=4.2 Hz), 7.06 (s, 1H). ^{13}C NMR (75 MHz, D_2O): δ 22.5, 22.6, 22.9, 23.6, 27.5, 27.6, 30.7, 33.8, 35.8, 36.4, 39.0, 39.1, 43.4, 44.4, 46.6, 46.9, 50.7, 103.5, 104.4, 106.6, 116.1, 122.9, 124.1, 138.6, 149.6, 171.4, 172.1, 174.5, 175.9. FABHRMS calcd for $C_{29}H_{49}N_8O_5$ $(M+H)^+$ 589.3810, found 589.3849. ESI-MS/MS: Supplementary data 4.

4.1.13. *N*-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-*N*′-(2-nitrobenzenesulfonyl)-1,4-diaminobutane (19). To a stirred solution of *N*-(2-nitrobenzenesulfonyl)-1,4-diaminobutane (500 mg, 1.83 mmol) in DMF (5 mL) was added Boc-L-Asn-ONp (647 mg, 1.83 mmol) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ solution (5×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (1–12% MeOH/CH_2Cl_2) gave the title compound **19** (845 mg, 95%) as a white powder. $[\alpha]_D^{22}$ −2.13 (*c* 2.44, DMF). IR (Nujol) 1681, 1659, 1543, 1461, 1161 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.35 (m, 13H), 2.34 (m, 2H), 2.86 (m, 2H), 2.96 (quin, 2H, *J*=6.0 Hz), 4.13 (m, 1H), 6.79 (d, 1H, *J*=8.1 Hz), 6.86 (br s, 1H), 7.24 (br s, 1H), 7.69 (br s, 1H), 7.83 (m, 2H), 7.92 (m, 2H), 8.04 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 26.3, 26.6, 28.4, 37.7, 38.2, 42.6, 51.7, 78.4, 124.6, 129.6, 132.8, 133.0, 134.2, 148.0, 155.3, 171.5, 175.8. HRMS calcd for $C_{14}H_{20}N_5O_6S$ (M^+−Boc) 386.1134, found 386.1113.

4.1.14. *N*-(2-Nitrobenzenesulfonyl)-*N*′-(benzyloxycarbonyl)-1,3-diaminopropane. To a cold (0 °C) and stirred suspension of *N*-(2-nitrobenzenesulfonyl)-1,3-diaminopropane[22] (**20**) (400 mg, 1.54 mmol) in CH_2Cl_2 (10 mL) were added CbzCl (30% in toluene, 876 mg, 1.54 mmol) and TEA (156 mg, 1.54 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated NH_4Cl solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (30–40% EtOAc/hexane) gave the title compound (530 mg, 88%) as a colorless oil. IR (film) 1702, 1540, 1442, 1364, 1259, 1166, 854 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.71 (quin, 2H, *J*=5.7 Hz), 3.15 (q, 2H, *J*=5.7 Hz), 3.28 (q, 2H, *J*=5.7 Hz), 5.06 (br s, 1H), 5.08 (s, 2H), 5.86 (br s, 1H), 7.33 (m, 5H), 7.71 (m, 2H), 7.83 (m, 1H), 8.10 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.2, 37.6, 40.7, 66.8, 125.3, 128.0, 128.1, 128.5, 130.8, 132.7, 133.5, 133.8, 136.4, 148.0, 156.8. HRMS calcd for $C_{11}H_{15}N_2O_2$ (M^+−Ns) 207.1133, found 207.1118.

4.1.15. 7-Benzyloxycarbonylamino-4-(2-nitrobenzenesulfonyl)-4-azaheptan-1-yl bromide (21). To a solution of the above sulfonamide (420 mg, 1.07 mmol) and 1,3-dibromopropane (648 mg, 3.21 mmol) in DMF (5 mL) was added Cs_2CO_3 (525 mg, 1.61 mmol). After being stirred for 0.5 h at 50 °C, the reaction mixture was diluted with EtOAc (100 mL) and washed with H_2O (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (20–50% EtOAc/hexane) gave the title compound **21** (501 mg, 91%) as a colorless oil. IR (film) 1718, 1543, 1455, 1372, 1247, 1161, 852 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.79 (quin, 2H, *J*=6.6 Hz), 2.08 (quin, 2H, *J*=7.2 Hz), 3.23 (m, 2H), 3.38 (m, 6H), 5.09 (s, 2H), 5.14 (br s, 1H), 7.35 (m, 5H), 7.65 (m, 3H), 7.99 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.2, 29.8, 31.2, 37.7, 45.4, 46.1, 66.6, 124.3, 128.0, 128.1, 128.5, 130.9, 131.8, 132.8, 133.8, 136.5, 148.0, 156.4. HRMS calcd for $C_{14}H_{20}N_2O_2Br$ (M^+−Ns) 327.0708, found 327.0710.

4.1.16. *N*-(*N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-asparaginyl)-12-benzyloxycarbonylamino-5,9-[di-(2-nitrobenzenesulfonyl)]-5,9-diaza-1-aminoundecane (22). To a solution of sulfonamide **19** (244 mg, 500 μmol) and bromide **21** (387 mg, 752 μmol) in DMF (3 mL) were added Cs_2CO_3 (489 mg, 1.50 mmol) and TBAI (19.0 mg, 51.4 μmol). After being stirred for 1 h at 70 °C, the reaction mixture was diluted with EtOAc (200 mL) and washed with H_2O (3×40 mL) and brine (3×40 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–4% MeOH/CH_2Cl_2) gave the title compound **22** (433 mg, 94% from **19**) as a colorless solid. $[\alpha]_D^{24}$ −2.43 (*c* 1.48, MeOH). IR (Nujol) 1712, 1670, 1544, 1459, 1372, 1161, 852 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H), 1.53 (m, 4H), 1.78 (m, 4H), 2.51 (dd, 1H, *J*=6.0, 15.6 Hz), 2.88 (dd, 1H, *J*=4.8, 15.6 Hz), 3.25 (m, 12H), 4.41 (m, 1H), 5.08 (s, 2H), 5.50 (br s, 1H), 5.68 (br s, 1H), 6.08 (br s, 2H), 6.85 (br s, 1H), 7.35 (m, 5H), 7.61 (m, 2H), 7.68 (m, 4H), 7.96 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.1, 26.7, 27.6, 28.3, 28.4, 38.0, 38.4, 45.1, 45.4, 45.5, 47.6, 51.2, 66.6, 80.3, 124.2, 124.3, 128.1, 128.5, 130.6, 131.8, 131.9, 132.7, 132.9, 133.6, 133.7, 136.6, 148.0, 155.7, 156.6,

171.4, 173.4. FABHRMS calcd for $C_{39}H_{52}N_8O_{14}S_2Na$ $(M+Na)^+$ 943.2942, found 943.2951.

4.1.17. *N*-[N^{α}-(Indoleacetyl)-L-asparaginyl]-12-benzyloxycarbonylamino-5,9-[di-(2-nitrobenzenesulfonyl)]-5,9-diaza-1-aminoundecane (23). To a cold (0 °C) and stirred suspension of polyamine **22** (156 mg, 170 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (18 mg, 178 μmol) and indoleacetic acid succinimide ester[45] (70 mg, 257 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–8% EtOH/CH_2Cl_2) gave the title compound **23** (139 mg, 84%, two steps) as a white powder. $[\alpha]_D^{22}$ +5.06 (*c* 1.76, acetone). IR (Nujol) 1666, 1542, 1461, 1376, 1256, 1160, 852 cm^{-1}. ^1H NMR (300 MHz, CD_3COCD_3): δ 1.21 (quin, 2H, *J*=6.3 Hz), 1.39 (quin, 2H, *J*=7.2 Hz), 1.75 (quin, 2H, *J*=6.9 Hz), 1.78 (quin, 2H, *J*=6.6 Hz), 2.52 (dd, 1H, *J*=6.0, 15.6 Hz), 2.72 (dd, 1H, *J*=5.1, 15.6 Hz), 3.02 (m, 2H), 3.13 (m, 2H), 3.20 (m, 2H), 3.23 (m, 2H), 3.30 (m, 2H), 3.37 (m, 2H), 3.69 (s, 2H), 4.65 (m, 1H), 5.06 (s, 2H), 6.31 (br s, 1H), 6.43 (br s, 1H), 6.94 (br s, 1H), 7.01 (t, 1H, *J*=6.9 Hz), 7.09 (t, 1H, *J*=6.9 Hz), 7.33 (m, 7H), 7.53 (br s, 1H), 7.59 (d, 1H, *J*=6.9 Hz), 7.83 (m, 6H), 8.00 (m, 3H), 10.13 (br s, 1H). ^{13}C NMR (75 MHz, CD_3COCD_3): δ 25.1, 26.6, 27.2, 33.3, 36.7, 38.1, 38.2, 38.4, 44.8, 45.3, 45.7, 47.4, 50.2, 65.9, 109.2, 111.7, 118.8, 119.2, 121.8, 124.1, 124.3, 124.4, 124.5, 127.8, 128.0, 128.1, 128.6, 130.4, 130.5, 132.4, 132.5, 132.9, 133.1, 134.3, 134.5, 137.0, 137.8, 148.4, 156.7, 171.9, 172.3, 173.8. FABHRMS calcd for $C_{44}H_{51}N_9O_{13}S_2Na$ $(M+Na)^+$ 1000.2946, found 1000.2936.

4.1.18. NPTX-473 (4). To a stirred solution of polyamine **23** (40 mg, 40.9 μmol) in DMF (1 mL) were added 2-mercaptoethanol (20 mg, 256 μmol) and DBU (40 mg, 263 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. NPTX-473 (**4**) was eluted at 12.02 min and was obtained as a colorless TFA salt (20 mg, 60%, two steps). $[\alpha]_D^{22}$ −3.81 (*c* 0.42, H_2O). IR (Nujol) 3300, 2924, 1670, 1549 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 1.32 (m, 4H), 1.87 (quin, 2H, *J*=8.1 Hz), 1.93 (quin, 2H, *J*=6.6 Hz), 2.56 (dd, 1H, *J*=8.1, 15.3 Hz), 2.64 (dd, 1H, *J*=7.2, 15.3 Hz), 2.78 (m, 4H), 2.98 (m, 8H), 3.69 (s, 2H), 4.47 (dd, 1H, *J*=7.2, 8.1 Hz), 7.06 (t, 1H, *J*=8.1 Hz), 7.16 (t, 1H, *J*=8.1 Hz), 7.22 (s, 1H), 7.41 (d, 1H, *J*=8.1 Hz), 7.48 (d, 1H, *J*=8.1 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.6, 22.7, 23.9, 25.5, 32.4, 36.2, 36.7, 38.6, 44.1, 44.7, 45.5, 47.2, 51.3, 107.8, 112.2, 118.6, 119.8, 122.4, 125.2, 126.8, 136.5, 172.8, 174.6, 175.4. FABHRMS calcd for $C_{24}H_{39}N_7O_3$ $(M+H)^+$ 474.3193, found 474.3171. ESI-MS/MS: Supplementary data 5.

4.1.19. *N*-(2-Nitrobenzenesulfonyl)-*N*′-(benzyloxycarbonyl)-1,4-diaminobutane. To a cold (0 °C) and stirred suspension of *N*-(2-nitrobenzenesulfonyl)-1,4-diaminobutane[22] (**12**) (400 mg, 1.46 mmol) in CH_2Cl_2 (10 mL) were added CbzCl (30% in toluene, 830 mg, 1.46 mmol) and TEA (148 mg, 1.46 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated NH_4Cl solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (30–50% EtOAc/hexane) gave the title compound (564 mg, 95%) as a colorless oil. IR (film) 1703, 1540, 1442, 1364, 1252, 1166, 854 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.55 (m, 4H), 3.10 (m, 2H), 3.16 (m, 2H), 4.89 (br s, 1H), 5.08 (s, 2H), 5.46 (br s, 1H), 7.35 (m, 5H), 7.72 (m, 2H), 7.84 (m, 1H), 8.12 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.8, 26.9, 40.3, 43.3, 66.6, 125.3, 128.0, 128.5, 131.0, 132.8, 133.6, 136.5, 148.0, 156.5. HRMS calcd for $C_{12}H_{17}N_2O_2$ (M^+−Ns) 221.1290, found 221.1299.

4.1.20. Methyl 7-benzyloxycarbonylamino-3-(2-nitrobenzenesulfonyl)-3-azaheptanoate. To a solution of the above sulfonamide (514 mg, 1.26 mmol) and methyl bromoacetate (289 mg, 1.89 mmol) in DMF (4 mL) was added Cs_2CO_3 (616 mg, 1.89 mmol). After being stirred for 0.5 h at 50 °C, the reaction mixture was diluted with EtOAc (150 mL) and washed with H_2O (3×30 mL) and brine (3×30 mL). Aqueous layers were extracted with EtOAc (3×30 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (30–50% EtOAc/hexane) gave the ester (520 mg, 86%) as a colorless oil. IR (film) 1752, 1714, 1546, 1439, 1372, 1254, 1218, 1164, 853 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.54 (m, 4H), 3.17 (m, 2H), 3.41 (t, 2H, *J*=7.2 Hz), 3.65 (s, 3H), 4.16 (s, 2H), 5.00 (br s, 1H), 5.08 (s, 2H), 7.35 (m, 5H), 7.60 (m, 1H), 7.68 (m, 2H), 8.06 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.8, 26.8, 40.3, 47.6, 48.1, 52.3, 66.6, 124.1, 128.0, 128.1, 128.5, 130.8, 131.7, 133.2, 133.6, 136.6, 147.9, 156.5, 162.8. HRMS calcd for $C_{13}H_{18}N_3O_6S$ (M^+−Cbz) 344.0917, found 344.0918.

4.1.21. Methyl 7-benzyloxycarbonylamino-3-(2-nitrobenzenesulfonyl)-3-azaheptanoic acid (24). To a cold (0 °C) and stirred solution of the above ester (500 mg, 1.04 mmol) in MeOH (5 mL) was added 3.0 M aqueous NaOH solution (1.04 mL, 3.12 mmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was adjusted to pH 2.0 by concentrated HCl. The resultant

mixture was diluted with EtOAc (100 mL) and washed with H_2O (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–40% MeOH/CH_2Cl_2) gave the title compound **24** (414 mg, 86%) as a colorless oil. IR (film) 1729, 1714, 1548, 1455, 1371, 1259, 1162, 853 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.34 (m, 2H), 1.41 (m, 2H), 3.02 (m, 2H), 3.31 (m, 2H), 4.04 (s, 2H), 5.02 (s, 2H), 5.07 (br s, 1H), 7.29 (m, 5H), 7.54 (m, 3H), 8.00 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.5, 26.6, 40.3, 48.1, 49.0, 66.6, 124.1, 127.9, 128.0, 128.4, 130.7, 132.0, 133.0, 133.5, 136.5, 147.7, 156.6, 174.4. HRMS calcd for $C_{14}H_{19}N_2O_4$ (M^+−Ns) 279.1345, found 279.1347.

4.1.22. *N*-[N^{α}-(Indoleacetyl)-L-asparaginyl]-N'-[7-benzyloxycarbonylamino-3-(2-nitrobenzenesulfonyl)-3-azaheptanoyl]-1,5-diaminopentane (26). To a cold (0 °C) and stirred suspension of carboxylic acid **24** (186 mg, 400 μmol) and HOSu (92 mg, 799 μmol) in CH_2Cl_2 (5 mL) was added DCC (124 mg, 601 μmol) slowly. After being stirred for 4 h at 0 °C, the reaction mixture was filtrated through a pad of silica gel with EtOAc. The filtrate was concentrated in vacuo to give the crude succinimidyl ester as a colorless oil.

To a stirred solution of obtained ester in DMF (3 mL) was added amine **11** (127 mg, 401 μmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–8% MeOH/CH_2Cl_2) gave the polyamine **25** (252 mg), which was used in the next step without further purification. ^1H NMR (300 MHz, CD_3OD): δ 1.29 (m, 2H), 1.43 (s, 9H), 1.45 (m, 8H), 2.57 (dd, 1H, J=6.3, 13.2 Hz), 2.66 (dd, 1H, J=6.3, 13.2 Hz), 3.06 (t, 2H, J=6.6 Hz), 3.12 (t, 2H, J=6.0 Hz), 3.15 (t, 2H, J=6.6 Hz), 3.39 (t, 2H, J=6.6 Hz), 4.02 (s, 2H), 4.36 (t, 1H, J=6.3 Hz), 5.04 (s, 2H), 7.32 (m, 5H), 7.75 (m, 3H), 8.10 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.0, 26.0, 27.9, 28.7, 29.9, 38.4, 40.3, 41.2, 50.4, 53.1, 67.3, 80.9, 125.4, 128.8, 129.0, 129.5, 131.9, 133.1, 133.9, 135.3, 138.5, 149.5, 157.5, 158.9, 170.2, 173.8, 175.1.

To a cold (0 °C) and stirred suspension of polyamine **25** (120 mg, 157 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (16 mg, 158 μmol) and indoleacetic acid succinimide ester[54] (65 mg, 239 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/CH_2Cl_2) gave the title compound **26** (113 mg, 73%, four steps) as a white powder. $[\alpha]_D^{22}$ +5.59 (*c* 2.22, DMF). IR (Nujol) 1660, 1538, 1456, 1376, 1257, 1158, 852 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.14 (m, 2H), 1.28 (m, 6H), 1.45 (m, 2H), 2.37 (dd, 1H, J=7.8, 15.3 Hz), 2.48 (dd, 1H, J=6.0, 15.3 Hz), 2.94 (m, 6H), 3.27 (m, 2H), 3.54 (s, 2H), 3.93 (s, 2H), 4.50 (dd, 1H, J=6.0, 7.8 Hz), 4.98 (s, 2H), 6.84 (br s, 1H), 6.94 (t, 1H, J=6.9 Hz), 7.05 (t, 1H, J=6.9 Hz), 7.18 (br s, 1H), 7.21 (t, 1H, J=5.4 Hz), 7.33 (m, 7H), 7.51 (d, 1H, J=8.1 Hz), 7.59 (t, 1H, J=5.4 Hz), 7.80 (m, 2H), 7.91 (m, 2H), 8.10 (m, 2H), 10.84 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.7, 24.7, 26.7, 28.8, 32.7, 37.5, 38.7, 48.2, 48.7, 50.1, 65.4, 108.9, 111.5, 118.5, 118.9, 121.2, 124.0, 124.3, 127.4, 127.9, 128.0, 128.6, 130.3, 132.3, 132.4, 134.5, 136.3, 137.5, 147.8, 156.3, 167.1, 170.8, 170.9, 171.7. FABHRMS calcd for $C_{39}H_{48}N_8O_{10}SNa$ $(M+Na)^+$ 843.3112, found 843.3115.

4.1.23. NPTX-501 (5). To a stirred solution of polyamine **26** (40 mg, 48.7 μmol) in DMF (1 mL) were added 2-mercaptoethanol (12 mg, 154 μmol) and DBU (24 mg, 158 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. NPTX-501 (**5**) was eluted at 13.21 min and was obtained as a colorless TFA salt (23 mg, 65%, two steps). $[\alpha]_D^{22}$ −0.41 (*c* 1.22, H_2O). IR (Nujol) 3554, 2924, 1698, 1541 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 0.99 (quin, 2H, J=7.2 Hz), 1.23 (m, 4H), 1.55 (m, 4H), 2.51 (dd, 1H, J=7.8, 15.3 Hz), 2.60 (dd, 1H, J=6.0, 15.3 Hz), 2.84 (m, 4H), 2.97 (m, 4H), 3.56 (s, 2H), 3.66 (s, 2H), 4.48 (dd, 1H, J=6.0, 7.8 Hz), 7.03 (t, 1H, J=8.1 Hz), 7.13 (t, 1H, J=8.1 Hz), 7.19 (s, 1H), 7.38 (d, 1H, J=8.1 Hz), 7.44 (d, 1H, J=8.1 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.4, 22.9, 23.7, 27.6, 32.1, 36.1, 38.6, 39.0, 39.1, 46.8, 47.7, 50.8, 107.4, 111.9, 118.2, 119.5, 122.1, 124.8, 126.5, 136.2, 165.4, 172.1, 174.4, 174.9. FABHRMS calcd for $C_{25}H_{40}N_7O_4$ $(M+H)^+$ 502.3142, found 502.3108. ESI-MS/MS: Supplementary data 6.

4.1.24. *N*-[N^{α}-(*tert*-Butoxycarbonyl)-L-asparaginyl]-N'-[8-(N^{α},N^{δ},N^{ω}-tribenzyloxycarbonylarginyl)-amino-4-benzyloxycarbonyl-4-azaoctanoyl]-1,5-diaminopentane (27). To a stirred solution of polyamine **14** (300 mg, 386 μmol) in DMF (1 mL) were added 2-mercaptoethanol (91 mg, 1.16 mmol) and DBU (176 mg, 1.16 mmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) to afford the secondary amine as a white powder.

To a stirred solution of obtained amine in DMF (1 mL) was added tri-Cbz-arginine succinimide ester[48] (389 mg, 577 μmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–8% EtOH/CH_2Cl_2) to afford the title compound **27** (337 mg, 76%) as a white powder. $[\alpha]_D^{22}$ +6.36 (*c* 1.32, DMSO). IR (Nujol) 1725, 1688, 1652, 1259, 1103 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.25 (m, 2H), 1.35 (m, 19H), 1.55 (m, 4H), 2.29 (t, 2H, *J*=6.9 Hz), 2.35 (m, 2H), 2.98 (m, 6H), 3.15 (m, 2H), 3.42 (m, 2H), 3.84 (m, 3H), 4.16 (dd, 1H, *J*=5.2, 6.6 Hz), 4.97 (s, 2H), 5.03 (s, 4H), 5.20 (s, 2H), 6.81 (d, 1H, *J*=8.4 Hz), 6.87 (s, 1H), 7.31 (m, 20H), 7.67 (br s, 1H), 7.85 (m, 2H), 9.14 (br s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.8, 25.3, 26.5, 28.4, 28.9, 29.5, 34.4, 35.2, 37.6, 38.5, 38.7, 43.3, 44.0, 44.6, 46.6, 46.7, 51.7, 54.8, 65.6, 66.2, 66.3, 68.4, 78.4, 127.5, 127.8, 127.9, 128.1, 128.4, 128.5, 128.6, 128.7, 135.5, 137.2, 137.3, 155.2, 155.3, 155.4, 156.1, 159.9, 163.1, 170.2, 171.4, 171.7, 171.8. FABHRMS calcd for $C_{59}H_{79}N_{10}O_{14}$ $(M+H)^+$ 1151.5778, found 1151.5778.

4.1.25. *N*-[N^{α}-(*tert*-Butoxycarbonyl)-L-asparaginyl]-N'-[8-(N^{α},N^{δ},N^{ω}-tribenzyloxycarbonylarginyl)-amino-4-benzyloxycarbonyl-4-azaoctanoyl]-1,5-diaminopentane (28). To a cold (0 °C) and stirred suspension of polyamine **27** (100 mg, 86.9 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (9 mg, 89.0 μmol) and 2,4-dibenzyloxyphenylacetic acid succinimide ester[28] (58 mg, 130 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–8% MeOH/CH_2Cl_2) to afford the title compound **28** (104 mg, 87%) as a white powder. $[\alpha]_D^{22}$ +2.57 (*c* 1.05, DMF). IR (Nujol) 1691, 1664, 1645, 1614, 1260 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.17 (m, 2H), 1.31 (m, 8H), 1.50 (m, 4H), 2.29 (t, 2H, *J*=6.6 Hz), 2.36 (dd, 1H, *J*=6.3, 15.9 Hz), 2.43 (dd, 1H, *J*=6.0, 15.9 Hz), 2.96 (m, 6H), 3.14 (m, 2H), 3.27 (m, 2H), 3.41 (s, 2H), 3.84 (m, 3H), 4.53 (m, 1H), 4.97 (s, 2H), 5.03 (s, 6H), 5.08 (s, 2H), 5.20 (s, 2H), 6.52 (d, 1H, *J*=9.3 Hz), 6.68 (s, 1H), 6.85 (s, 1H), 7.07 (d, 1H, *J*=8.4 Hz), 7.35 (m, 32H), 7.60 (t, 1H, *J*=7.2 Hz), 7.83 (m, 2H), 7.96 (d, 1H, *J*=8.1 Hz)), 9.14 (br s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.8, 23.8, 25.3, 26.5, 28.8, 28.9, 29.5, 36.4, 37.5, 38.5, 38.6, 38.7, 44.6, 46.5, 49.4, 50.0, 54.7, 65.6, 66.2, 66.3, 68.3, 69.4, 69.5, 100.7, 105.9, 117.3, 127.3, 127.5, 127.9, 128.0, 128.6, 128.7, 131.1, 135.5, 137.2, 137.3, 137.4, 155.1, 155.3, 156.1, 157.1, 158.5, 159.9, 163.1, 170.1, 170.5, 170.8, 171.6, 171.7. FABHRMS calcd for $C_{76}H_{88}N_{10}O_{15}Na$ $(M+Na)^+$ 1403.6328, found 1403.6323.

4.1.26. NSTX-3 (6). A solution of the polyamine **28** (26 mg, 18.8 μmol) in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 4 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. NSTX-3 (**6**) was eluted at 9.66 min and was obtained as a colorless TFA salt (14 mg, 74%). $[\alpha]_D^{22}$ +2.95 (*c* 1.73, H_2O). IR (Nujol) 3444, 2925, 1696, 1523 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 1.03 (quin, 2H, *J*=7.5 Hz), 1.29 (m, 4H), 1.52 (m, 6H), 1.77 (dt, 2H, *J*=9.6, 6.9 Hz), 2.50 (t, 2H, *J*=6.9 Hz), 2.52 (dd, 1H, *J*=7.8, 15.0 Hz), 2.63 (dd, 1H, *J*=5.7, 15.0 Hz), 2.93 (t, 4H, *J*=8.1 Hz), 3.08 (t, 4H, *J*=6.9 Hz), 3.13 (t, 4H, *J*=6.9 Hz), 3.34 (d, 1H, *J*=15.3 Hz), 3.43 (d, 1H, *J*=15.3 Hz), 3.81 (t, 1H, *J*=6.9 Hz), 4.46 (dd, 1H, *J*=5.7, 7.8 Hz), 6.31 (m, 2H), 6.94 (d, 1H, *J*=9.0 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.8, 23.0, 23.5, 25.3, 27.6, 27.7, 27.9, 30.8, 35.9, 36.8, 38.7, 39.1, 39.2, 40.2, 43.3, 46.9, 50.8, 52.9, 102.7, 107.4, 113.6, 132.3, 155.1, 156.1, 156.7, 169.2, 171.5, 172.2, 174.6. FABHRMS calcd for $C_{30}H_{53}N_{10}O_7$ $(M+H)^+$ 665.4107, found 665.4083. ESI-MS/MS: Supplementary data 7.

4.1.27. Methyl 7-(2-nitrobenzenesulfonyl)-amino-4-benzyloxycarbonyl-4-azahenpanoate. A solution of methyl acrylate (166 mg, 1.93 mmol) in EtOH (10 mL) was added at room temperature over 5 h to a stirring solution of *N*-(2-nitrobenzenesulfonyl)-1,3-diaminopropane[18] (**20**) (500 mg, 1.93 mmol) in EtOH (20 mL). The solvent was evaporated in vacuo, and the residue was subjected to silica gel chromatography (5–20% MeOH/CH_2Cl_2) to give the crude secondary amine as a colorless oil.

To a cold (0 °C) and stirred solution of obtained secondary amine in CH_2Cl_2 (20 mL) were added CbzCl (30% in toluene, 1.10 g, 1.93 mmol) and Et_3N (195 mg, 1.93 mmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated NH_4Cl solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (20–50% EtOAc/hexane) gave the methyl ester (796 mg, 86%, two steps) as a colorless oil. IR (film) 1732, 1695, 1539, 1480, 1368, 1125, 854 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.73 (quin, 2H, *J*=6.6 Hz), 2.57 (m, 2H), 3.11 (m, 2H), 3.37 (t, 2H, *J*=6.3 Hz), 3.49 (t, 2H, *J*=7.2 Hz), 3.64 (s, 3H), 5.12 (s, 2H), 5.44 (br s, 1H), 7.33 (m, 5H), 7.72 (m, 2H), 7.83 (m, 1H), 8.09 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5, 33.6, 40.6, 43.1, 44.7, 51.8, 67.4, 125.2, 127.9, 128.1, 128.5, 130.7, 132.6, 133.3, 134.2, 136.3, 148.0, 155.6, 171.9. HRMS calcd for $C_{20}H_{22}N_3O_7S$ (M^+-OMe) 448.1179, found 448.1183.

4.1.28. 7-(2-Nitrobenzenesulfonyl)-amino-4-benzyloxycarbonyl-4-azahenpanoic acid (29). To a cold (0 °C) and stirred solution of the above ester (600 mg, 1.25 mmol) in MeOH (4 mL) was added 3.0 M aqueous NaOH solution (1.25 mL, 3.75 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was adjusted to pH 2.0 by concentrated HCl. The resultant mixture was diluted with EtOAc (200 mL) and washed with H_2O (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (1–40% MeOH/CH_2Cl_2) gave the title compound **29** (494 mg, 85%) as a colorless oil. IR (film) 1694, 1540, 1484, 1424, 1365, 1166, 854 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.75 (quin, 2H, J=6.6 Hz), 2.59 (m, 2H), 3.09 (m, 2H), 3.37 (m, 2H), 3.50 (t, 2H, J=6.6 Hz), 5.12 (s, 2H), 5.47 (br s, 1H), 7.33 (m, 5H), 7.70 (m, 2H), 7.83 (m, 1H), 8.08 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.4, 33.4, 40.7, 42.9, 43.7, 67.4, 125.2, 127.8, 128.0, 128.5, 130.7, 132.6, 133.3, 136.2, 147.9, 156.5, 175.9. HRMS calcd for $C_{12}H_{16}N_3O_6S$ (M^+-Na) 330.0760, found 330.0757.

4.1.29. *N*-[N^{α}-(*tert*-Butoxycarbonyl)-L-asparaginyl]-*N*′-[7-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaheptanoyl]-1,5-diaminopentane (30). To a cold (0 °C) and stirred suspension of carboxylic acid **29** (377 mg, 810 μmol) and HOSu (186 mg, 1.62 mmol) in CH_2Cl_2 (5 mL) was added DCC (252 mg, 1.22 mmol) slowly. After being stirred for 5 h at 0 °C, the reaction mixture was filtrated through a pad of silica gel with EtOAc. The filtrate was concentrated in vacuo to give the crude succinimidyl ester as a colorless oil.

To a stirred solution of obtained ester in DMF (6 mL) was added amine **11** (256 mg, 0.81 mmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/CH_2Cl_2) gave the title compound **30** (505 mg, 82%, two steps) as a colorless solid. $[\alpha]_D^{24}$ −2.53 (*c* 2.96, MeOH). IR (Nujol) 1685, 1636, 1540, 1480, 1367, 1164, 854 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD): δ 1.26 (m, 2H), 1.39 (s, 9H), 1.43 (m, 4H), 1.69 (quin, 2H, J=7.2 Hz), 2.36 (t, 2H, J=6.6 Hz), 2.52 (dd, 1H, J=5.7, 16.2 Hz), 2.59 (dd, 1H, J=5.7, 16.2 Hz), 2.84 (m, 2H), 3.07 (m, 2H), 3.13 (t, 2H, J=6.6 Hz), 3.26 (m, 2H), 3.44 (m, 2H), 4.32 (dd, 1H, J=5.1, 5.7 Hz), 5.05 (s, 2H), 7.28 (m, 5H), 7.75 (m, 3H), 7.99 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 25.1, 28.7, 29.8, 29.9, 35.8, 36.4, 38.4, 40.3, 41.8, 41.9, 45.0, 46.2, 53.1, 63.4, 80.9, 125.9, 128.9, 129.1, 129.6, 131.5, 133.6, 134.8, 135.0, 138.1, 150.0, 157.5, 158.1, 173.5, 173.8, 175.1. FABHRMS calcd for $C_{34}H_{49}N_7O_{11}SNa$ (M^++Na) 786.3129, found 786.3119.

4.1.30. *N*-[N^{α}-(4-Benzyloxyphenylacetyl)-L-asparaginyl]-*N*′-[7-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaheptanoyl]-1,5-diaminopentane (31). To a cold (0 °C) and stirred suspension of polyamine **30** (170 mg, 223 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added Et_3N (23 mg, 228 μmol) and 4-benzyloxyphenylacetic acid succinimide ester[31] (114 mg, 336 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/CH_2Cl_2) gave the title compound **31** (174 mg, 88%, two steps) as a white powder. $[\alpha]_D^{22}$ −3.88 (*c* 2.55, DMF). IR (Nujol) 1641, 1542, 1457, 1376, 1239, 1164, 853 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.14 (m, 2H), 1.32 (m, 4H), 1.62 (m, 2H), 2.27 (t, 2H, J=7.2 Hz), 2.34 (dd, 1H, J=7.8, 15.6 Hz), 2.43 (dd, 1H, J=6.3, 15.6 Hz), 2.87 (m, 2H), 2.96 (m, 4H), 3.18 (m, 2H), 3.36 (m, 4H), 4.47 (dd, 1H, J=6.3, 7.8 Hz), 5.02 (s, 2H), 5.05 (s, 2H), 6.85 (br s, 1H), 6.90 (d, 2H, J=8.4 Hz), 7.14 (d, 2H, J=8.4 Hz), 7.32 (m, 10H), 7.41 (m, 1H), 7.67 (t, 1H, J=5.7 Hz), 7.84 (m, 3H), 7.95 (m, 2H), 8.07 (br s, 1H), 8.15 (d, 1H, J=8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.8, 28.0, 28.8, 28.9, 34.3, 35.0, 37.6, 38.6, 38.7, 40.7, 41.4, 43.5, 44.8, 50.1, 66.3, 69.4, 114.7, 124.6, 127.5, 127.8, 127.9, 128.0, 128.5, 128.6, 129.6, 130.3, 132.7, 132.8, 134.3, 137.2, 137.4, 148.0, 157.1, 170.1, 170.5, 170.9, 171.6. FABHRMS calcd for $C_{44}H_{54}N_7O_{11}S$ $(M+H)^+$ 888.3602, found 888.3604.

4.1.31. Joramine (7). To a stirred solution of polyamine **31** (40 mg, 45.1 μmol) in DMF (1 mL) were added 2-mercaptoethanol (11 mg, 141 μmol) and DBU (22 mg, 145 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the primary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 3 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing 0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. Joramine (**7**) was eluted at 10.24 min and was obtained as a colorless TFA salt (22 mg, 69%). $[\alpha]_D^{22}$ −10.29 (*c* 0.35, H_2O). IR (Nujol) 3479, 2924, 1696, 1515 cm^{-1}. ^{1}H NMR (300 MHz, D_2O): δ 1.05 (quin, 2H, J=6.9 Hz), 1.28 (quin, 4H, J=6.9 Hz), 1.96 (quin, 2H, J=8.1 Hz), 2.53 (t, 2H, J=6.9 Hz), 2.57 (dd, 1H, J=8.1, 15.3 Hz), 2.64 (dd, 1H, J=6.0, 15.3 Hz), 3.00 (m, 8H), 3.18 (t, 2H, J=6.9 Hz), 3.42 (s, 2H), 4.46 (dd, 1H, J=6.0, 8.1 Hz), 6.74 (d, 2H, J=8.1 Hz), 7.05 (d, 2H, J=8.1 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.8, 23.3, 27.4, 27.5, 30.6, 36.0, 36.2, 38.9, 39.0, 41.0, 43.4, 44.2, 50.7, 115.4, 126.4, 130.2, 154.4, 171.2, 171.9, 174.2, 174.6. FABHRMS calcd for $C_{23}H_{38}N_6O_5$ $(M+H)^+$ 479.3008, found 479.2959. ESI-MS/MS: Supplementary data 8.

4.1.32. 7-Allyloxycarbonylamino-4-(2-nitrobenzenesulfonyl)-4-azaheptan-1-yl bromide (32). To a solution of *N*-(2-nitrobenzenesulfonyl)-*N*′-(allyloxycarbonyl)-1,3-diaminopropane (600 mg, 1.75 mmol), which was prepared from sulfonamide **20** in 93% yield,[18] and 1,3-dibromopropane (1.06 g, 5.20 mmol) in DMF (10 mL) was added Cs_2CO_3 (855 mg, 2.62 mmol). After being stirred for 0.5 h at 50 °C, the reaction mixture was diluted with EtOAc (200 mL) and washed with H_2O (3×40 mL) and brine (3×40 mL). Aqueous layers were extracted with EtOAc (3×50 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (20–40% EtOAc/hexane) gave the title

compound **32** (730 mg, 90%) as a colorless oil. IR (film) 1715, 1546, 1440, 1373, 1247, 1162, 931, 852 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.80 (quin, 2H, J=6.3 Hz), 2.10 (quin, 2H, J=6.3 Hz), 3.25 (q, 2H, J=6.3 Hz), 3.40 (m, 6H), 4.56 (d, 2H, J=5.4 Hz), 5.08 (br s, 1H), 5.22 (dd, 1H, J=1.8, 10.8 Hz), 5.31 (dd, 1H, J=1.8, 15.9 Hz), 5.92 (ddd, 1H, J=5.4, 10.8, 15.9 Hz), 7.66 (m, 1H), 7.72 (m, 2H), 8.04 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1, 29.7, 31.1, 37.5, 45.3, 45.6, 65.4, 117.5, 124.2, 130.9, 131.6, 132.7, 133.6, 147.9, 156.2. HRMS calcd for $C_{10}H_{18}N_2O_2Br$ (M^+-Ns) 277.0551, found 277.0564.

4.1.33. *N*-(N^{α}-*tert*-Butoxycarbonyl-L-asparaginyl)-12-(N^{α},N^{δ},N^{ω}-tribenzyloxycarbonylarginyl)-amino-6,10-[di-(2-nitrobenzenesulfonyl)]-6,10-diaza-1-aminoundecane (34). To a solution of sulfonamide **10** (200 mg, 399 μmol) and bromide **32** (278 mg, 599 μmol) in DMF (2 mL) were added Cs_2CO_3 (391 mg, 1.20 mmol) and TBAI (15.0 mg, 40.6 μmol). After being stirred for 1 h at 70 °C, the reaction mixture was diluted with EtOAc (150 mL) and washed with H_2O (3×30 mL) and brine (3×30 mL). Aqueous layers were extracted with EtOAc (3×30 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–4% MeOH/CH_2Cl_2) gave the title compound **33** (332 mg), which was used in the next step without further purification. ^1H NMR (300 MHz, CD_3COCD_3): δ 1.27 (m, 2H), 1.40 (s, 9H), 1.48 (m, 4H), 1.78 (quin, 2H, J=6.9 Hz), 1.86 (quin, 2H, J=7.2 Hz), 2.59 (dd, 1H, J=6.6, 15.9 Hz), 2.73 (dd, 1H, J=4.8, 15.9 Hz), 3.16 (m, 4H), 3.36 (m, 8H), 4.35 (dd, 1H, J=4.8, 6.6 Hz), 4.50 (d, 2H, J=5.4 Hz), 5.14 (dd, 1H, J=1.2, 10.8 Hz), 5.27 (dd, 1H, J=1.2, 16.2 Hz), 5.92 (ddd, 1H, J=5.4, 10.8, 16.2 Hz), 6.36 (m, 3H), 6.98 (br s, 1H), 7.33 (br s, 1H), 7.87 (m, 6H), 8.06 (m, 2H). ^{13}C NMR (75 MHz, CD_3COCD_3): δ 24.6, 28.3, 28.8, 28.9, 30.1, 38.4, 39.3, 39.8, 46.1, 46.3, 46.7, 48.8, 52.7, 65.8, 79.9, 117.5, 125.5, 125.6, 131.5, 133.3, 133.4, 135.1, 135.4, 135.5, 149.5, 156.6, 157.4, 172.3, 173.9.

To a cold (0 °C) and stirred solution of polyamine **33** (135 mg, 153 μmol) in CH_2Cl_2 (4 mL) were added PPh_3 (8 mg, 30.5 μmol), $Pd(PPh_3)_4$ (9 mg, 7.8 μmol), and pyrrolidine (54 mg, 759 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was subjected to silica gel chromatography (2–10% EtOH/CH_2Cl_2, then 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) to afford the primary amine as a colorless oil.

To a stirred solution of amine obtained in DMF (1 mL) was added tri-Cbz-arginine succinimide ester[48] (154 mg, 229 μmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by chromatography on silica gel (2–6% MeOH/CH_2Cl_2) gave the title compound **34** (146 mg, 66%, three steps from **10**) as a white powder. $[\alpha]_D^{22}$ +3.06 (*c* 1.11, DMSO). IR (Nujol) 1727, 1681, 1646, 1544, 1456, 1376, 1255, 1162, 852 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.09 (m, 2H), 1.34 (m, 13H), 1.55 (m, 8H), 2.32 (dd, 1H, J=6.3, 15.0 Hz), 2.39 (dd, 1H, J=6.0, 15.0 Hz), 2.69 (m, 4H), 3.17 (m, 8H), 3.84 (m, 3H), 4.16 (dd, 1H, J=6.0, 6.3 Hz), 4.97 (s, 2H), 5.02 (s, 2H), 5.20 (s, 2H), 6.81 (d, 1H, J=7.5 Hz), 6.86 (br s, 1H), 7.32 (m, 17H), 7.67 (br s, 1H), 7.87 (m, 9H), 9.14 (br s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.3, 25.3, 26.9, 27.5, 28.3, 28.4, 28.7, 29.3, 36.2, 37.6, 38.6, 44.6, 44.8, 45.0, 45.6, 47.3, 51.7, 54.8, 65.6, 66.3, 68.4, 78.3, 124.5, 124.6, 127.8, 127.9, 128.1, 128.4, 128.5, 128.7, 129.9, 131.8, 131.9, 132.7, 134.7, 134.8, 135.5, 137.2, 137.3, 147.7, 155.2, 155.3, 156.2, 159.9, 163.1, 171.4, 171.8. FABHRMS calcd for $C_{62}H_{78}N_{12}O_{19}S_2Na$ $(M+Na)^+$ 1381.4845, found 1381.4835.

4.1.34. *N*-(N^{α}-2,4-Dibenzyloxyphenylacetyl-L-asparaginyl)-12-(N^{α},N^{δ},N^{ω}-tribenzyloxycarbonylarginyl)-amino-6,10-[di-(2-nitrobenzenesulfonyl)]-6,10-diaza-1-aminoundecane (35). To a cold (0 °C) and stirred suspension of polyamine **34** (90 mg, 66.2 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added Et_3N (7 mg, 69.2 μmol) and 2,4-dibenzyloxyphenylacetic acid succinimide ester[28] (45 mg, 101 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–10% MeOH/CH_2Cl_2) to afford the title compound **35** (84 mg, 80%, two steps) as a white powder. $[\alpha]_D^{24}$ −0.43 (*c* 0.70, DMSO). IR (Nujol) 1721, 1658, 1545, 1457, 1377, 1262, 1162, 852 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 1.06 (m, 2H), 1.23 (m, 2H), 1.33 (m, 2H), 1.54 (m, 8H), 2.35 (dd, 1H, J=7.2, 15.0 Hz), 2.42 (dd, 1H, J=6.3, 15.0 Hz), 2.89 (m, 2H), 2.97 (m, 2H), 3.16 (m, 8H), 3.37 (m, 2H), 3.83 (m, 3H), 4.51 (m, 1H), 4.96 (s, 2H), 5.01 (s, 2H), 5.02 (s, 2H), 5.07 (s, 2H), 5.18 (s, 2H), 6.51 (s, 2H), 6.67 (br s, 1H), 6.85 (br s, 1H), 7.07 (m, 2H), 7.33 (m, 26H), 7.61 (m, 1H), 7.85 (m, 10H), 9.14 (br s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 23.4, 25.3, 26.8, 27.5, 28.2, 28.7, 29.3, 36.2, 36.4, 37.5, 38.7, 44.6, 44.7, 45.0, 45.6, 47.3, 50.0, 54.8, 65.6, 66.3, 68.4, 69.4, 69.5, 127.3, 127.8, 127.9, 128.0, 128.1, 128.5, 128.6, 128.7, 129.9, 131.2, 131.8, 131.9, 132.7, 134.7, 134.8, 135.5, 137.2, 137.3, 137.4, 147.7, 155.2, 156.2, 157.1, 158.5, 160.0, 163.1, 170.5, 170.9, 171.7, 171.8. FABHRMS calcd for $C_{79}H_{88}N_{12}O_{20}S_2Na$ $(M+Na)^+$ 1611.5577, found 1611.5571.

4.1.35. Argiotoxin-636 (8). To a stirred solution of polyamine **35** (40 mg, 25.2 μmol) in DMF (1 mL) were added 2-mercaptoethanol (39 mg, 499 μmol) and DBU (77 mg, 506 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/CH_2Cl_2, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% H_2O/MeCN containing

0.1% TFA to 50% H_2O/MeCN containing 0.1% TFA in 20 min. Arg-636 (**8**) was eluted at 9.06 min and was obtained as a colorless TFA salt (14 mg, 51%, two steps). $[\alpha]_D^{22}$ +4.69 (*c* 0.98, H_2O). IR (Nujol) 3394, 2923, 1674 cm^{-1}. ^1H NMR (300 MHz, D_2O): δ 1.07 (quin, 2H, *J*=7.2 Hz), 1.32 (m, 2H), 1.45 (quin, 2H, *J*=7.2 Hz), 1.50 (quin, 2H, *J*=7.2 Hz), 1.77 (m, 4H), 1.95 (m, 2H), 2.56 (dd, 1H, *J*=6.9, 15.0 Hz), 2.62 (dd, 1H, *J*=6.9, 15.0 Hz), 2.95 (m, 8H), 3.10 (m, 6H), 3.33 (d, 1H, *J*=15.6 Hz), 3.43 (d, 1H, *J*=15.6 Hz), 3.83 (t, 1H, *J*=7.2 Hz), 4.44 (t, 1H, *J*=6.9 Hz), 6.30 (m, 2H), 6.95 (d, 1H, *J*=8.7 Hz). ^{13}C NMR (75 MHz, D_2O): δ 22.5, 22.7, 23.5, 24.9, 25.3, 27.5, 27.9, 35.8, 36.4, 36.8, 38.8, 40.2, 44.1, 44.4, 45.2, 47.5, 50.9, 52.9, 102.7, 107.4, 113.7, 132.4, 155.1, 156.1, 156.7, 169.6, 172.4, 174.6, 174.9. FABHRMS calcd for $C_{29}H_{53}N_{10}O_6$ (M^++H) 665.4162, found 665.4127. ESI-MS/MS: Supplementary data 9.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.051.

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Chiral ligand-controlled asymmetric conjugate amination of enoates with lithium mesitylmethyl(trimethylsilyl)amide

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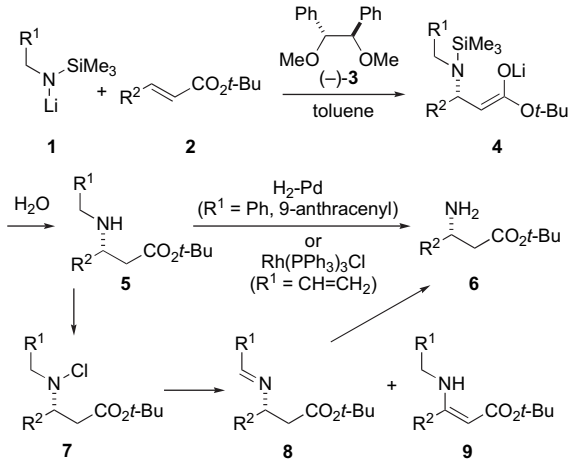
Abstract—Lithium mesitylmethyl(trimethylsilyl)amide behaved as a nice amination agent in a chiral ligand-controlled conjugate addition reaction of *tert*-butyl cinnamate to give the conjugate amination product with 99% ee in 90% yield. Other acyclic and cyclic enoates were also aminated in reasonably high enantioselectivity, while the deprotonation of abstractable proton of enoates caused yield loss of the conjugate amination products, due to the bulkiness and enriched basicity of the lithium amide. Although such steric bulkiness made hard the hydrogenolytic cleavage of a mesitylmethyl–N bond of the adducts, a new protocol comprising N-chlorination–regioselective dehydrochlorination–transoximation was developed for N-dearylmethylation, giving 3-aminoalkanoates in reasonably good yields.

1. Introduction

Chiral β-amino acids have been established to be the critical skeleton unit of biologically potent peptidic natural products,[1] medicinally important class of nonpeptidic β-lactams,[2] and pharmaceuticals.[3] Among several strategies for the synthesis of chiral β-amino acid derivatives,[4] the conjugate addition of nitrogen nucleophiles to α,β-unsaturated carboxylic acid derivatives is one of the most attractive and versatile methods as has been shown by the elegant reactions using chiral amine nucleophiles,[5,6] chiral enoates,[7] and chiral catalysts.[8] As part of our studies directed toward the development of asymmetric conjugate addition reactions of lithiated nucleophiles,[9] organocoppers,[10] and organoboranes,[11] we have been engaged in the asymmetric conjugate addition of nitrogen nucleophiles to enoates providing chiral β-amino acid equivalents.[12] Our methodology relies on the chiral ligand-mediated asymmetric conjugate addition[13] of lithiated arylmethyl(trimethylsilyl)amines **1** (R^1=Ar)[14] or allyl(trialkylsilyl)amines **1** (R^1=CH=CH_2)[15] to acyclic and cyclic enoates **2**, giving the corresponding β-alkylaminoalkanoates **5** in high enantioselectivity via protonation of enolates **4**, which were then converted to 3-aminoalkanoates **6** by hydrogenolysis of an arylmethylamino group or rhodium-catalyzed isomerization of an allylamino group into an imine followed by hydrolysis (Scheme 1). This two-step procedure to **6** from **2** seems very promising; however, hydrogenolysis and isomerization at the second step are not applicable to the substrates **5** bearing such sensitive functional groups. In our further studies toward the asymmetric conjugate amination of a bulky lithium amide, we developed another more general method for the removal of *N*-arylmethyl group of **5**, which comprised three successive steps, N-chlorination of **5** to **7**, regioselective dehydrochlorination to imines **8**, and finally transoximation to **6**. The problem to be solved is the regioselective dehydrochlorination of **7** to **8**, not to **9**.



Scheme 1. Asymmetric conjugate amination of **2** and subsequent conversion of **5** to 3-aminoalkanoates **6**.

2. Results and discussion

2.1. Steric tuning of arylmethyl(trimethylsilyl)amine

The chiral diether (−)-**3**[9]-controlled asymmetric conjugate addition reaction of a lithium amide **1a** (R^1=Ph) with

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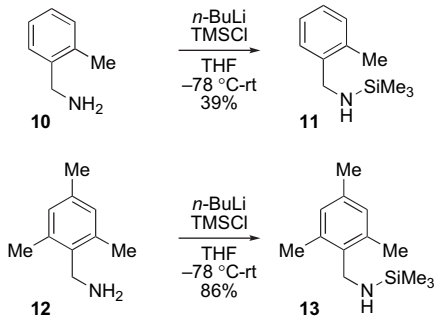
enoates **2** in the presence of trimethylsilylchloride (TMSCl) afforded **5** (R^1=Ph) with high enantioselectivity up to 99% ee (Scheme 1).[14a] However, the presence of TMSCl sometimes results in poorer chemical yield, and the reaction in the absence of TMSCl afforded **5** with poorer ee. TMSCl was added to the reaction for the conversion of the corresponding lithium enolate **4** to its TMS enol ether and consequently to avoid mixed aggregate formation with a lithium amide **1**,[12] which caused decreased enantioselectivity. Since the bulky R^1 group of **4** and **1** is another possibility in avoiding mixed aggregate formation due to the steric reason and such bulky lithium amide **1** may behave as a more powerful nucleophile due to the deaggregation,[16] we focused our study on the reaction of lithium amide **1** derived from bulkier amines **11** and **13** (Scheme 2).



Scheme 2. Trimethylsilylation of amines **10** and **12**.

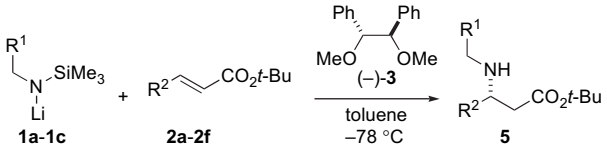
The reaction of **1b** (R^1=2-MeC_6H_4) bearing an *ortho*-methylphenyl group, prepared by the *n*-BuLi treatment of **11**, with *tert*-butyl cinnamate **2a** (R^2=Ph) in the presence of stoichiometric amount of (−)-**3** in toluene at −78 °C proceeded to completion within 0.5 h to give **5ba**[17] with 81% ee, disappointingly poorer ee by comparing with 93% ee of the reaction of lithium benzyl(TMS)amide **1a** (R^1=Ph) (Table 1, entries 1 and 2). However, it was very satisfactory to find that the reaction of **1c** (R^1=2,4,6-$Me_3C_6H_2$) bearing a mesityl group, prepared from **13**, gave **5ca** with 99% ee in 90% yield (entry 3). Prolonged reaction time, 2.5 h, indicated that the bulkiness of **1c** caused the loss of nucleophilic reactivity, and this bulkiness, in turn, helps to avoid the mixed aggregate formation of a resulting lithium enolate **4ca** with **1c**, giving excellently high enantioselectivity. It is also possible to speculate that the bulkiness of lithium amide **1c** itself prevents the self-aggregation of **1c**–**3** complex and exerts high stereoselectivity.

Other acyclic and cyclic enoates **2** were converted to the corresponding conjugate amination products **5** with satisfactorily high 93–96% ee, excepting **2c** that has a bulky isopropyl group at the reaction site (entries 4–8). However, the drawback of bulky **1c** became apparent by the moderate chemical yield, which was caused by the deprotonation of **2** bearing an abstractable proton, because of the stronger basicity of **1c**.

2.2. Unsuccessful hydrogenolysis of bulky arylmethyl group

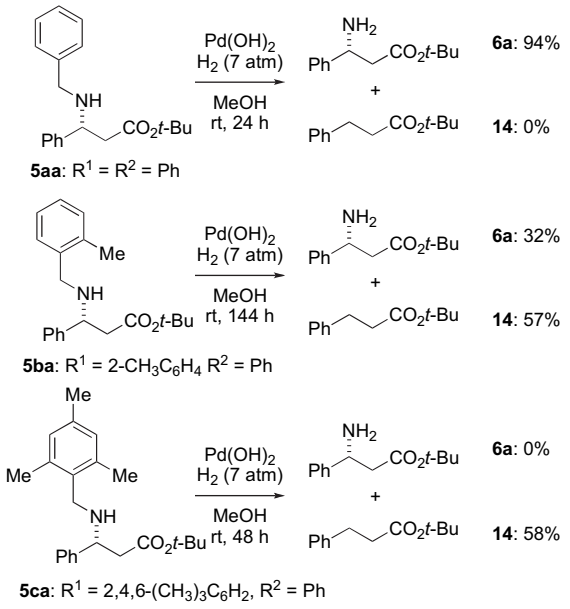
Another severe disaster came from the difficulty in the hydrogenolytic cleavage of a mesitylmethyl–N bond of **5** (Scheme 3). Hydrogenolysis of benzylamine adduct **5aa** with Perlman's catalyst in methanol under 7 atm hydrogen pressure successfully gave a debenzylation product **6a** in 94% yield without concomitant formation of phenylpropanoate **14** that arose from hydrogenolysis at the 3-position of **5aa**. *ortho*-Methylbenzyl group of **5ba** was hydrogenolyzed to give a mixture of **6a** in 32% yield and **14** in 57% yield. The mesitylmethyl group of **5ca** was extremely hard to be hydrogenolyzed to give **14** in 58% yield without formation of **6a**. The attempted N-demesitylmethylation of **5cb** (R^2=Me) was also totally unsuccessful, giving a complex mixture. A similar difficulty in the hydrogenolysis of substituted benzyl group has been also reported by Davies group.[19]

Table 1. Asymmetric conjugate amination of **2** with **1**[a]



Entry	1	R^1	2	R^2	Time (h)	5	Yield (%)	ee (%)[b]
1	**1a**	Ph	**2a**	Ph	0.7	**5aa**	92	93[c]
2	**1b**	2-Tol	**2a**	Ph	0.5	**5ba**	76	81
3	**1c**	Mes	**2a**	Ph	2.5	**5ca**	90	99
4	**1c**	Mes	**2b**	Me	0.1	**5cb**	62	93
5	**1c**	Mes	**2c**	*i*-Pr	15.0	**5cc**	61	75
6	**1c**	Mes	**2d**	*E*-Propenyl	12.0	**5cd**	26	94
7	**1c**	Mes	**2e**	1-Naph	2.0	**5ce**	52	96
8	**1c**	Mes	**2f**		1.5	*cis*-**5cf**	90	96

[a] Three equivalents of **1** were used.
[b] The ee was determined by chiral stationary phase HPLC. For entry 8, the ee was determined by ^{1}H NMR using (*S*)-(−)-1,1′-bi-2,2′-naphthol as a chiral shift reagent.[18]
[c] Quoted from Ref. 14a.

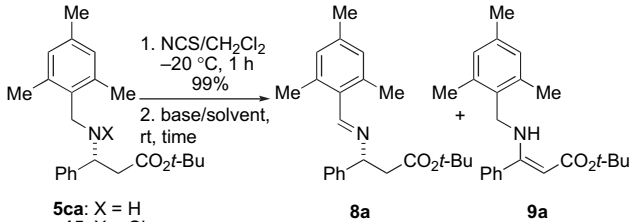


Scheme 3. Successful and unsuccessful hydrogenolysis of R^1CH_2 group.

2.3. Regioselective imine formation and transoximation for dearylmethylation

The problem in hydrogenolysis was circumvented by an oxidative imine formation from an amine **5ca**.[20] Chlorination of secondary amine of **5ca** with NCS in methylene chloride at −20 °C for 1 h gave an *N*-chlorinated product **15**, which was stable in silica gel chromatography, quantitatively (Table 2). Dehydrochlorination was then attempted by using a variety of bases. Treatment with KOH in ethanol[21] gave a mixture of desired imine and undesired enamine **8a** and **9a**, and **5ca** in a 32:20:48 ratio (Table 2, entry 1).

Table 2. Regioselective dehydrochlorination of **15** to imine **8a** and **9a** from **5ca**[a]

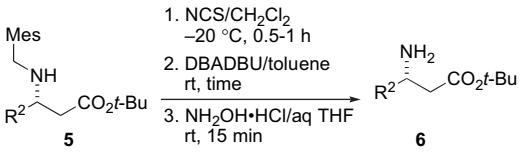


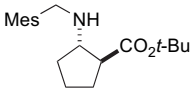
Entry	Base	Solvent	Time (h)	**8a** (%)	**9a** (%)	**5ca** (%)
1	KOH	EtOH	0.5	32	20	48
2	KO*t*-Bu	THF	0.5	0	0	0[b]
3	Et_3N	PhH	48	0	0	100
4	*i*-Pr_2NEt	PhH	144	0	0	100
5	Proton sponge	PhH	144	0	0	100
6	DABCO	PhH	96	48	38	14
7	$HN{=}C(NMe_2)_2$	PhH	17	29	71	0
8	DBN	PhH	12	62	38	0
9	DBU	PhH	17	79	21	0
10	DBADBU	PhH	84	85	15	0
11	DBADBU	PhMe	72	90	10	0

[a] Relative ratio determined by ^1H NMR of crude product.
[b] A complex mixture was obtained.

Potassium *tert*-butoxide,[22] triethylamine,[23] diisopropylethylamine,[23] and proton sponge as bases gave back **5ca** without formation of any imine and enamine (entries 2–5). Promising result was first obtained by treating with DABCO in benzene to give a 48:38:14 mixture (entry 6). Guanidine base gave a 29:71 mixture without recovery of **5ca** (entry 7). DBN and DBU[24] were found to give the desired **8a** as the major product in the mixture in a good recovery (entries 8 and 9). Much more improvement was obtained by using commercially available dibutylamino-DBU (DBADBU)[25] in benzene to give a 85:15 mixture of **8a** and **9a**, and the best result was obtained by the reaction in toluene at rt for 72 h to give a 90:10 mixture of **8a** and **9a** (entries 10 and 11). Further treatment of the mixture of imine and enamine with hydroxylamine hydrochloride[26] in aqueous THF at rt for 15 min gave **6a** without any racemization[27] in 81% three-step isolated yield from **5ca**, thus succeeding in the development of a generally applicable N-dearylmethylation protocol (Table 3, entry 1). (*E*)- and (*Z*)-Oximes of mesitylaldehyde were isolated in 73% combined yield, indicating transoximation of an imine. It is notable that DBADBU was recovered in 91% yield and was reusable.

Table 3. Chlorination of **5**, regioselective dehydrochlorination, and transoximation to 3-aminoalkanoates **6**



Entry	**5**	R^2	Time (h)	**6**	Yield (%)
1	**5ca**	Ph	72	(+)-(*S*)-**6a**	81
2	**5cb**	Me	72	(−)-(*S*)-**6b**	67[a]
3	**5cc**	*i*-Pr	48	**6c**	62
4	**5cd**	*E*-Propenyl	48	**6d**	71
5	**5ce**	1-Naph	72	**6e**	69
6	*trans*-**5cf**		48	(−)-(1*S*,2*S*)-**6f**	53

[a] The yield after conversion to Cbz-**6b** with the established absolute configuration.[28]

The established chlorination–regioselective dehydrochlorination with DBADBU–transoximation protocol was applicable to **5cb**–**5cf** giving 3-aminoalkanoates **6** in reasonably good yields (Table 3).

3. Conclusion

Lithium mesitylmethyl(trimethylsilyl)amide was developed as a new conjugate amination agent of enoates, giving the products in excellently high enantioselectivity, while bulkiness of the lithium amide decreased its nucleophilicity and enhanced basic character. For the N-dearylmethylation of the conjugate amination products, a new protocol, N-chlorination–regioselective dehydrochlorination–transoximation was successfully developed to give 3-aminoalkanoates in reasonably good yields.

4. Experimental

4.1. General

All melting points are uncorrected. IR spectra were expressed in cm^{-1}. ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ at 500 and 125 MHz, respectively, unless otherwise noted. Chemical shift values are expressed in parts per million relative to internal TMS. *J* values are presented in hertz. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

4.1.1. *N*-(2-Methylbenzyl)-*N*-trimethylsilylamine (11). The same procedure for **13** (vide infra) and distillation (106 °C/10 mmHg) gave **11** as a colorless oil in 39% yield. ^1H NMR (C_6D_6): 0.06 (9H, s), 0.28 (1H, br s), 2.15 (3H, s), 3.77 (2H, d, *J*=6.7), 7.03–7.12 (4H, m), 7.36 (1H, m). ^{13}C NMR (C_6D_6): −0.1, 18.7, 43.9, 126.2, 126.8, 127.6, 130.3, 135.6, 141.9. IR (neat): 3387, 1250. MS (EI) *m/z*: 193 (M^+), 120 (M^+-TMS). HRMS (EI) *m/z*: calcd for $C_{11}H_{19}NSi$: 193.1287. Found: 193.1296.

4.1.2. Mesitylmethylamine (12) hydrobromide.[29] To a solution of liquid NH_3 (135 g, 7.9 mol) in EtOH (220 mL) was added a solution of mesitylmethylbromide[30] (12.0 g, 56.4 mmol) in EtOH (120 mL) over 20 min at −78 °C. After stirring for 0.5 h under reflux, the mixture was concentrated. To the residue was added chloroform in which bismesitylmethylamine hydrobromide was dissolved and the mixture was filtrated. Recrystallization from water gave hydrobromide of **12** (6.64 g, 50% yield) as colorless needles of mp>270 °C. ^1H NMR (DMSO-d_6): 2.21 (3H, s), 2.34 (6H, s), 3.97 (2H, s), 6.89 (2H, s), 8.02 (3H, br s). ^{13}C NMR (DMSO-d_6): 19.4, 20.6, 36.2, 127.6, 129.0, 137.8, 138.1. IR (Nujol): 3550, 1570, 1460, 852. MS (EI) *m/z*: 149 (M^+-HBr). Anal. Calcd for $C_{10}H_{16}BrN\cdot 1/8H_2O$: C, 51.68; H, 7.05; N, 6.03. Found: C, 52.19; H, 7.01; N, 6.09.

4.1.3. Mesitylmethylamine (12).[31] The suspension of hydrobromide of **12** (21 g, 91 mmol) in 10% NaOH (100 mL) was extracted with ether. Organic layers were washed with brine and dried over sodium sulfate. Concentration and distillation (107 °C/10 mmHg) gave **12** (12.4 g, 91% yield) as a colorless oil. ^1H NMR (C_6D_6): 0.66 (2H, br s), 2.15 (3H, s), 2.20 (6H, s), 3.01 (2H, s), 6.76 (2H, s). ^{13}C NMR (C_6D_6): 19.2, 20.9, 40.2, 129.3, 135.8, 136.2, 137.0. IR (neat): 3368, 3294, 1612, 852. MS (EI) *m/z*: 149 (M^+), 132 (M^+-NH_2).

4.1.4. *N*-Mesitylmethyl-*N*-trimethylsilylamine (13). Under Ar atmosphere, to a solution of **12** (4.7 g, 31.5 mmol) in THF (30 mL) was added a 1.6 M hexane solution of *n*-BuLi (23.6 mL, 37.8 mmol) at −78 °C over 10 min. After 1 h stirring, TMSCl (5.3 mL, 37.8 mmol) was added over 3 min. The mixture was stirred for 2 h at −78 °C, and then stirred for additional 0.5 h at rt. After addition of benzene (10 mL), the mixture was stood for 10 min. The supernatant was concentrated and distilled (92 °C/1 mmHg) to give **13** as a colorless oil in 86% yield. ^1H NMR (C_6D_6): 0.07 (1H, br s), 0.09 (9H, s), 2.17 (3H, s), 2.30 (6H, s), 3.79 (2H, d, *J*=5.8), 6.79 (2H, s). ^{13}C NMR (C_6D_6): −0.2, 19.4, 21.0, 39.7, 129.3, 136.0, 136.4, 136.9. IR (neat): 3406, 1250. MS (EI) *m/z*: 221 (M^+), 149 (M^+-TMS). Anal. Calcd for $C_{13}H_{23}NSi$: C, 70.52; H, 10.47; N, 6.33. Found: C, 70.61; H, 10.70; N, 6.28.

4.1.5. (+)-*tert*-Butyl (*R*)-3-(mesitylmethylamino)-3-phenylpropanoate (5ca). Under Ar atmosphere, a 1.6 M hexane solution of *n*-BuLi (1.8 mL, 3.0 mmol) was added to a solution of an amine **13** (3.0 mmol) in toluene (8 mL) at −78 °C over 5 min. After stirring for 0.5 h, a solution of (−)-**3** (873 mg, 3.6 mmol) in toluene (6 mL) was added and the mixture was stirred for 0.5 h at −78 °C. A toluene solution of *tert*-butyl cinnamate **2a** (1.0 mmol) was added over 5 min and the mixture was stirred at −78 °C for 2.5 h, and then quenched with satd ammonium chloride (3 mL). After addition of 10% potassium carbonate (6 mL), the mixture was extracted with AcOEt. The organic layer was washed with brine and dried over sodium sulfate. Concentration and silica gel column chromatography (benzene/hexane/AcOEt=2/7/1) gave **5ca** (318 mg, 90% yield) as a colorless oil of $[\alpha]_D^{25}$+27.7 (*c* 1.26, $CHCl_3$) and recovered (−)-**3** (870 mg, quant yield); 99% ee (Daicel Chiralcel OD-H, hexane/2-PrOH=100/1, 0.5 mL/min, 254 nm, major 9.9 min and minor 12.5 min). ^1H NMR: 1.37 (9H, s), 1.61 (1H, br s), 2.23 (3H, s), 2.24 (6H, s), 2.51 (1H, dd, *J*=4.9, 15.6), 2.62 (1H, dd, *J*=9.2, 15.6), 3.45 and 3.53 (each 1H, d, *J*=11.3), 4.10 (1H, dd, *J*=4.9, 9.2), 6.80 (2H, s), 7.28 (1H, m), 7.33–7.36 (2H, m), 7.41–7.43 (2H, m). ^{13}C NMR: 19.3, 20.9, 28.0, 44.1, 45.9, 60.6, 80.6, 127.2, 127.4, 128.4, 128.8, 133.7, 136.4, 137.0, 143.1, 171.2. IR (neat): 3300, 1720. MS (EI) *m/z*: 353 (M^+), 296 (M^+-t-Bu). Anal. Calcd for $C_{23}H_{31}NO_2$: C, 78.15; H, 8.84; N, 3.96. Found: C, 77.99; H, 9.03; N, 4.02.

Recrystallization of recovered (−)-**3** from hexane gave pure (−)-**3** quantitatively and was reusable in an asymmetric reaction.

4.1.6. (+)-*tert*-Butyl (*R*)-3-(2-methylbenzylamino)-3-phenylpropanoate (5ba). Column chromatography (AcOEt/hexane=1/20) gave a white solid of mp 57–59 °C and $[\alpha]_D^{25}$ +23.6 (*c* 1.38, $CHCl_3$) in 76% yield; 81% ee (Daicel Chiralpak AD-H, hexane/2-PrOH=500/1, 1.0 mL/min, 254 nm, major 17.7 min and minor 19.7 min). ^1H NMR: 1.37 (9H, s), 1.61 (1H, br s), 2.25 (3H, s), 2.53 (1H, dd, *J*=5.2, 15.3), 2.64 (1H, dd, *J*=8.9, 15.3), 3.56 (2H, s), 4.10 (1H, dd, *J*=5.2, 8.9), 7.13–7.39 (9H, m). ^{13}C NMR: 18.8, 27.9, 44.3, 49.5, 59.9, 80.6, 125.9, 127.0, 127.3, 127.4, 128.5, 128.8, 130.3, 136.6, 138.3, 142.9, 171.2. IR (Nujol): 3321, 1713. MS (EI) *m/z*: 325 (M^+). Anal. Calcd for $C_{21}H_{27}NO_2$: C, 77.50; H, 8.36; N, 4.30. Found: C, 77.64; H, 8.48; N, 4.26.

4.1.7. (+)-*tert*-Butyl (*S*)-3-(mesitylmethylamino)butanoate (5cb). Column chromatography (hexane/AcOEt=10/1) gave a colorless oil of $[\alpha]_D^{25}$ +23.7 (*c* 0.99, $CHCl_3$) in 62% yield; 93% ee (Daicel Chiralpak AD, hexane/2-PrOH=100/1, 0.5 mL/min, 254 nm, major 9.3 min and minor 11.5 min). ^1H NMR: 1.17 (3H, d, *J*=6.4), 1.31 (1H, br s), 1.44 (9H, s), 2.24 (3H, s), 2.27 (1H, dd, *J*=5.5, 15.0), 2.35 (6H, s), 2.43 (1H, dd, *J*=7.3, 15.0), 3.17 (1H, ddq, *J*=5.5, 7.3, 6.4), 3.66 and 3.75 (each 1H, d, *J*=11.3), 6.83 (2H, s). ^{13}C NMR: 19.3, 20.4, 20.8, 28.0, 42.9, 45.1, 51.0, 80.3, 128.9, 133.8, 136.3, 136.8, 171.9. IR (neat): 3333, 1728, 1157. MS (EI) *m/z*: 291 (M^+), 234 (M^+-t-Bu).

Anal. Calcd for $C_{18}H_{29}NO_2$: C, 74.18; H, 10.03; N, 4.81. Found: C, 74.31; H, 9.91; N, 4.84.

4.1.8. (+)-*tert*-Butyl 3-(mesitylmethylamino)-4-methylpentanoate (5cc). Column chromatography (hexane/AcOEt=10/1) gave a colorless oil of $[\alpha]_D^{25}$ +27.0 (*c* 0.96, $CHCl_3$) in 61% yield; 75% ee (Daicel Chiralpak AD, hexane/2-PrOH=500/1, 0.3 mL/min, 254 nm, major 15.0 min and minor 16.6 min). ^{1}H NMR: 0.91 and 0.92 (each 3H, d, *J*=6.7), 1.42 (1H, br s), 1.43 (9H, s), 1.94 (1H, dqq, *J*=4.3, 6.7, 6.7), 2.24 (3H, s), 2.26 (1H, dd, *J*=8.3, 15.3), 2.35 (1H, dd, *J*=4.3, 15.3), 2.36 (6H, s), 2.90 (1H, ddd, *J*=4.3, 4.3, 8.3), 3.69 (2H, s), 6.83 (2H, s). ^{13}C NMR: 17.4, 18.8, 19.3, 20.8, 28.0, 30.1, 37.0, 45.9, 60.8, 80.2, 128.9, 134.1, 136.3, 137.1, 172.7. IR (neat): 3341, 1728, 1153. MS (EI) *m/z*: 319 (M^+), 276 (M^+-t-Bu). Anal. Calcd for $C_{20}H_{33}NO_2$: C, 75.19; H, 10.41; N, 4.38. Found: C, 75.31; H, 10.16; N, 4.31.

4.1.9. (−)-*tert*-Butyl 3-(mesitylmethylamino)-4-hexenoate (5cd). Column chromatography (benzene/AcOEt=10/1) gave a colorless oil of $[\alpha]_D^{25}$ −14.0 (*c* 1.04, $CHCl_3$) in 26% yield; 94% ee (Daicel Chiralpak AD+AS-H, hexane/2-PrOH=200/1, 0.5 mL/min, 254 nm, major 15.6 min and minor 17.3 min). ^{1}H NMR: 1.42 (9H, s), 1.43 (1H, br s), 1.72 (3H, dd, *J*=1.6, 6.4), 2.23 (3H, s), 2.328 (1H, dd, *J*=5.8, 15.3), 2.329 (6H, s), 2.42 (1H, dd, *J*=7.6, 15.3), 3.47 (1H, ddd, *J*=5.8, 7.6, 8.3), 3.53 and 3.71 (each 1H, d, *J*=11.3), 5.36 (1H, ddd, *J*=1.6, 8.3, 15.0), 5.68 (1H, qd, *J*=6.4, 15.0), 6.82 (2H, s). ^{13}C NMR: 17.6, 19.3, 20.8, 28.0, 42.2, 45.2, 58.4, 80.3, 127.3, 128.9, 132.8, 133.9, 136.3, 136.9, 171.4. IR (neat): 3333, 1728, 1157. MS (EI) *m/z*: 317 (M^+), 260 (M^+-t-Bu). Anal. Calcd for $C_{20}H_{31}NO_2$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.85; H, 9.63; N, 4.33.

4.1.10. (+)-*tert*-Butyl 3-(mesitylmethylamino)-3-(1-naphthyl)propanoate (5ce). Column chromatography (hexane/AcOEt=20/1) gave a colorless amorphous solid of $[\alpha]_D^{25}$ +47.0 (*c* 1.05, $CHCl_3$) in 52% yield; 96% ee (Daicel Chiralcel OD-H, hexane/2-PrOH=200/1, 1.0 mL/min, 254 nm, major 9.7 min and minor 19.9 min). ^{1}H NMR: 1.38 (9H, s), 1.81 (1H, br s), 2.24 (3H, s), 2.27 (6H, s), 2.70 (2H, d, *J*=6.4), 3.59 (2H, s), 5.02 (1H, t, *J*=6.4), 6.82 (2H, s), 7.48–7.55 (3H, m), 7.79–7.81 (2H, m), 7.88 (1H, m), 8.36 (1H, m). ^{13}C NMR: 19.3, 20.8, 28.0, 43.5, 46.1, 80.7, 123.2, 123.9, 125.47, 125.53, 126.0, 127.7, 128.9, 129.0, 131.4, 133.7, 134.1, 136.5, 137.2, 138.6, 171.6. IR (neat): 3337, 1724, 1150. MS (EI) *m/z*: 403 (M^+), 346 (M^+-t-Bu). Anal. Calcd for $C_{27}H_{33}NO_2$: C, 80.36; H, 8.24; N, 3.47. Found: C, 80.26; H, 8.28; N, 3.44.

4.1.11. (+)-*tert*-Butyl (1*R*,2*S*)-2-(mesitylmethylamino)-cyclopentanecarboxylate (*cis*-5cf). Column chromatography (toluene/AcOEt=30/1–10/1) gave a colorless oil of $[\alpha]_D^{25}$ +3.9 (*c* 0.98, $CHCl_3$) in 90% yield; 96% ee (^{1}H NMR (C_6D_6) using (*S*)-(−)-1,1′-bi-2,2′-naphthol as a chiral shift reagent,[18] judged by the integral areas of the peaks of the C2 protons (the major peak at 2.70 ppm and the minor peak at 2.64 ppm)). ^{1}H NMR: 1.37 (1H, br s), 1.40 (9H, s), 1.59 (1H, m), 1.72–1.88 (4H, m), 1.97 (1H, m), 2.24 (3H, s), 2.34 (6H, s), 2.79 (1H, m), 3.32 (1H, m), 3.68 and 3.71 (each 1H, d, *J*=11.6), 6.81 (2H, s). ^{13}C NMR: 19.4, 20.8, 22.0, 27.0, 28.1, 31.5, 46.2, 49.1, 62.3, 80.0, 128.8, 134.1, 136.2, 136.9, 173.8. IR (neat): 3341, 1720, 1150. MS (EI) *m/z*: 317 (M^+), 260 (M^+-t-Bu). Anal. Calcd for $C_{20}H_{31}NO_2$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.91; H, 9.81; N, 4.27.

4.1.12. (+)-*tert*-Butyl (1*S*,2*S*)-2-(mesitylmethylamino)-cyclopentanecarboxylate (*trans*-5cf). A colorless oil of $[\alpha]_D^{25}$ +73.4 (*c* 1.15, $CHCl_3$) in 3% yield; 95% ee (^{1}H NMR (C_6D_6) using (*S*)-(−)-1,1′-bi-2,2′-naphthol as a chiral shift reagent, judged by the integral areas of the peaks of the C2 protons (the major peak at 2.50 ppm and the minor peak at 2.43 ppm)). ^{1}H NMR: 1.44 (9H, s), 1.40–1.52 (2H, m), 1.66–1.75 (2H, m), 1.85 (1H, m), 1.96 (1H, m), 2.05 (1H, m), 2.24 (3H, s), 2.36 (6H, s), 2.50 (1H, m), 3.32 (1H, m), 3.70 (2H, s), 6.84 (2H, s). ^{13}C NMR: 19.3, 20.8, 23.5, 28.0, 28.7, 33.2, 46.4, 51.9, 64.1, 80.1, 129.0, 133.8, 136.5, 136.8, 175.3. IR (neat): 3325, 1724, 1150. MS (EI) *m/z*: 317 (M^+), 260 (M^+-t-Bu). HRMS (EI) *m/z*: calcd for $C_{20}H_{31}NO_2$: 317.2355. Found: 317.2359.

4.1.13. Epimerization of *cis*-5cf to *trans*-5cf.[32] To a solution of *tert*-butanol (1.03 mL, 10.9 mmol) in THF (25 mL) was added a 0.5 M toluene solution of potassium bis(trimethylsilyl)amide (12.5 mL, 6.2 mmol) at 0 °C over 5 min. After stirring for 15 min, *cis*-**5cf** (500 mg, 1.6 mmol) in toluene (5 mL) was added and the mixture was stirred for 15 min at rt. The mixture was quenched with satd ammonium chloride (10 mL). After addition of 10% potassium carbonate (25 mL), the mixture was extracted with AcOEt. The organic layers were washed with brine and dried over sodium sulfate. Concentration and column chromatography (acetone/hexane=1/30) gave *trans*-**5cf** (387 mg, 77% yield) and *cis*-**5cf** (21 mg, 4% yield).

4.1.14. (−)-*tert*-Butyl (*R*)-3-(*N*-chloro-*N*-mesitylmethylamino)-3-phenylpropanoate (15). To a solution of **5ca** (0.62 mmol) in methylene chloride (13 mL) was added NCS (249 mg, 1.87 mmol) at −20 °C. The mixture was stirred for 14 h at −20 °C and then washed with brine and dried over sodium sulfate. Concentration and column chromatography (AcOEt/hexane=1/20) gave **15** (238 mg, 99% yield) as a pale yellow oil of $[\alpha]_D^{25}$ −10.4 (*c* 0.91, $CHCl_3$). ^{1}H NMR: 1.31 (9H, s), 2.24 (3H, s), 2.25 (6H, s), 2.87 (1H, dd, *J*=8.3, 15.3), 3.27 (1H, dd, *J*=6.5, 15.3), 3.89 and 3.95 (each 1H, d, *J*=13.5), 4.53 (1H, dd, *J*=6.5, 8.3), 6.81 (2H, s), 7.33–7.39 (3H, m), 7.43–7.44 (2H, m). ^{13}C NMR: 20.1, 20.9, 27.8, 39.7, 57.9, 70.6, 80.8, 128.2, 128.3, 129.06, 129.10, 130.5, 137.2, 138.0, 138.3, 170.5. IR (neat): 1732, 1150. MS (FAB) *m/z*: 388 (M^++H). Anal. Calcd for $C_{23}H_{30}ClNO_2$: C, 71.21; H, 7.79; N, 3.61. Found: C, 71.03; H, 8.00; N, 3.38.

4.1.15. (+)-*tert*-Butyl (*S*)-3-(*N*-chloro-*N*-mesitylmethylamino)butanoate from 5cb. Column chromatography (AcOEt/hexane=1/10) gave a pale yellow oil of $[\alpha]_D^{25}$ +18.6 (*c* 1.03, $CHCl_3$) in quantitative yield. ^{1}H NMR: 1.29 (3H, d, *J*=6.2), 1.43 (9H, s), 2.26 (3H, s), 2.360 (1H, dd, *J*=7.4, 15.3), 2.361 (6H, s), 2.74 (1H, dd, *J*=6.2, 15.3), 3.55 (1H, ddq, *J*=6.2, 7.4, 6.2), 4.03 and 4.15 (each 1H, d, *J*=13.5), 6.84 (2H, s). ^{13}C NMR: 15.4, 20.0, 20.9, 28.0, 40.3, 57.8, 60.3, 80.5, 129.1, 130.6, 137.3, 138.2, 171.2. IR (neat): 1732, 1157. MS (FAB) *m/z*: 326 (M^++H). Anal.

Calcd for $C_{18}H_{28}ClNO_2$: C, 66.34; H, 8.66; N, 4.30. Found: C, 66.55; H, 8.61; N, 4.13.

4.1.16. (+)-*tert*-Butyl 3-(*N*-chloro-*N*-mesitylmethylamino)-4-methylpentanoate from 5cc. Column chromatography (AcOEt/hexane=1/10) gave a colorless oil of $[\alpha]_D^{25}$ +32.7 (*c* 1.11, $CHCl_3$) in 89% yield. ^1H NMR: 0.93 and 1.00 (each 3H, d, *J*=6.8), 1.47 (9H, s), 1.91 (1H, m), 2.26 (3H, s), 2.37 (6H, s), 2.45 (1H, dd, *J*=6.1, 16.8), 2.90 (1H, dd, *J*=3.7, 16.8), 3.06 (1H, m), 3.94 and 4.20 (each 1H, d, *J*=13.4), 6.85 (2H, s). ^{13}C NMR: 19.4, 20.1, 20.4, 20.9, 28.0, 32.6, 35.2, 58.4, 70.8, 80.7, 129.1, 130.6, 137.3, 138.3, 172.5. IR (neat): 1728, 1157. MS (FAB) *m/z*: 353 (M^++H). Anal. Calcd for $C_{20}H_{32}ClNO_2$: C, 67.87; H, 9.11; N, 3.96. Found: C, 67.89; H, 8.95; N, 3.96.

4.1.17. (−)-*tert*-Butyl 3-(*N*-chloro-*N*-mesitylmethylamino)hex-4-enoate from 5cd. Column chromatography (AcOEt/hexane=1/10) gave a colorless oil of $[\alpha]_D^{25}$ −27.1 (*c* 1.03, $CHCl_3$) in 94% yield. ^1H NMR: 1.42 (9H, s), 1.79 (3H, d, *J*=5.8), 2.25 (3H, s), 2.34 (6H, s), 2.49 (1H, dd, *J*=7.6, 15.0), 2.80 (1H, dd, *J*=6.4, 15.0), 3.86 (1H, ddd, *J*=6.4, 7.6, 8.0), 3.88 and 4.16 (each 1H, d, *J*=13.4), 5.69 (1H, dd, *J*=8.0, 15.3), 5.77 (1H, dq, *J*=15.3, 5.8), 6.83 (2H, s). ^{13}C NMR: 17.9, 20.1, 20.9, 28.0, 40.2, 58.0, 67.9, 80.5, 127.4, 129.1, 130.6, 131.2, 137.2, 138.3, 170.6. IR (neat): 1732, 1157. MS (FAB) *m/z*: 352 (M^++H). Anal. Calcd for $C_{20}H_{30}ClNO_2$: C, 68.26; H, 8.59; N, 3.98. Found: C, 68.20; H, 8.67; N, 3.93.

4.1.18. (−)-*tert*-Butyl 3-(*N*-chloro-*N*-mesitylmethylamino)-3-(1-naphthyl)propanoate from 5ce. Column chromatography (AcOEt/hexane=1/10) gave a colorless amorphous solid of $[\alpha]_D^{25}$ −19.1 (*c* 1.45, $CHCl_3$) in 89% yield. ^1H NMR: 1.18 (9H, s), 2.12 (6H, s), 2.22 (3H, s), 3.17 (1H, dd, *J*=9.2, 15.3), 3.49 (1H, dd, *J*=5.5, 15.3), 3.98 and 4.03 (each 1H, d, *J*=13.1), 5.43 (1H, dd, *J*=5.5, 9.2), 6.77 (2H, s), 7.46–7.50 (3H, m), 7.69 (1H, m), 7.82–7.86 (2H, m), 8.14 (1H, m). ^{13}C NMR: 20.0, 20.8, 27.6, 37.9, 56.8, 80.8, 123.9, 124.8, 125.5, 125.6, 128.2, 128.7, 128.98, 129.04, 130.4, 132.3, 133.9, 134.8, 137.3, 138.3, 170.4. IR (neat): 1728, 1146. MS (FAB) *m/z*: 438 (M^++H). Anal. Calcd for $C_{27}H_{32}ClNO_2$: C, 74.04; H, 7.36; N, 3.20. Found: C, 73.80; H, 7.14; N, 3.21.

4.1.19. (−)-*tert*-Butyl (1*R*,2*S*)-2-(*N*-chloro-*N*-mesitylmethylamino)cyclopentane-1-carboxylate from *cis*-5cf. Column chromatography (AcOEt/hexane=1/10) gave a pale yellow oil of $[\alpha]_D^{25}$ −47.0 (*c* 0.89, $CHCl_3$) in 85% yield. ^1H NMR: 1.43 (9H, s), 1.73 (1H, m), 1.85 (1H, m), 1.96–2.13 (3H, m), 2.19 (1H, m), 2.26 (3H, s), 2.38 (6H, s), 3.09 (1H, m), 3.58 (1H, m), 4.02 and 4.13 (each 1H, d, *J*=12.8), 6.83 (2H, s). ^{13}C NMR: 20.3, 20.9, 22.9, 27.3, 28.0, 29.3, 49.4, 60.1, 76.6, 80.0, 129.0, 130.8, 137.2, 138.3, 173.5. IR (neat): 1724, 1150. MS (FAB) *m/z*: 352 (M^++H). Anal. Calcd for $C_{20}H_{30}ClNO_2$: C, 68.26; H, 8.59; N, 3.98. Found: C, 68.00; H, 8.44; N, 3.84.

4.1.20. (+)-*tert*-Butyl (1*S*,2*S*)-2-(*N*-chloro-*N*-mesitylmethylamino)cyclopentane-1-carboxylate from *trans*-5cf. Column chromatography (AcOEt/hexane=1/20) gave a colorless oil of $[\alpha]_D^{25}$ +87.0 (*c* 1.00, $CHCl_3$) in 95% yield. ^1H NMR: 1.42 (9H, s), 1.70–1.80 (3H, m), 1.95–2.05 (3H, m), 2.26 (3H, s), 2.37 (6H, s), 2.97 (1H, m), 3.89 (1H, m), 4.08 (2H, s), 6.84 (2H, s). ^{13}C NMR: 20.1, 20.9, 24.9, 27.9, 29.1, 30.6, 48.8, 59.6, 74.0, 80.3, 129.1, 130.7, 137.3, 138.1, 175.2. IR (neat): 1724, 1150. MS (FAB) *m/z*: 352 (M^++H). Anal. Calcd for $C_{20}H_{30}ClNO_2$: C, 68.26; H, 8.59; N, 3.98. Found: C, 68.38; H, 8.81; N, 4.07.

4.1.21. (−)-*tert*-Butyl (*R*)-3-(*N*-benzyl-*N*-chloroamino)-3-phenylpropanoate from 5aa. Column chromatography (AcOEt/hexane=1/10) gave a colorless oil of $[\alpha]_D^{25}$ −10.4 (*c* 1.02, $CHCl_3$) in 97% yield. ^1H NMR: 1.32 (9H, s), 2.84 (1H, dd, *J*=8.6, 15.3), 3.26 (1H, dd, *J*=6.5, 15.3), 3.87 and 3.93 (each 1H, d, *J*=13.7), 4.53 (1H, dd, *J*=6.5, 8.6), 7.26–7.43 (10H, m). ^{13}C NMR: 27.8, 40.4, 64.3, 70.1, 80.7, 127.7, 128.3, 128.36, 128.43, 128.9, 129.0, 137.4, 137.7, 170.3. IR (neat): 1728, 1150. MS (FAB) *m/z*: 346 (M^++H). Anal. Calcd for $C_{20}H_{24}ClNO_2$: C, 69.45; H, 6.99; N, 4.05. Found: C, 69.30; H, 7.07; N, 4.02.

4.1.22. *tert*-Butyl (*Z*)-3-(*N*-mesitylmethylamino)cinnamate (9a) (Table 2, entry 9). Column chromatography (Et_2O/hexane=1/10) of the dehydrochlorination products with DBU gave **9a** as colorless plates of mp 106–107 °C in 18% yield. ^1H NMR: 1.43 (9H, s), 2.227 (6H, s), 2.235 (3H, s), 4.11 (2H, d, *J*=4.6), 4.52 (1H, s), 6.81 (2H, s), 7.41–7.45 (5H, m), 8.14 (1H, br s). ^{13}C NMR: 19.5, 20.8, 28.5, 43.3, 78.2, 87.4, 127.8, 128.4, 129.1, 129.2, 131.7, 136.9, 137.0, 137.1, 163.8, 170.0. IR (KBr): 3290, 1639, 1593, 1570, 1150. MS (EI) *m/z*: 351 (M^+), 294 (M^+−*t*-Bu). Anal. Calcd for $C_{23}H_{29}NO_2$: C, 78.59; H, 8.32; N, 3.99. Found: C, 78.64; H, 8.44; N, 3.96. Stereochemistry was determined to be (*Z*) by NOE (6.4%) between a vinyl α-proton (4.52 ppm) and the *ortho* protons of phenyl group (7.42 ppm).

4.1.23. (+)-*tert*-Butyl (*R*)-3-amino-3-phenylpropanoate (6a).[28] To a solution of **15** (122 mg, 0.31 mmol) in toluene (5 mL) was added a solution of 6-(dibutylamino)-1,8-diazabicyclo[5.4.0]undec-7-ene (DBADBU) (958 mg, 3.4 mmol) in toluene (3 mL) under Ar at rt. The mixture was stirred for 72 h at rt. The mixture was washed with 1 N HCl and brine, and dried over sodium sulfate. Concentration gave an imine as a pale yellow oil. DBADBU was recovered by extraction with chloroform from acidic water layer after alkalization with 10% NaOH. Organic layers were washed with brine and dried over sodium sulfate. Concentration and distillation gave DBADBU (872 mg, 91% yield).

Hydroxylamine hydrochloride (77 mg, 1.1 mmol) was added to a solution of the above imine in a 1:1 mixture of THF and water (1 mL). After 15 min stirring at rt, 10% HCl was added. The whole was extracted with AcOEt to remove oximes (vide infra). The aqueous layer was treated with potassium carbonate to be pH 10 and then extracted with ether. The organic layer was dried over potassium carbonate. Concentration and column chromatography (AcOEt/hexane=1/2) gave **6a** (56 mg, 82% yield) as a colorless oil of $[\alpha]_D^{20}$ +20.0 (*c* 0.71, $CHCl_3$); 99% ee (Daicel Chiralcel OD-H, hexane/2-PrOH/Et_2NH=100/10/0.1, 0.5 mL/min, 254 nm, major 11.8 min and minor 14.4 min). ^1H NMR: 1.42 (9H, s), 1.69 (2H, br s), 2.58 (2H, d, *J*=6.7), 4.37 (1H, t, *J*=6.7), 7.24–7.37 (5H, m). ^{13}C NMR: 28.0, 45.3, 52.8, 80.7, 126.3, 127.3, 128.6, 144.8, 171.4. IR (neat): 3379, 1724, 1150. MS (EI) *m/z*: 221 (M^+), 164 (M^+−*t*-Bu).

Column chromatography (Et_2O/hexane=1/10) of organic layers above including oximes gave (*E*)- and (*Z*)-mesitaldehyde oximes in 51% (26 mg) and 22% (11 mg) yields, respectively. (*E*)-oxime: colorless prisms of mp 126–127 °C.[33] ^1H NMR: 2.29 (3H, s), 2.38 (6H, s), 6.89 (2H, s), 8.00 (1H, br s), 8.42 (1H, s). ^{13}C NMR: 21.01, 21.04, 126.4, 129.4, 137.6, 138.9, 150.0. IR (Nujol): 3252, 1609. MS (EI) *m/z*: 163 (M^+), 146 (M^+-OH). (*Z*)-oxime: colorless needles of mp 178–179 °C.[33] ^1H NMR: 2.25 (6H, s), 2.29 (3H, s), 6.89 (2H, s), 7.62 (1H, s), 8.17 (1H, br s). ^{13}C NMR: 19.7, 21.0, 128.1, 135.7, 138.7, 149.0. IR (Nujol): 3209, 1612. MS (EI) *m/z*: 163 (M^+), 146 (M^+-OH).

4.1.24. (−)-*tert*-Butyl (*S*)-*N*-(benzyloxycarbonyl)-3-aminobutanoate (Cbz-6b).[28] To a solution of a crude imine in a 1:1 mixture of THF and water, hydroxylamine hydrochloride was added. After the mixture was stirred for 20 min at rt, sodium bicarbonate and CbzCl were added. After stirring for 16 h, to the mixture was added satd ammonium chloride and satd sodium bicarbonate (5 mL). The mixture was extracted with AcOEt, and organic layers were washed with brine and then dried over sodium sulfate. Concentration and column chromatography (Et_2O/hexane=1/15, then acetone/hexane=1/10) gave Cbz-**6b** (67% yield) as a colorless oil of $[\alpha]_D^{20}$ −11.0 (*c* 1.05, CH_2Cl_2). ^1H NMR: 1.23 (3H, d, *J*=6.8), 1.44 (9H, s), 2.42 (2H, d, *J*=5.8), 4.08 (1H, qt, *J*=6.8, 5.8), 5.09 (2H, s), 5.26 (1H, br s), 7.29–7.36 (5H, m). ^{13}C NMR: 20.2, 27.9, 41.6, 44.1, 66.3, 80.9, 128.0, 128.4, 136.6, 155.5, 170.7. IR (neat): 1724. MS (EI) *m/z*: 293 (M^+), 237 (M^+-t-Bu).

4.1.25. (+)-*tert*-Butyl 3-amino-4-methylpentanoate (6c). Column chromatography (AcOEt/MeOH=10/1) gave **6c** as a colorless oil of $[\alpha]_D^{25}$ +19.1 (*c* 0.68, $CHCl_3$) in 72% yield. ^1H NMR: 0.91 and 0.92 (each 3H, d, *J*=6.7), 1.46 (9H, s), 1.50 (2H, br s), 1.62 (1H, dqq, *J*=4.5, 6.7, 6.7), 2.15 (1H, dd, *J*=9.8, 15.3), 2.38 (1H, dd, *J*=3.7, 15.3), 2.98 (1H, ddd, *J*=3.7, 4.5, 9.8). ^{13}C NMR: 17.7, 18.7, 28.1, 33.3, 40.9, 53.6, 80.4, 172.6. IR (neat): 3387, 1728, 1153. MS (FAB) *m/z*: 188 (M^++H). HRMS (FAB) *m/z*: calcd for $C_{10}H_{21}NO_2$+H: 188.1651. Found: 188.1659.

4.1.26. (−)-*tert*-Butyl 3-amino-4-hexenoate (6d). Column chromatography (AcOEt/MeOH=10/1) gave **6d** as a colorless oil of $[\alpha]_{405}^{25}$ −13.8 (*c* 0.93, MeOH) in 76% yield. ^1H NMR: 1.45 (9H, s), 1.61 (2H, br s), 1.67 (3H, d, *J*=6.4), 2.30 (1H, dd, *J*=8.3, 15.3), 2.37 (1H, dd, *J*=4.9, 15.3), 3.70 (1H, ddd, *J*=4.9, 7.1, 8.3), 5.44 (1H, dd, *J*=7.1, 15.3), 5.61 (1H, dq, *J*=15.3, 6.4). ^{13}C NMR: 17.6, 28.1, 43.8, 50.6, 80.5, 125.3, 134.4, 171.5. IR (neat): 3375, 1728, 1153. MS (EI) *m/z*: 185 (M^+), 128 (M^+-t-Bu). HRMS (EI) *m/z*: calcd for $C_{10}H_{19}NO_2$: 185.1416. Found: 185.1420.

4.1.27. (+)-*tert*-Butyl 3-amino-3-(1-naphthyl)propanoate (6e). Column chromatography (AcOEt/hexane=1/1) gave **6e** as a colorless solid of mp 74–75 °C and $[\alpha]_D^{25}$ +41.3 (*c* 1.09, $CHCl_3$) in 78% yield. ^1H NMR: 1.45 (9H, s), 1.81 (2H, br s), 2.65 (1H, dd, *J*=9.8, 15.9), 2.81 (1H, dd, *J*=3.4, 15.9), 5.24 (1H, dd, *J*=3.4, 9.8), 7.46–7.67 (3H, m), 7.67 (1H, m), 7.77 (1H, m), 7.88 (1H, m), 8.17 (1H, m). ^{13}C NMR: 28.1, 44.6, 48.0, 80.9, 122.7, 122.8, 125.6, 126.2, 127.8, 129.0, 130.6, 133.9, 140.4, 171.7. IR (KBr): 3248, 3167, 1728, 1161. MS (EI) *m/z*: 271 (M^+), 214 (M^+-t-Bu). Anal. Calcd for $C_{17}H_{21}NO_2$: C, 75.25; H, 7.80; N, 5.16. Found: C, 74.99; H, 7.70; N, 5.19.

4.1.28. (−)-*tert*-Butyl (1*R*,2*S*)-2-aminocyclopentane-1-carboxylate (*cis*-6f)[32] from *cis*-5cf. Column chromatography (AcOEt/hexane=1/2) gave *cis*-**6f** as a colorless oil of $[\alpha]_D^{25}$ −1.7 (*c* 0.35, $CHCl_3$) in 12% yield. ^1H NMR: 1.47 (9H, s), 1.50–2.00 (8H, m), 2.70 (1H, m), 3.57 (1H, br s). ^{13}C NMR: 22.3, 26.2, 28.2, 34.8, 51.1, 54.9, 80.3, 173.7. IR (neat): 3383, 1724, 1366, 1153. MS (EI) *m/z*: 185 (M^+).

4.1.29. (+)-*tert*-Butyl (1*S*,2*S*)-2-aminocyclopentane-1-carboxylate (*trans*-6f) from *trans*-5cf. Column chromatography (Et_2O/MeOH=5/1) gave *trans*-**6f** as a colorless oil of $[\alpha]_D^{20}$ +50.8 (*c* 1.15, $CHCl_3$) in 56% yield. ^1H NMR: 1.30–2.05 (8H, m), 1.46 (9H, s), 2.32 (1H, m), 3.39 (1H, m). ^{13}C NMR: 22.5, 28.0, 28.1, 35.1, 54.6, 57.0, 80.2, 174.7. IR (neat): 3368, 1720, 1153. MS (EI) *m/z*: 185 (M^+), 169 (M^+-NH_2), 128 (M^+-t-Bu). HRMS (EI) *m/z*: calcd for $C_{10}H_{19}NO_2$: 185.1416. Found: 185.1407.

4.1.30. Determination of the absolute configuration of *trans*-6f by conversion to (+)-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid.[32] A solution of *trans*-**6f** (21.6 mg, 0.11 mmol) in trifluoroacetic acid (0.5 mL) was stirred at rt for 12 h. Concentration gave a pale yellow oil. After addition of MeOH (0.5 mL) and 4.7 N HCl in ether (0.5 mL), the mixture was concentrated to give a solid, which was recrystallized from a mixture of EtOH and AcOEt to give (1*S*,2*S*)-2-aminocyclopentanecarboxylic acid (7.2 mg, 40% yield) as colorless needles of mp 146–147 °C and $[\alpha]_D^{25}$ +67.2 (*c* 0.61, H_2O). ^1H NMR (D_2O): 1.73–1.89 (4H, m), 2.18–2.22 (2H, m), 3.00 (1H, m), 3.76 (3H, s), 3.91 (1H, m). ^{13}C NMR (D_2O): 23.6, 29.4, 31.4, 49.2, 55.0, 176.8. IR (KBr): 3445, 2955, 1728. MS (EI) *m/z*: 128 (M^+-HCl), 112 ($M^+-HCl-NH_2$).

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Microwave-assisted tandem Wittig–intramolecular Diels–Alder cycloaddition. Product distribution and stereochemical assignment

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Abstract—The IMDA cycloadditions of 10 different ester-tethered 1,3,8-nonatrienes have been examined under controlled microwave heating (MeCN, 180 °C, 30 min), giving 90–99% yields, and the stereochemical outcome of the *exo* and *endo* adducts established together with X-ray crystal structural analysis. A microwave-assisted tandem Wittig–IMDA cycloaddition protocol has been established for a modular synthesis of the bicyclic lactones starting from α-bromoacetates of 2,4-pentadien-1-ols and α-oxo carbonyl compounds in the presence of PPh_3 and 2,6-lutidine (MeCN, 180 °C, 30 min). The overall yields of the tandem reactions are 68–80% and the stereoselectivity of the major adducts formed from *E*-substituted 1,3,8-nonatriene is the same as that observed for the purified 1,3,8-nonatrienes.

1. Introduction

The Diels–Alder cycloaddition remains the most powerful and efficient synthetic tool for accessing highly functionalized carbocycles, possibly generating up to four continuous stereogenic centers in one operation.[1] In particular, the intramolecular Diels–Alder (IMDA) cycloaddition has been extensively used for assembly of complex molecular architectures of designed or natural products origin.[2] Biosynthetic pathways incorporating IMDA reactions have been recognized and examples of biomimetic total synthesis of natural products by using a key IMDA cycloaddition are known.[2i] A good understanding on stereocontrol is fundamental to application of IMDA reactions in organic synthesis. A number of theoretical[3,4b–e,5e–h,j,7d] and experimental[4–8] studies have addressed the stereoselectivity of IMDA cycloadditions of the ester-tethered 1,3,8-nonatrienes[9] (Chart 1). The type **I** substrates have been extensively studied for formation of bicyclic lactones **A**. The acrylates **Ia**[4] undergo IMDA reactions at 132–250 °C while the doubly activated *E*- and *Z*-substituted 1,3,8-nonatrienes **Ib**,**c** cyclize, respectively, in PhMe (100–130 °C for **Ib**)[5,6] and in refluxing PhMe or xylene (110–140 °C for **Ic**).[7] As similar to the reactivity of **Ia**, the substrates **II**[8] afford the IMDA adducts **B** at temperatures of 135–250 °C depending on the nature of substituents R^1 and R^2. Synthetically useful stereoselectivity has been achieved for the lactones **A** by using a stereogenic substituent R^1 or R^4 at the position C1 or C5,[4a,b,5a–d,f,g,j,k,6p] a cooperative effect[4d–h] of two substituents R^3 and R^4 at the positions C3 and C5, and an internal hydrogen bonding interaction[5h] with CH_2OH (=R^2) at the position C2. We report here our original results on the IMDA cycloadditions of the type **Ib** substrates under controlled microwave heating.[10]

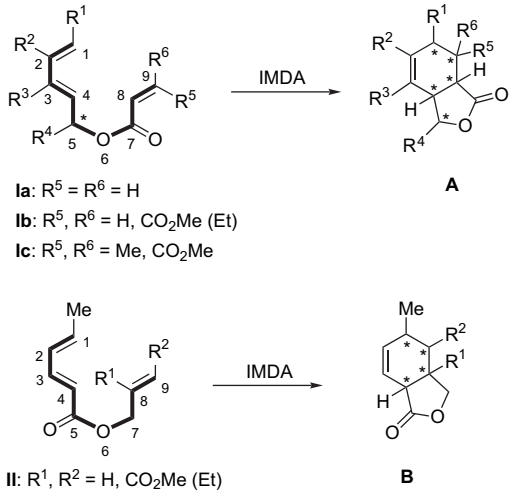

Chart 1. Ester-tethered 1,3,8-nonatrienes **I** and **II** and the IMDA adducts **A** and **B**.

Keywords: Wittig; IMDA; Microwave; Lactones; Tandem reactions.
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We also disclose results on the tandem Wittig–IMDA reactions for a modular synthesis of the bicyclic lactones **C** by using α-bromoacetates **III** and α-oxo carbonyl compounds **IV** as the building blocks (Chart 2).

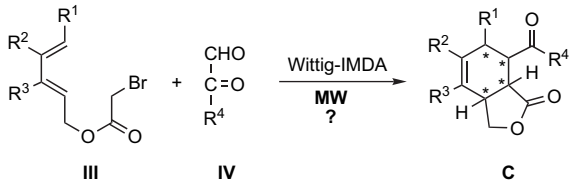
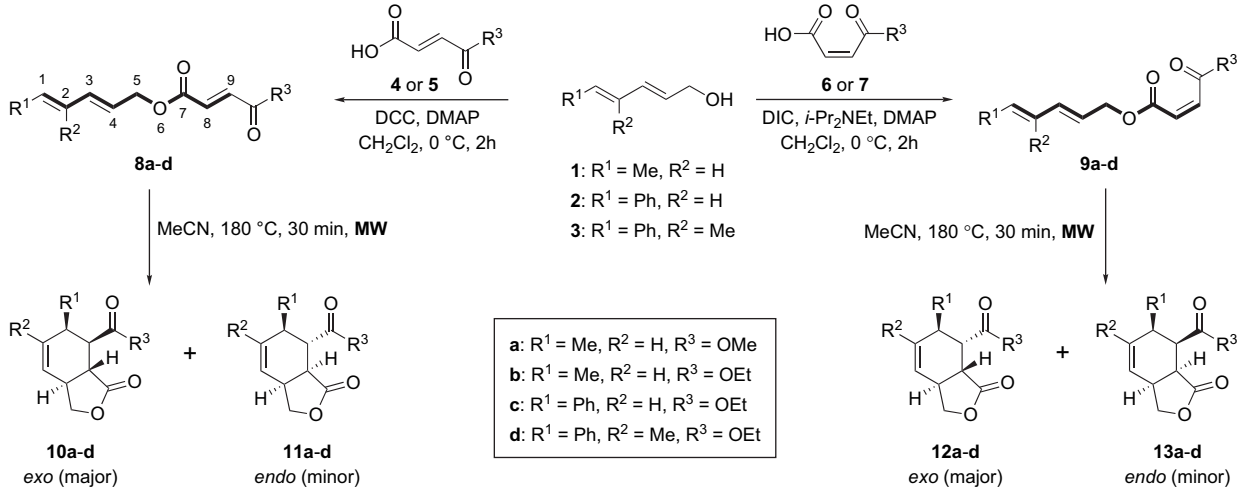


Chart 2. Proposed tandem Wittig–IMDA reactions of **III** under controlled microwave heating.

2. Results and discussion

2.1. Microwave-assisted IMDA cycloadditions of ester-tethered 1,3,8-nonatrienes

As reported by Arseniyadis et al.[5b] and Paddon-Row et al.,[5e,j] the terminally activated *E*-isomers of 1,3,8-nonatrienes **8a**,**b** underwent the IMDA reactions in PhMe at 100–110 °C for 23–24 h to provide the bicyclic lactones *exo*-**10a**,**b** and *endo*-**11a**,**b** in 78–85% combined yields and with *exo*:*endo* ratios of 60:40–65:35 (Scheme 1; Table 1, entries 2 and 5). The *Z*-substituted 1,3,8-nonatrienes **9a** showed a higher reactivity toward cycloaddition (110 °C, 2 h) and gave the bicyclic lactones *exo*-**12a** and *endo*-**13a** in 79% combined yield and with an *exo*:*endo* ratio of 79:21 (Table 1, entry 12).[5e,j] In connection with our interest in applying controlled microwave heating in closed reaction vials to solution[11] and solid-phase[12] organic synthesis, we assumed that the IMDA cycloadditions of the ester-linked 1,3,8-nonatrienes could be facilitated at higher temperatures, which are applicable on a technical microwave reactor with temperature and pressure regulation capability. The specific question we need to address centers on whether stereocontrol in the microwave-assisted IMDA reactions decreases due to a temperature effect.[13] As illustrated in Schemes 1 and 2, we prepared 10 different *E*- and *Z*-substituted 1,3,8-nonatrienes **8a–e** and **9a–e** and the yields are listed in Table 1. Condensation of **1–3** with fumaric acid monomethyl ester **4** or monoethyl ester **5**[14] in the presence of DCC–DMAP at 0 °C for 2 h afforded the *E*-substituted **8a–d** in 85–90% yields. It was found that the combination of DIC–*i*-Pr_2NEt–DMAP[15] was more suitable for the formation of the *Z*-substituted trienes **9a–d**, which were obtained in 65–71% yields from **1–3** and maleic acid monomethyl ester **6** or monoethyl ester **7**.[16] The ketoesters **8e** and **9e** were prepared by a modified Wittig olefination procedure by using MeOH as the solvent[17] in order to get much more amount of the *Z*-isomer **9e**. The α-bromoacetate **14** was treated with PPh_3 in MeCN at room temperature for overnight to give quantitatively the phosphonium salt **20**, which reacts with phenylglyoxal monohydrate in the presence of Et_3N in MeOH (0 °C, 30 min), giving **8e** and **9e** in 37 and 18% yields. We used MeCN as the solvent for the microwave-assisted IMDA reactions[18] due to its better microwave energy absorption property than PhMe[10b] and easy workup with its low boiling point. Moreover, it is advantageous to use MeCN in IMDA cycloadditions of the ester-linked substrates because a polar solvent effect was known, resulting in a significant rate acceleration.[13c–e] After heating a MeCN solution of **8a** in a closed pressurized reaction vial at 180 °C for 30 min, the adducts *exo*-**10a** and *endo*-**11a** were isolated in 91% combined yield and in a 67:33 isomer ratio (Table 1, entry 1). Similarly, *exo*-**10b** and *endo*-**11b** were isolated from the triene **8b** in 90% combined yield and in a 66:34 isomer ratio (Table 1, entry 3). For the purpose of the tandem Wittig–IMDA cycloadditions discussed below, we applied the same microwave heating conditions for the IMDA reactions of *Z*-isomers **9a**,**b** although they could cyclize at a lower temperature. The expected *exo*-**12a**,**b** and *endo*-**13a**,**b** were produced in 98% combined yield for each and in 68:32–76:24 isomer ratios (Table 1, entries 11 and 13). These results confirm two findings: (a) higher product yields with significantly shortened reaction time can be achieved by applying controlled microwave heating; and (b) the same level of stereoselectivity can be maintained for the IMDA cycloadditions in different solvents (MeCN vs PhMe) and at a higher reaction temperature (180 °C vs 100–110 °C) although an exceptional case was



Scheme 1. Synthesis and microwave-assisted IMDA of 1,3,8-nonatrienes **8a–d** and **9a–d**.

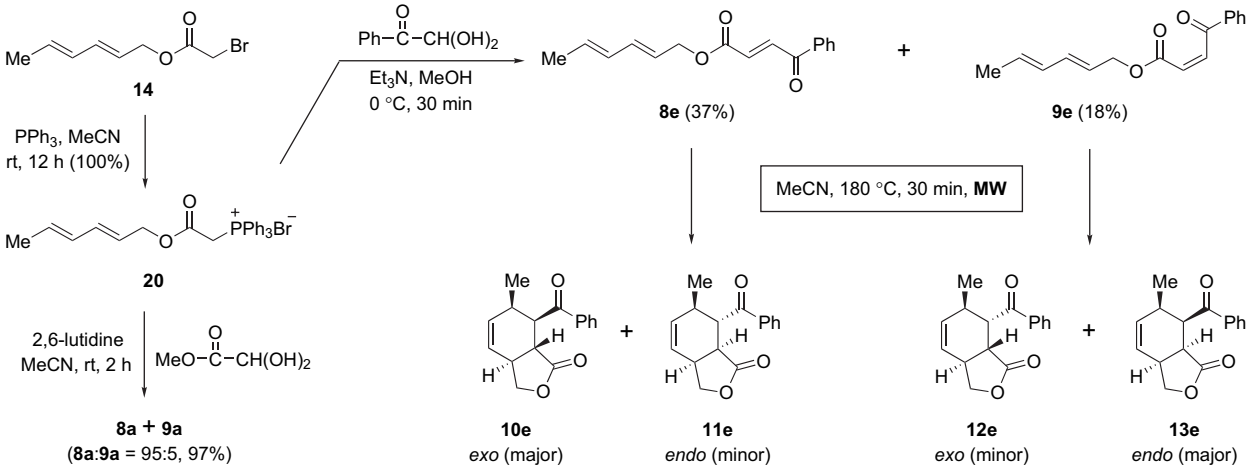
Table 1. Synthesis and IMDA of *E*- and *Z*-substituted 1,3,8-nonatrienes **8a–e** and **9a–e**[a]

Entry	Esters	Yield (%)	Solvent	Lactones	Yield (%)[b]	*exo*:*endo* Ratio[c]
1	(*E*)-**8a**	88	MeCN	**10a+11a**	91	67:33[d] (73:27)
2	(*E*)-**8a**		PhMe	**10a+11a**	85[f]	65:35[f]
3	(*E*)-**8b**	90	MeCN	**10b+11b**	90	66:34[d] (66:34)
4	(*E*)-**8b**		MeCN[h]	**10b+11b**	88	64:36[d]
5	(*E*)-**8b**		PhMe	**10b+11b**	78[g]	60:40[g]
6	(*E*)-**8c**	83	MeCN	**10c+11c**	95	76:24[d] (74:26)
7	(*E*)-**8c**		PhMe[i]	**10c+11c**	55	71:29[d]
8	(*E*)-**8d**	85	MeCN	**10d+11d**	91	68:32[d] (67:33)
9	(*E*)-**8e**	37	MeCN	**10e+11e**	99	73:27[e] (71:29)
10	(*E*)-**8e**		PhMe[i]	**10e+11e**	99	74:26[d]
11	(*Z*)-**9a**	65	MeCN	**12a+13a**	98	76:24[e] (67:33)
12	(*Z*)-**9a**		PhMe	**12a+13a**	79[f]	79:21[f]
13	(*Z*)-**9b**	69	MeCN	**12b+13b**	98	68:32[d] (NA)[j]
14	(*Z*)-**9c**	70	MeCN	**12c+13c**	97	71:29[e] (77:23)
15	(*Z*)-**9d**	71	MeCN	**12d+13d**	97	71:29[e] (NA)[j]
16	(*Z*)-**9e**	18	MeCN	**12e+13e**	99	45:55[d] (47:53)
17	(*Z*)-**9e**		MeCN[h]	**12e+13e**	ND[k]	44:56[d]
18	(*Z*)-**9e**		PhMe[i]	**12e+13e**	(100)[l]	65:35[d]

[a] Except for otherwise stated, all IMDA cycloadditions were carried out in MeCN at 180 °C for 30 min in closed pressurized vials with the reaction temperature measured by an IR sensor.
[b] Combined isolated yields.
[c] The numbers in the parentheses are taken from Table 3 for the tandem reactions.
[d] The ratio was determined by ^{1}H NMR of the product mixtures.
[e] The ratio was calculated based on the weights of the isolated products.
[f] Data taken from Ref. 5e. Compounds **8a** and **9a** were heated in PhMe at 110 °C for 23 and 2 h, respectively.
[g] Data taken from Ref. 5b. Compound **8b** was heated in PhMe at 100 °C for 24 h.
[h] 2,6-Lutidine (1.3 equiv) was added.
[i] Heated in PhMe in an oil bath at 110 °C for 80 and 1 h for **8c** and **9e**, respectively, or in a microwave reactor at 150 °C for 1 h for **8e**.
[j] Not available.
[k] Not determined.
[l] Conversion of **9e** as determined by ^{1}H NMR of the product mixture.

found for the reaction of *Z*-substituted 1,3,8-nonatriene **9e** (vide infra).

With the above encouraging results, we turned our attention to the IMDA reactions of the 1,3,8-nonatrienes **8c–e** and **9c–e**, which were not covered in a recent study by Paddon-Row and Sherburn.[5j] For comparison, the triene **8c** possessing a C1-phenyl group was subjected to cycloaddition in PhMe at 110 °C for 80 h, giving the adducts **10c** and **11c** in 55% combined yield and in a 71:29 *exo*:*endo* ratio (Table 1, entry 7). The stereoselectivity of **8c** is slightly better than the C1-methyl analog **8b** (Table 1, entry 5)[5g] although **8c** showed a diminished reactivity toward cycloaddition. For the microwave-heated cycloadditions, the paired adducts **10c–e/11c–e** and **12c,d/13c,d** were obtained in 91–99% combined yields and in 68:32–76:24 isomer ratios in favor of the *exo* isomers (Table 1, entries 6, 8, 9, 14, and 15), except for the adducts **12e** and **13e**, whose ratio is 45:55 (Table 1, entry 16). We repeated the cycloaddition of **9e** in MeCN at 180 °C for several times and obtained the same isomer ratio in all cases. It was reported that treatment of the *trans*-fused lactone **A** with a catalytic NaOMe gave the corresponding cis-fused isomer.[7b] We carried out the microwave-assisted IMDA reactions of **8b** and **9e** in the presence of 2,6-lutidine and obtained almost the same results in both cases (Table 1, entries 4 and 17). Moreover, we did not find any structural



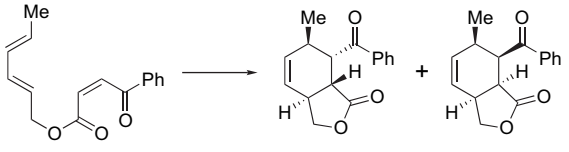
Scheme 2. Synthesis and microwave-assisted IMDA of 1,3,8-nonatrienes **8e** and **9e**.

change after heating each pure adducts **10e**, **11e**, **12e**, and **13e** at 180 °C for 30 min in MeCN in the presence of 2,6-lutidine. The results confirmed that isomerization of the adducts did not happen even in the presence of a base. When **9e** was heated in PhMe at 110 °C for 1 h, the adducts **12e** and **13e** were formed in a 65:35 ratio in favor of the *exo* isomer **12e** (Table 1, entry 18). Similarly, the IMDA reaction of the *E*-substituted **8e**, after heated in PhMe at 150 °C for 1 h under microwave irradiation, afforded a 74:26 ratio of **10e**:**11e** in 99% combined yield (Table 1, entry 10). The results indicated that the solvent-dependent stereoselectivity is unique to the (*Z*)-9-acyl-substituted 1,3,8-nonatrienes.

We decided to examine the solvent and temperature effects on the IMDA cycloaddition of the 9-benzoyl-substituted triene **9e**. The results are summarized in Table 2. The triene **9e** was the most reactive among the 10 substrates we studied and it underwent the IMDA reaction at ambient temperature (Table 2, entries 1–3). After stirring at 20 °C for 70 h, the conversion of **9e** was measured by ^{1}H NMR spectroscopy of the reaction mixtures. The reactivity of **9e** parallels with the polarity of the solvent in the order of PhMe< $CDCl_3$<MeCN and the conversions are 60, 85, and 95%, respectively. The results consist with the rate enhancement observed in polar solvents.[13c–e] Moreover, it is interesting to find that the nonpolar solvent, PhMe favored for the *exo* isomer **12e** while the polar solvent, MeCN promoted the formation of the *endo* isomer **13e**. We observed that the ratios of **12e**:**13e** increased from 56:44 to 65:35 in PhMe at 110 °C (Table 2, entry 1 vs entry 4) and from 36:64 to 45:55 in MeCN at 82 °C (Table 2, entry 3 vs entry 5). We attempted to modify the preformed adduct ratios by switching solvent or temperature without success (Table 2, entries 6 and 7). It suggests that an equilibrium through IMDA–retro-IMDA pathways does not exist.

We carefully secured the stereochemistry of **12e** with the help of X-ray crystal structural analysis. As shown in Figure 1, the compound **12e** features a trans-fused bicyclic

Table 2. Solvent effect on IMDA of **9e**



9e *exo* **-12e** *endo* **-13e**

Entry	Solvent	*T* (°C); *t* (h)	Conversion (%)[a]	**12e**:**13e**[a]
1	PhMe	20; 70	60	56:44
2	$CDCl_3$	20; 70	85	54:46
3	MeCN	20; 70	95	36:64
4	PhMe	110; 1	100[b]	65:35[b]
5	MeCN	82; 1	100	45:55
6	PhMe[c]	150; 0.5	—	45:55
7	MeCN[d]	180; 0.5	—	35:65

[a] The conversion of **9e** and the ratio of the adducts **12e**:**13e** were determined by ^{1}H NMR of the crude reaction mixture.
[b] Data taken from entry 18 of Table 1.
[c] The 45:55 adduct mixture of entry 5, instead of **9e**, was heated on the microwave reactor.
[d] The 36:64 adduct mixture of entry 3, instead of **9e**, was heated on the microwave reactor.

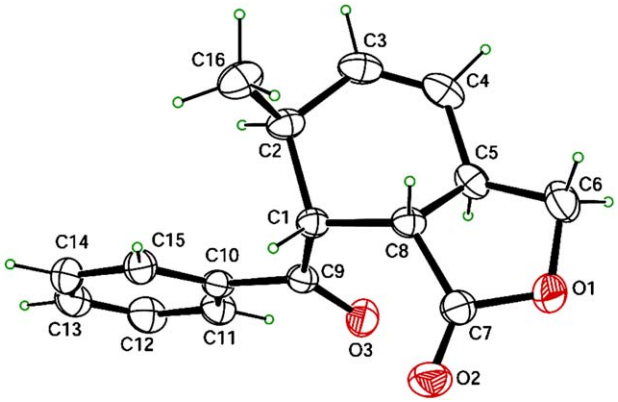


Figure 1. X-ray crystal structure of **12e** (shown as the enantiomer).

skeleton with both trans-oriented substituents sitting at the axial positions of the half-chair-like cyclohexene ring. Indeed, **12e** is the expected *exo* adduct of the *Z*-substituted 1,3,8-nonatriene system. The IMDA cycloadditions of the related *E*-substituted 1,3,8-nonatrienes, similar to **8e**, possessing a C9-keto unit were reported to yield the adducts in the *exo*:*endo* ratios of 61:39–80:20 (PhMe, 120–140 °C, 12–20 h).[6o] Our observation for the reaction of **8e** is consistent with the reported stereoselectivity (Table 1, entry 9). We also carried out X-ray crystal structural analysis for the adducts **10c** and **13d** derived from the 1-phenyl-substituted[6g,19] 1,3,8-nonatrienes. Figure 2 is the drawing of the *exo* adduct **10c**, depicting a trans-fused bicycle with the phenyl group placing in the pseudo axial and the ester moiety in the pseudo equatorial positions. The structural drawing of the *cis*-fused bicyclic lactone **13d** is shown in Figure 3. The phenyl group is placed in the pseudo axial position while the ester moiety occupies the pseudo equatorial orientation. On the basis of our results on IMDA cycloadditions of the 10 different *E*- and *Z*-substituted 1,3,8-nonatrienes **8a–e** and **9a–e** possessing an ester linkage, *exo* selectivity is generally observed irrespective of the nature of C1-substituent (Me vs Ph) and C9-activator (ester vs keto).[5i] The 'abnormal' *exo*:*endo* ratio for the adducts **12e**/**13e** obtained from the (*Z*)-9-acyl-substitued **9e** in MeCN originates from a polar solvent effect, which favors formation of the *endo* isomer. Its nature is not fully understood.[13c,e]

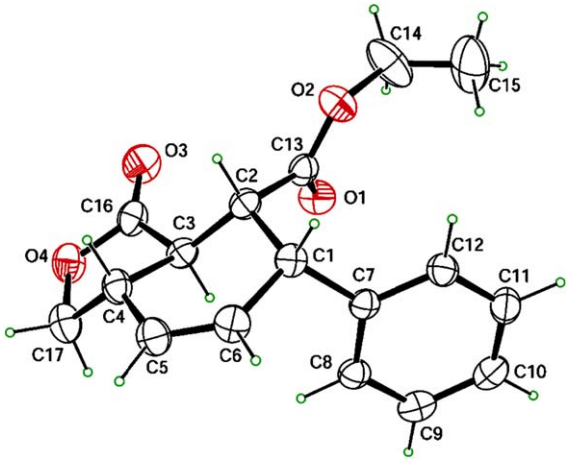


Figure 2. X-ray crystal structure of **10c**.

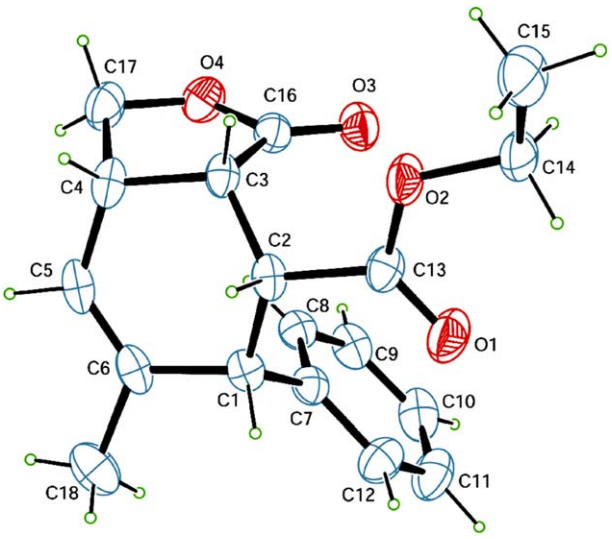
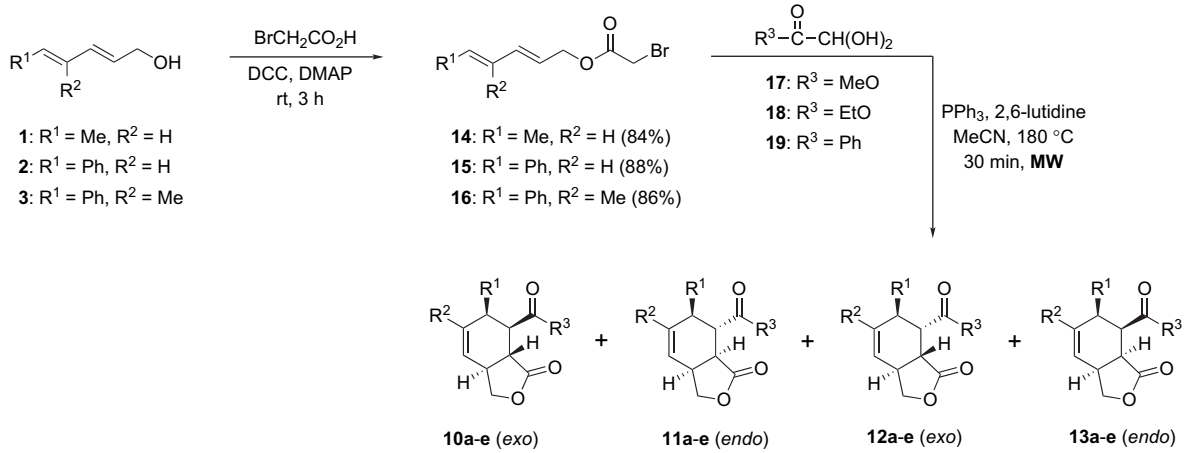


Figure 3. X-ray crystal structure of **13d**.

2.2. Microwave-assisted tandem Wittig–IMDA cycloadditions

The Wittig olefination[20] is another powerful methodology for synthesis of functionalized olefins from halides and carbonyl compounds. The stabilized phosphorus ylides are easily prepared and purified in pure forms. However, the reactivity of stabilized ylides is relatively lower and heating conditions are frequently required with prolonged reaction times. The microwave-assisted Wittig reactions of stabilized phosphorus ylides with aldehydes,[21] ketones,[11a,d,22] and lactones[23] have been explored although the majority was done in domestic microwave ovens. Mechanistically speaking, the Wittig reaction consists of three steps: (a) phosphonium salt formation; (b) ylide formation via deprotonation; and (c) olefination of carbonyl substrates. The step (a) is often carried out under forced conditions such as microwave heating.[24] The most efficient way to conduct a Wittig reaction should begin with the ylide precursor but not the preformed ylide. Development of the so-called 'one-pot' Wittig reaction has been the focus of considerable research efforts,[25] including the work of Westman[25g] for demonstrating the one-pot Wittig reaction of a resin-bound phosphine under controlled microwave heating. As a continuation of our previous studies on the Wittig reactions with microwave irradiation,[11a,d] in aqueous media,[26c,d] and in the asymmetric versions,[24b,26a,b] we proposed to establish a microwave-assisted tandem Wittig–IMDA cycloaddition protocol[27] for a modular synthesis of the bicyclic lactones **C**, starting from the α-bromoacetates **III** and the α-oxo carbonyl compounds **IV** (Chart 2). To the best of our knowledge, no prior example of this sort is known in the literature. As a proof-of-concept study, we selected three each of the α-bromoacetates **14**–**16** and the hydrate forms of α-oxo carbonyl compounds **17**–**19** in our current work. The results are summarized in Scheme 3 and Table 3.

According to the synthesis of **8a** and **9a** shown in Scheme 2, formation of the phosphonium salt **20** from **14** and subsequent olefination could proceed at room temperature. Therefore, the rate-limiting step for our proposed tandem process should be the IMDA cycloaddition. To our delight, the cascade reaction sequence took place at 180 °C consisting of alkylation of PPh_3 with the bromides **14**–**16**, deprotonation of the phosphonium salts with 2,6-lutidine, olefination of the ylides with the α-oxo carbonyls **17**–**19**, and finally the IMDA cycloadditions. Thus, after heating on a technique microwave reactor in a closed vial at 180 °C for 30 min in MeCN, the bicyclic lactones were produced in 68–80% isolated yields by a single operation. Other bases such as Et_3N could be used for the Wittig reaction as shown in Scheme 2. In order to avoid formation of a quaternary ammonium salt from Et_3N at the high temperature, 2,6-lutidine was selected. Four isomeric adducts were formed in all reactions and the *exo*:*endo* ratios are almost identical to those obtained for the IMDA reactions of the purified 1,3,8-nonatrienes **8a**–**e** and **9a**–**e** (Table 1, entries 1, 3, 6, 8, 9, 11, 14, and 16). On the basis of the ratios of (**10**+**11**):(**12**+**13**) in the entries 1, 3, and 5 of Table 3, it is estimated that the olefins **8**:**9** are formed in 78:22–88:12 mixtures of *E*- and *Z*-isomers at 180 °C. It is clear that the stereoselectivity of the Wittig olefination at high temperature somewhat deteriorated as compared to the room temperature version, which gave a 95:5 ratio for the *E*- and *Z*-isomers **8a** and **9a** (Scheme 2). Aside from the olefination stereoselectivity, our tandem Wittig–IMDA cycloaddition protocol, in combination with controlled microwave heating,



Scheme 3. Synthesis of α-bromoacetates **14**–**16** and tandem Wittig–IMDA under microwave heating.

Table 3. Microwave-assisted tandem Wittig–IMDA of **14–16** with **17–19**[a]

Entry	Substrates	Lactones	Yield (%)[b]	Isomer ratio (**10a**:**11a**:**12a**:**13a**)	*exo*:*endo* Ratios (**10**:**11**; **12**:**13**)
1	**14+17**	**10a+11a+12a+13a**	68	64:24:8:4[c]	73:27; 67:33
2	**14+18**	**10b+11b+12b+13b**	71	57:29:14:0[d,e]	66:34; NA[g]
3	**15+18**	**10c+11c+12c+13c**	80	58:20:17:5[c]	74:26; 77:23
4	**16+18**	**10d+11d+12d+13d**	76	56:28:16:0[d,e]	67:33; NA[g]
5	**14+19**	**10e+11e+12e+13e**	77	59:24:8:9[c,f]	71:29; 47:53

[a] All IMDA were carried out in closed pressurized vials with the reaction temperature measured by an IR sensor.
[b] Combined isolated yields.
[c] The ratio was determined by ^{1}H NMR of the crude product mixtures. Copies of the ^{1}H NMR charts are found in Supplementary data.
[d] Due to overlapping of ^{1}H NMR signals of the stereoisomers, the ratio was calculated based on the weights of the isolated products.
[e] The minor isomer **13** was not isolated, probably lost during purification.
[f] Average values of two runs.
[g] Not available.

provides a high-throughput synthesis of the bicyclic lactones by using the appropriate bromide and aldehyde building blocks in a combinatorial approach. Structural complexity and functional groups of the lactones **A** (Chart 1) can be easily introduced according to the known chemistry developed for their syntheses over the years.[4–7]

3. Conclusion

We have studied the IMDA cycloaddition of the ester-tethered 1,3,8-nonatrienes possessing substituents at both C1 and C9 under controlled microwave heating at 180 °C for 30 min in a polar solvent, MeCN. In general, the predicted *exo* stereoselectivity[5j] for both pentadienyl maleates (**8b–d**) and fumarates (**9a–d**) has been confirmed. A similar *exo* stereoselectivity is obtained for IMDA cycloaddition of the C1-substituted pentadienyl (*E*)-4-oxobut-2-enoate **8e**[6o] although (*Z*)-4-oxobut-2-enoate **9e** demonstrates an unusual solvent-dependent stereoselectivity. Our findings suggest that the IMDA cycloadditions can be significantly accelerated with microwave irradiation without deteriorating stereoselectivity. We have also realized a tandem Wittig–IMDA cycloaddition protocol illustrated in Chart 2 for an expedite synthesis of the bicyclic lactones **C** starting from the bromide and aldehyde building blocks. Our synthetic strategy in combination with controlled microwave heating offers an efficient synthesis of the bicyclic lactones, whose applications can be amplified subject to improvement of the stereoselectivity.

4. Experimental

4.1. General information and the microwave reactor

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 (300, 400, or 500 MHz for ^{1}H, 75, 100, or 125 MHz for ^{13}C, and 121 MHz for ^{31}P). IR spectra were taken on an FTIR spectrophotometer. Mass spectra (MS) were measured by the +ESI or +EI method. Melting points are uncorrected. Silica gel plates pre-coated on glass were used for thin-layer chromatography using UV light, or 7% ethanolic phosphomolybdic acid and heating as the visualizing methods. Silica gel was used for flash column chromatography. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Petroleum ether of 60–90 °C fraction was used in this work. *trans*,*trans*-2,4-Hexadien-1-ol **1**, fumaric acid monoethyl ester **5**, and phenylglyoxal hydrate **19**, and other reagents were obtained commercially and used as received. Fumaric acid monomethyl ester **4**,[14] maleic acid monomethyl ester **6**,[16] and monoethyl ester **7**,[16] methyl glyoxalate hydrate **17**,[28] and ethyl glyoxalate hydrate **18**[28] were prepared according to the reported procedures. All microwave-assisted reactions were carried out on an Emrys creator from Personal Chemistry AB (now under Biotage AB, Uppsala, Sweden) with temperature measured by an IR sensor. The microwave-assisted reaction time is the hold time at the final temperature.

4.2. General procedure for synthesis of alcohols 2 and 3

To a solution of the dienonate (5 mmol) in dry CH_2Cl_2 (15 mL) cooled in an ice-water bath under a nitrogen atmosphere was added DIBAL-H (11 mL, 1.0 M in hexane) dropwise. The resultant mixture was stirred for 45 min at the same temperature and the reaction was quenched with saturated aqueous sodium potassium tartrate (Rochelle's salt). The mixture was stirred at ambient temperature for 2 h, and then diluted with Et_2O (10 mL). The aqueous layer was extracted with Et_2O (10 mL×2) and the combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 20% EtOAc in petroleum ether) to give the 2,4-pentadien-1-ols.

4.2.1. (2*E*,4*E*)-5-Phenylpenta-2,4-dien-1-ol (2). Prepared from ethyl (2*E*,4*E*)-5-phenylpenta-2,4-dienoate[29,30] in 95% yield as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.22 (m, 5H), 6.79 (dd, J=15.6, 10.6 Hz, 1H), 6.57 (d, J=15.6 Hz, 2H), 6.43 (dd, J=15.2, 10.6 Hz, 1H), 5.97 (dt, J=15.2, 6.0 Hz, 1H), 4.26 (d, J=5.6 Hz, 2H), 1.75–1.50 (br s, 1H).

4.2.2. (2*E*,4*E*)-4-Methyl-5-phenylpenta-2,4-dien-1-ol (3). Prepared from ethyl (2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dienoate[30] in 95% yield as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.27 (m, 5H), 6.60 (s, 1H), 6.52 (d, J=15.2 Hz, 1H), 6.00 (dt, J=15.6, 6.0 Hz, 1H), 4.34 (d, J=5.6 Hz, 2H), 2.89 (s, 1H), 2.08 (s, 3H).

4.3. General procedure for synthesis of *E*-substituted 1,3,8-nonatrienes 8a–d

To a solution of the alcohol (1.5 mmol), 4-dimethylaminopyridine (DMAP, 0.15 mmol), and fumaric acid monomethyl

ester **4**[14] or monoethyl ester **5**[16] (2 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C under a nitrogen atmosphere was added *N*,*N*′-dicyclohexylcarbodiimide (DCC, 2.0 mmol) in one portion. After stirring for 30 min at the same temperature, the reaction mixture was allowed to warm up to room temperature followed by stirring for another 3 h. Celite was added to the reaction vessel and the mixture, after stirring for 30 min, was then filtered with washing by CH_2Cl_2. The combined filtrate was evaporated under reduced pressure to give the crude product, which was purified by silica gel chromatography (3% EtOAc in petroleum ether) to provide the pure product. The yields are given in Table 1.

4.3.1. Methyl (2*E*,4*E*)-hexa-2,4-dien-1-yl fumarate (8a).[5b] Prepared from (2*E*,4*E*)-hexa-2,4-dien-1-ol **1** and fumaric acid monomethyl ester **4** in 88% yield as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (d, J=0.4 Hz, 2H), 6.27 (dd, J=15.2, 10.4 Hz, 1H), 6.03 (ddd, J=14.8, 10.0, 1.6 Hz, 1H), 5.75 (dq, J=14.8, 6.8 Hz, 1H), 5.62 (dt, J=15.2, 6.8 Hz, 1H), 4.68 (d, J=7.2 Hz, 2H), 3.78 (s, 3H), 1.75 (d, J=6.8 Hz, 3H).

4.3.2. Ethyl (2*E*,4*E*)-hexa-2,4-dien-1-yl fumarate (8b).[5e] Prepared from (2*E*,4*E*)-hexa-2,4-dien-1-ol **1** and fumaric acid monoethyl ester **5** in 90% yield as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (s, 2H), 6.29 (dd, J=15.6, 10.4 Hz, 1H), 6.06 (dd, J=15.6, 10.0 Hz, 1H), 5.78 (dq, J=15.2, 6.4 Hz, 1H), 5.65 (dt, J=15.6, 6.4 Hz, 1H), 4.70 (d, J=6.8 Hz, 2H), 4.26 (q, J=7.2 Hz, 2H), 1.76 (d, J=6.4 Hz, 3H), 1.32 (t, J=7.2 Hz, 3H).

4.3.3. Ethyl (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-yl fumarate (8c). Prepared from (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-ol **2** and fumaric acid monoethyl ester **5** in 83% yield as a colorless oil; R_f=0.35 (4.8% EtOAc in hexane); IR (film) 3026, 2984, 1721, 1645, 1294, 1258, 1153, 988 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.18 (m, 5H), 6.85 (s, 2H), 6.74 (dd, J=15.6, 10.4 Hz, 1H), 6.57 (d, J=15.6 Hz, 1H), 6.45 (dd, J=15.2, 10.4 Hz, 1H), 5.84 (dt, J=14.8, 6.8 Hz, 1H), 4.75 (d, J=6.8 Hz, 2H), 4.23 (q, J=7.2 Hz, 2H), 1.28 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 164.7, 136.7, 135.1, 134.2, 134.0, 133.3, 128.6 (×2), 127.9, 127.4, 126.5 (×2), 125.9, 65.5, 61.3, 14.1; MS (+ESI) m/z 595 (2M+Na$^+$, 100), 309 (M+Na$^+$, 82); HRMS (+ESI) calcd for $C_{17}H_{18}O_4Na$ (M+Na$^+$), 309.1097; found, 309.1085.

4.3.4. Ethyl (2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dien-1-yl fumarate (8d). Prepared from (2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dien-1-ol **3** and fumaric acid monoethyl ester **5** in 85% yield as a colorless oil; R_f=0.38 (4.8% EtOAc in hexane); IR (film) 2984, 1721, 1645, 1294, 1258, 1153, 966 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.20 (m, 5H), 6.88 (d, J=0.8 Hz, 2H), 6.55 (s, 1H), 6.50 (d, J=15.6 Hz, 1H), 5.84 (dt, J=15.6, 6.4 Hz, 1H), 4.80 (d, J=6.8 Hz, 2H), 4.24 (q, J=6.8 Hz, 2H), 1.99 (s, 3H), 1.30 (td, J=7.2, 0.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 164.8, 140.3, 137.3, 134.5, 134.0, 133.4, 133.0, 129.2 (×2), 128.1 (×2), 126.8, 121.5, 66.0, 61.3, 14.1, 13.8; MS (+ESI) m/z 623 (2M+Na$^+$, 87), 323 (M+Na$^+$, 100); HRMS (+ESI) calcd for $C_{18}H_{20}O_4Na$ (M+Na$^+$), 323.1254; found, 323.1244.

4.4. General procedure for synthesis of *Z*-substituted 1,3,8-nonatrienes 9a–d

To a solution of maleic acid monomethyl ester **6** or monoethyl ester **7**[16] (3.7 mmol) in dry CH_2Cl_2 was added *N*,*N*′-diisopropylcarbodiimide (DIC, 1.85 mmol) and the mixture was stirred at 0 °C for 1 h. The insoluble urea was filtered off and the filtrate was added to a solution of the alcohol (1.23 mmol) followed by addition of *i*-Pr_2NEt (DIEA, 3.70 mmol) and 4-dimethylaminopyridine (DMAP, cat.). The resultant mixture was stirred at 0 °C for 1 h and at ambient temperature for another 1 h. The reaction mixture was diluted with CH_2Cl_2 and successively washed with saturated aqueous NH_4Cl and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to give the crude product, which was purified by silica gel chromatography (9% EtOAc in petroleum ether) to provide the pure product. The yields are given in Table 1.

4.4.1. Methyl (2*E*,4*E*)-hexa-2,4-dien-1-yl maleate (9a).[5b] Prepared from (2*E*,4*E*)-hexa-2,4-dien-1-ol **1** and maleic acid monomethyl ester **6** in 65% yield as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (s, 2H), 6.27 (dd, J=15.2, 10.4 Hz, 1H), 6.05 (ddd, J=15.2, 10.0, 1.2 Hz, 1H), 5.76 (dq, J=15.2, 6.8 Hz, 2H), 4.68 (d, J=6.8 Hz, 2H), 3.77 (s, 3H), 1.75 (d, J=6.8 Hz, 3H).

4.4.2. Ethyl (2*E*,4*E*)-hexa-2,4-dien-1-yl maleate (9b). Prepared from (2*E*,4*E*)-hexa-2,4-dien-1-ol **1** and maleic acid monoethyl ester **7** in 69% yield as a colorless oil; R_f=0.43 (9.1% EtOAc in hexane); IR (film) 2984, 1735, 1725, 1642, 1403, 1209, 1160, 990 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.25 (dd, J=15.6, 10.8 Hz, 1H), 6.21 (s, 2H), 6.07–5.98 (m, 1H), 5.73 (dq, J=15.2, 6.4 Hz, 1H), 5.61 (dt, J=14.8, 6.8 Hz, 1H), 4.65 (d, J=6.8 Hz, 2H), 4.21 (q, J=7.2 Hz, 2H), 1.73 (d, J=6.4 Hz, 3H), 1.27 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.1, 164.8, 135.4, 131.4, 130.2, 130.0, 129.3, 122.8, 65.6, 61.1, 18.0, 13.8; MS (+ESI) m/z 471 (2M+Na$^+$, 100), 247 (M+Na$^+$, 49); HRMS (+ESI) calcd for $C_{12}H_{16}O_4Na$ (M+Na$^+$), 247.0941; found, 247.0934.

4.4.3. Ethyl (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-yl maleate (9c). Prepared from (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-ol **2** and maleic acid monoethyl ester **7** in 70% yield as a colorless oil; R_f=0.34 (9.1% EtOAc in hexane); IR (film) 2983, 1728, 1643, 1403, 1210, 1162, 989 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.22 (m, 5H), 6.70 (dd, J=15.6, 10.4 Hz, 1H), 6.52 (d, J=15.6 Hz, 1H), 6.41 (dd, J=14.8, 10.0 Hz, 1H), 6.19 (s, 2H), 5.83 (dt, J=15.2, 6.8 Hz, 1H), 4.78 (d, J=6.4 Hz, 2H), 4.25 (q, J=7.6 Hz, 2H), 1.31 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 164.9, 136.8, 135.1, 134.0, 130.2, 129.4, 128.6 (×2), 127.9, 127.5, 126.5 (×2), 126.1, 65.5, 61.2, 14.0; MS (+ESI) m/z 595 (2M+Na$^+$, 100), 309 (M+Na$^+$, 57); HRMS (+ESI) calcd for $C_{17}H_{18}O_4Na$ (M+Na$^+$), 309.1097; found, 309.1086.

4.4.4. Ethyl (2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dien-1-yl maleate (9d). Prepared from (2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dien-1-ol **3** and maleic acid monoethyl ester **7** in 71% yield as a colorless oil; R_f=0.4 (9.1% EtOAc in hexane); IR (film) 2983, 1728, 1643, 1403, 1210, 1161 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46–7.31 (m, 5H), 6.66

(s, 1H), 6.61 (d, J=15.5 Hz, 1H), 6.36 (s, 2H), 5.96 (dt, J=15.5, 6.5 Hz, 1H), 4.90 (d, J=6.5 Hz, 2H), 4.35 (q, J=7.0 Hz, 2H), 2.10 (d, J=0.5 Hz, 3H), 1.40 (t, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.1, 164.9, 140.1, 137.3, 134.5, 132.8, 130.1, 129.4, 129.1 (×2), 128.1 (×2), 126.7, 121.5, 65.9, 61.2, 13.9, 13.7; MS (+ESI) m/z 623 (2M+Na$^+$, 79), 323 (M+Na$^+$, 100); HRMS (+ESI) calcd for $C_{18}H_{20}O_4Na$ (M+Na$^+$), 323.1254; found, 323.1239.

4.5. General procedure for synthesis of the α-bromoacetates 14–16

To a suspension of the alcohols **1–3** (1.5 mmol), 4-dimethylaminopyridine (DMAP, 0.15 mmol), and bromoacetic acid (2.0 mmol) in dry CH_2Cl_2 (15 mL) cooled in an ice-water bath (0 °C) under a nitrogen atmosphere was added *N*,*N*′-dicyclohexylcarbodiimide (DCC, 2.0 mmol) in one portion. After stirring for 30 min at 0 °C, the reaction was allowed to warm up to ambient temperature followed by stirring for 3 h. Celite was added to the reaction mixture and, after stirring for 30 min, the mixture was filtered with washing by CH_2Cl_2. The combined filtrate was evaporated under reduced pressure and the residue was purified by silica gel chromatography (3% EtOAc in petroleum ether) to provide the products **14–16**.

4.5.1. (2*E*,4*E*)-Hexa-2,4-dien-1-yl α-bromoacetate (14). Prepared from (2*E*,4*E*)-hexa-2,4-dien-1-ol **1** and α-bromoacetic acid in 84% yield as a colorless oil; R_f=0.44 (4.8% EtOAc in hexane); IR (film) 3026, 2959, 1739, 1678, 1449, 1276, 1159, 1123, 990 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (dd, J=15.2, 10.4 Hz, 1H), 6.06 (dd, J=15.2, 10.4 Hz, 1H), 5.79 (dq, J=15.2, 6.8 Hz, 1H), 5.63 (dt, J=15.2, 6.8 Hz, 1H), 4.67 (d, J=6.8 Hz, 1H), 3.85 (s, 3H), 1.77 (d, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 135.7, 131.8, 130.1, 122.4, 66.6, 25.8, 18.0; MS (+ESI) m/z 243 (M+2+Na$^+$, 100), 241 (M+Na$^+$, 97); HRMS (+EI) calcd for $C_8H_{11}BrO_2$ (M$^+$), 217.9937; found, 219.9921 (M$^+$+2), 217.9939 (M$^+$).

4.5.2. (2*E*,4*E*)-5-Phenylpenta-2,4-dien-1-yl α-bromoacetate (15). Prepared from (2*E*,4*E*)-5-phenylpenta-2,4-dien-1-ol **2** and α-bromoacetic acid in 88% yield as a colorless oil; R_f=0.53 (4.8% EtOAc in hexane); IR (film) 3026, 2947, 1736, 1276, 1159, 989, 741, 692 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.24 (m, 5H), 6.78 (dd, J=15.6, 10.8 Hz, 1H), 6.62 (d, J=15.2 Hz, 1H), 6.51 (dd, J=15.6, 10.4 Hz, 1H), 5.89 (dt, J=14.8, 6.8 Hz, 1H), 4.77 (d, J=6.8 Hz, 2H), 3.88 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 136.6, 135.4, 134.2, 128.5 (×2), 127.8, 127.3, 126.4 (×2), 125.5, 66.3, 25.8; MS (+ESI) m/z 305 (M+2+Na$^+$, 100), 303 (M+Na$^+$, 98); HRMS (+EI) calcd for $C_{13}H_{13}BrO_2$ (M$^+$), 280.0093; found, 282.0078 (M$^+$+2), 280.0100 (M$^+$).

4.5.3. (2*E*,4*E*)-4-Methyl-5-phenylpenta-2,4-dien-1-yl α-bromoacetate (16). Prepared from (2*E*,4*E*)-4-methyl-5-phenylpenta-2,4-dien-1-ol **3** and α-bromoacetic acid in 86% yield as a colorless oil; R_f=0.43 (4.8% EtOAc in hexane); IR (film) 3022, 2950, 1736, 1274, 1157, 1111, 962 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.23 (m, 5H), 6.59 (s, 1H), 6.54 (d, J=16.0 Hz, 1H), 5.85 (dt, J=16.0, 6.4 Hz, 1H), 4.80 (d, J=7.2 Hz, 2H), 3.88 (d, J=1.2 Hz, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 140.5, 137.2, 134.4, 133.1, 129.1 (×2), 128.1 (×2), 126.8, 121.0, 66.9, 25.9, 13.7; MS (+ESI) m/z 319 (M+2+Na$^+$, 100), 317 (M+Na$^+$, 97); HRMS (+EI) calcd for $C_{14}H_{15}BrO_2$ (M$^+$), 294.0250; found, 296.0235 (M$^+$+2), 294.0255 (M$^+$).

4.5.4. Preparation of phosphonium salt (20). A solution of (2*E*,4*E*)-penta-2,4-dien-1-yl α-bromoacetate **14** (0.3 mmol) and PPh_3 (0.33 mmol) in MeCN (10 mL) was stirred at ambient temperature overnight (12 h). The reaction mixture was evaporated under reduced pressure to give a white solid, which was washed with dry benzene (9 mL×3) and dried in vacuum to provide quantitatively the salt **20**. The phosphonium salt was used without further purification. Compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86–7.59 (m, 15H), 6.04 (dd, J=15.0, 10.2 Hz, 1H), 5.85 (dd, J=15.0, 10.2 Hz, 1H), 5.67 (dq, J=14.4, 6.9 Hz, 1H), 5.44 (d, J=13.8 Hz, 2H), 5.23 (dt, J=15.0, 7.2 Hz, 1H), 4.41 (d, J=7.2 Hz, 2H), 1.71 (d, J=6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.9 (d, J_{P-C}=4.6 Hz), 136.2, 135.0 (d, J_{P-C}=3.1 Hz), 133.8 (d, J_{P-C}=10.9 Hz, ×2), 132.1, 130.1 (d, J_{P-C}=13.6 Hz, ×2), 129.8, 121.4, 117.6 (d, J_{P-C}=88.9 Hz), 67.0, 33.0 (d, J_{P-C}=55.0 Hz), 18.1; ^{31}P NMR (121 MHz, $CDCl_3$) δ 22.2.

4.5.5. Synthesis of (2′*E*,4′*E*)-hexa-2′,4′-dien-1-yl (2*E*)-4-phenyl-4-oxo-2-butenoate (8e) and (2′*E*,4′*E*)-hexa-2′,4′-dien-1-yl (2*Z*)-4-phenyl-4-oxo-2-butenoate (9e). To a solution of the phosphonium salt **20** (2 mmol) and phenylglyoxal hydrate **19** (2.2 mmol) in MeOH (15 mL) cooled in an ice-water bath (0 °C) was added Et_3N (2.1 mmol). The resultant mixture was stirred for 30 min at 0 °C and the reaction mixture was extracted with EtOAc (30 mL×2). The combined organic layer was washed with 6% aqueous HCl and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 9% EtOAc in petroleum ether) to give **8e** (37%) and **9e** (18%).

Compound **8e**. A yellow oil; R_f=0.56 (9.1% EtOAc in hexane); IR (film) 3026, 2936, 1723, 1673, 1292, 1262, 1164, 989 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00–7.98 (m, 2H), 7.91 (d, J=16.0 Hz, 1H), 7.64–7.60 (m, 1H), 7.51 (t, J=7.6 Hz, 2H), 6.89 (d, J=15.2 Hz, 1H), 6.31 (dd, J=15.6, 11.2 Hz, 1H), 6.08 (ddd, J=15.2, 10.8, 1.6 Hz, 1H), 5.79 (dq, J=15.2, 6.8 Hz, 1H), 5.68 (dt, J=15.2, 6.8 Hz, 1H), 4.74 (d, J=6.8 Hz, 2H), 1.77 (d, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.2, 165.1, 136.4, 136.4, 135.4, 133.7, 132.2, 131.6, 130.2, 128.7 (×2), 128.7 (×2), 122.8, 65.7, 18.0; MS (+ESI) m/z 535 (2M+Na$^+$, 17), 279 (M+Na$^+$, 100), 257 (M+H$^+$, 92); HRMS (+ESI) calcd for $C_{16}H_{16}O_3Na$ (M+Na$^+$), 279.0992; found, 279.0982.

Compound **9e**. A yellow oil; R_f=0.24 (9.1% EtOAc in hexane); IR (film) 3026, 2933, 1722, 1673, 1203, 1165, 990 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95–792 (m, 2H), 7.61–7.44 (m, 3H), 6.89 (dd, J=11.7, 0.6 Hz, 1H), 6.29 (dd, J=12.6, 1.2 Hz, 1H), 6.11 (dd, J=15.0, 10.2 Hz, 1H), 5.96 (ddd, J=15.0, 10.5, 1.2 Hz, 1H), 5.70 (dq, J=15.0, 6.9 Hz, 1H), 5.40 (dt, J=15.0, 6.6 Hz, 1H), 4.49 (d, J=6.6 Hz, 2H), 1.75 (d, J=6.9 Hz, 3H); ^{13}C NMR (75 MHz,

$CDCl_3$) δ 194.0, 164.5, 141.3, 135.8, 135.4, 133.7, 131.5, 130.3, 128.8 (×2), 128.7 (×2), 125.9, 122.7, 65.7, 18.3.

*Note: The triene **9e** undergoes IMDA cycloaddition at ambient temperature and the adducts **12e** and **13e** were formed during the course of measurement of ^1H NMR. Therefore, mass data were not attempted for **9e**.*

4.6. General procedure for microwave-assisted intramolecular Diels–Alder reactions of *E*- and *Z*-substituted 1,3,8-nonatrienes 8a–e and 9a–e

To a 10-mL pressurized process vial was added the 1,3,8-nonatrienes (0.50 mmol) in CH_3CN (4 mL). The loaded vial was then sealed with a cap containing a silicon septum, and put into the microwave cavity and heated at 180 °C for 30 min. The reaction mixture was evaporated under reduced pressure and the residue was purified by flash column chromatography (silica gel, 20% EtOAc in petroleum ether) to give the desired products. The structures, isomer ratios, and yields are found in Schemes 1 and 2 and Table 1.

4.6.1. Methyl (3a*R,6*S**,7*R**,7a*S**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (10a).**[5e] The major isomer from IMDA reaction of **8a** was obtained as a colorless oil; ^1H NMR (300 MHz, $CDCl_3$) δ 5.78 (ddd, *J*=9.6, 1.5, 1.5 Hz, 1H), 5.70 (ddd, *J*=9.9, 3.0, 3.0 Hz, 1H), 4.46 (dd, *J*=8.1, 6.3 Hz, 1H), 3.93 (dd, *J*=11.4, 8.4 Hz, 1H), 3.77 (s, 3H), 2.97 (dd, *J*=11.1, 6.9 Hz, 1H), 2.91–2.61 (m, 2H), 2.59 (dd, *J*=13.2, 11.4 Hz, 1H), 0.98 (d, *J*=6.6 Hz, 3H).

4.6.2. Methyl (3a*R,6*S**,7*S**,7a*R**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (11a).**[5e] The minor isomer from IMDA reaction of **8a** was obtained as a colorless oil (this sample contains 8% **10a**); ^1H NMR (300 MHz, $CDCl_3$) δ 5.77 (ddd, *J*=9.9, 2.4, 2.4 Hz, 1H), 5.60 (ddd, *J*=10.5, 3.3, 2.1 Hz, 1H), 4.48 (dd, *J*=8.7, 7.5 Hz, 1H), 4.00 (dd, *J*=8.7, 6.3 Hz, 1H), 3.77 (s, 3H), 3.27–3.16 (m, 1H), 3.11 (dd, *J*=8.1, 8.1 Hz, 1H), 2.66 (dd, *J*=6.6, 6.6 Hz, 1H), 2.65–2.56 (m, 1H), 1.11 (d, *J*=7.2 Hz, 3H).

4.6.3. Methyl (3a*R,6*S**,7*R**,7a*R**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (12a).**[5e] The major isomer from IMDA reaction of **9a** was obtained as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.78 (ddd, *J*=13.2, 1.8, 1.8 Hz, 1H), 5.65 (ddd, *J*=9.6, 3.2, 3.2 Hz, 1H), 4.52 (dd, *J*=7.4, 7.4 Hz, 1H), 3.86 (dd, *J*=11.6, 8.0 Hz, 1H), 3.69 (s, 3H), 3.25–3.15 (m, 1H), 2.96 (d, *J*=4.0 Hz, 1H), 2.97–2.89 (m, 1H), 2.35 (dd, *J*=13.2, 3.6 Hz, 1H), 1.19 (d, *J*=7.2 Hz, 3H).

4.6.4. Methyl (3a*R,6*S**,7*S**,7a*S**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (13a).**[5e] The minor isomer from IMDA reaction of **9a** was obtained as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.82 (ddd, *J*=10.0, 4.0, 2.4 Hz, 1H), 5.60 (ddd, *J*=10.0, 2.4, 2.4 Hz, 1H), 4.44 (dd, *J*=8.4, 7.6 Hz, 1H), 4.17 (dd, *J*=8.4, 4.4 Hz, 1H), 3.75 (s, 3H), 3.34 (dd, *J*=9.6, 5.2 Hz, 1H), 3.24–3.17 (m, 1H), 3.09 (dd, *J*=5.2, 5.2 Hz, 1H), 2.75–2.65 (m, 1H), 1.15 (d, *J*=7.2 Hz, 3H).

4.6.5. Ethyl (3a*R,6*S**,7*R**,7a*S**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (10b).**[5b] The major isomer from IMDA reaction of **8b** was obtained as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.79 (ddd, *J*=9.6, 1.6, 1.6 Hz, 1H), 5.72 (ddd, *J*=9.6, 3.0, 3.0 Hz, 1H), 4.47 (dd, *J*=8.0, 6.4 Hz, 1H), 4.28 (q, *J*=7.0 Hz, 2H), 3.95 (dd, *J*=10.8, 7.6 Hz, 1H), 2.97 (dd, *J*=11.6, 7.2 Hz, 1H), 2.92–2.79 (m, 2H), 2.61 (dd, *J*=13.2, 11.6 Hz, 1H), 1.33 (t, *J*=7.2 Hz, 3H), 1.02 (d, *J*=7.6 Hz, 3H).

4.6.6. Ethyl (3a*R,6*S**,7*S**,7a*R**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (11b).**[5b] The minor isomer from IMDA reaction of **8b** was obtained as a colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.77 (ddd, *J*=10.4, 2.6, 2.6 Hz, 1H), 5.60 (ddd, *J*=10.4, 3.2, 2.4 Hz, 1H), 4.47 (dd, *J*=8.8, 8.0 Hz, 1H), 4.22 (qd, *J*=6.8, 1.2 Hz, 2H), 3.98 (dd, *J*=8.8, 6.4 Hz, 1H), 3.25–3.16 (m, 1H), 3.11 (dd, *J*=8.4, 8.4 Hz, 1H), 2.66–2.56 (m, 2H), 1.29 (t, *J*=7.2 Hz, 3H), 1.11 (d, *J*=7.2 Hz, 3H).

4.6.7. Ethyl (3a*R,6*S**,7*R**,7a*R**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (12b).** The major isomer from IMDA reaction of **9b** was obtained as a colorless oil; R_f=0.55 (25% EtOAc in hexane); IR (film) 2966, 1789, 1731, 1181, 1093, 993 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.76 (d, *J*=10.0 Hz, 1H), 5.63 (ddd, *J*=9.6, 3.0, 3.0 Hz, H), 4.49 (dd, *J*=7.6, 7.6 Hz, 1H), 4.20–4.08 (m, 2H), 3.83 (dd, *J*=11.6, 8.0 Hz, 1H), 3.19 (br dd, *J*=19.6, 12.4 Hz, 1H), 2.91 (br d, *J*=3.2 Hz, 2H), 2.33 (dd, *J*=13.6, 3.6 Hz, 1H), 1.22 (t, *J*=7.2 Hz, 3H), 1.17 (d, *J*=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 171.7, 134.6, 123.0, 70.5, 60.9, 42.6, 41.4, 36.2, 33.9, 21.9, 13.9; MS (+ESI) *m/z* 471 ($2M+Na^+$, 100), 247 ($M+Na^+$, 16); HRMS (+ESI) calcd for $C_{12}H_{16}O_4Na$ ($M+Na^+$), 247.0941; found, 247.0940.

4.6.8. Ethyl (3a*R,6*S**,7*S**,7a*S**)-6-methyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (13b).** The minor isomer from IMDA reaction of **9b** was obtained as a colorless oil; R_f=0.43 (25% EtOAc in hexane); IR (film) 2976, 2932, 1770, 1728, 1176, 1016 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.80 (ddd, *J*=9.6, 4.0, 1.6 Hz, 1H), 5.59 (ddd, *J*=10.0, 2.4, 2.4 Hz, 1H), 4.43 (dd, *J*=8.4, 8.4 Hz, 1H), 4.20 (q, *J*=6.8 Hz, 2H), 4.16 (dd, *J*=8.0, 4.0 Hz, 1H), 3.32 (dd, *J*=9.2, 5.2 Hz, 1H), 3.22–3.16 (m, 1H), 3.05 (dd, *J*=5.2, 5.2 Hz, 1H), 2.70–2.65 (m, 1H), 1.27 (t, *J*=7.2 Hz, 3H), 1.15 (d, *J*=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 171.3, 134.0, 124.4, 71.3, 60.7, 42.0, 38.5, 35.1, 30.2, 17.6, 14.1; MS (+ESI) *m/z* 471 ($2M+Na^+$, 46), 247 ($M+Na^+$, 100); HRMS (+ESI) calcd for $C_{12}H_{16}O_4Na$ ($M+Na^+$), 247.0941; found, 247.0936.

4.6.9. Ethyl (3a*R,6*S**,7*R**,7a*S**)-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (10c).** The major isomer from IMDA reaction of **8c** was obtained as colorless needles; mp 156–157 °C (EtOAc–hexane); R_f=0.33 (25% EtOAc in hexane); IR (KBr) 3034, 2984, 1784, 1735, 1321, 1180, 1085, 978 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.25 (m, 3H), 7.17–7.13 (m, 2H), 6.07 (ddd, *J*=10.0, 2.0, 2.0 Hz, 1H), 5.82 (ddd, *J*=10.0, 3.4, 3.4 Hz, 1H), 4.54 (dd, *J*=8.0, 6.0 Hz, 1H), 4.05 (dd, *J*=11.2, 8.4 Hz, 1H), 4.07–4.02 (m, 1H),

3.80–3.60 (m, 2H), 3.16 (dd, J=11.6, 8.0 Hz, 1H), 2.96–2.86 (m, 1H), 2.77 (dd, J=13.6, 11.6 Hz, 1H), 0.89 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 169.9, 139.2, 132.3, 129.4 (×2), 128.2 (×2), 127.6, 123.9, 70.3, 60.5, 45.5, 44.4, 40.6, 40.2, 30.9, 13.6; MS (+ESI) m/z 309 ($M+Na^+$, 98), 287 ($M+H^+$, 100). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.22; H, 6.33.

4.6.10. Ethyl (3a*R,6*S**,7*S**,7a*R**)-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (11c).** The minor isomer from IMDA reaction of **8c** was obtained as colorless needles; mp 117–118 °C (EtOAc–hexane); R_f=0.33 (25% EtOAc in hexane); IR (KBr) 2981, 1773, 1722, 1180, 1163 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.25 (m, 3H), 7.20–7.16 (m, 2H), 5.97 (ddd, J=10.0, 2.0, 2.0 Hz, 1H), 5.87 (ddd, J=10.4, 3.0, 3.0 Hz, 1H), 4.56 (dd, J=8.0, 8.0 Hz, 1H), 4.09 (d, J=8.4 Hz, 1H), 4.06 (q, J=6.4 Hz, 2H), 3.76 (ddd, J=8.0, 5.2, 2.0 Hz, 1H), 3.37–3.27 (m, 1H), 3.20 (dd, J=9.2, 9.2 Hz, 1H), 2.83 (dd, J=9.2, 8.4 Hz, 1H), 1.07 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 172.7, 141.4, 132.2, 128.6 (×2), 127.9 (×2), 127.3, 124.4, 71.5, 61.1, 46.1, 43.4, 40.1, 34.6, 13.9; MS (+ESI) m/z 309 ($M+Na^+$, 98), 287 ($M+H^+$, 100). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.36; H, 6.30.

4.6.11. Ethyl (3a*R,6*S**,7*R**,7a*R**)-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (12c).** The major isomer from IMDA reaction of **9c** was obtained as colorless needles; mp 69–71 °C (EtOAc–hexane); R_f=0.42 (25% EtOAc in hexane); IR (KBr) 2977, 1791, 1724, 1184, 987 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.20 (m, 5H), 6.08 (d, J=10.0 Hz, 1H), 5.81 (ddd, J=9.6, 3.4, 3.4 Hz, 1H), 4.53 (dd, J=7.4, 7.4 Hz, 1H), 4.27–4.14 (m, 3H), 3.90 (dd, J=11.2, 8.0 Hz, 1H), 3.32–3.20 (m, 1H), 3.17 (d, J=3.2 Hz, 1H), 2.39 (dd, J=13.6, 3.6 Hz, 1H), 1.27 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 171.2, 142.8, 130.5, 128.6 (×2), 127.7 (×2), 127.0, 125.3, 70.4, 61.1, 44.8, 44.1, 40.2, 36.1, 13.9; MS (+ESI) m/z 595 ($2M+Na^+$, 100), 309 ($M+Na^+$, 97). Anal. Calcd for $C_{17}H_{18}O_4$: C, 71.31; H, 6.34. Found: C, 71.36; H, 6.30.

4.6.12. Ethyl (3a*R,6*S**,7*S**,7a*S**)-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (13c).** The minor isomer from IMDA reaction of **9c** was obtained as a colorless oil; R_f=0.32 (25% EtOAc in hexane); IR (film) 2918, 1773, 1722, 1179, 1163, 1033, 1014 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.18 (m, 5H), 6.05 (ddd, J=10.4, 2.0, 2.0 Hz, 1H), 5.93 (ddd, J=10.0, 3.0, 3.0 Hz, 1H), 4.55 (dd, J=8.6, 8.6 Hz, 1H), 4.37 (dd, J=8.4, 8.4 Hz, 1H), 3.88–3.77 (m, 2H), 3.68–3.60 (m, 1H), 3.45 (dd, J=6.0, 6.0 Hz, 1H), 3.35–3.24 (m, 1H), 3.18 (dd, J=10.8, 6.4 Hz, 1H), 0.81 (t, J=7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 171.2, 140.8, 128.2 (×2), 128.2 (×2), 128.1, 127.0, 126.0, 71.6, 60.4, 45.1, 42.1, 39.4, 33.4, 13.4; MS (+ESI) m/z 595 ($2M+Na^+$, 16), 309 ($M+Na^+$, 100); HRMS (+ESI) calcd for $C_{17}H_{18}O_4Na$ ($M+Na^+$), 309.1097; found, 309.1085.

4.6.13. Ethyl (3a*R,6*S**,7*R**,7a*S**)-5-methyl-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (10d).** The major isomer from IMDA reaction of **8d** was obtained as colorless needles; mp 127–129 °C (EtOAc–hexane); R_f=0.36 (25% EtOAc in hexane); IR (KBr) 2983, 1786, 1736, 1315, 1195, 1178, 1139, 1080 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.23 (m, 3H), 7.11 (d, J=6.4 Hz, 2H), 5.79 (s, 1H), 4.49 (dd, J=7.4, 7.4 Hz, 1H), 4.01 (dd, J=11.2, 8.4 Hz, 1H), 3.82 (d, J=8.8 Hz, 1H), 3.84–3.74 (m, 1H), 3.67–3.58 (m, 1H), 3.09 (dd, J=11.6, 7.2 Hz, 1H), 2.96–2.82 (m, 1H), 2.76 (dd, J=13.6, 12.4 Hz, 1H), 1.54 (s, 3H), 0.88 (q, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, taken at 25 °C) δ 174.1, 169.9, 138.7, 138.7, 128.4 (×2), 127.5, 119.9, 70.7, 60.4, 49.4, 45.9, 41.6, 39.6, 22.4, 13.6 (two aromatic carbon atoms are missing due to slow conformational rotation); ^{13}C NMR (125 MHz, DMSO-d_6, taken at 80 °C) δ 174.1, 169.5, 139.4, 137.1, 129.4 (×2), 128.0 (×2), 127.1, 120.7, 70.4, 59.4, 49.0, 45.1, 40.7, 39.3, 21.9, 13.5; MS (+ESI) m/z 623 ($2M+Na^+$, 100), 323 ($M+Na^+$, 59). Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 72.18; H, 6.71.

4.6.14. Ethyl (3a*R,6*S**,7*S**,7a*R**)-5-methyl-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (11d).** The minor isomer from IMDA reaction of **8d** was obtained as an inseparable mixture with **10d**; R_f=0.36 (25% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$, *only partial signals shown*) δ 5.64 (s, 1H), 4.12–4.09 (m, 1H), 3.30–3.25 (m, 1H), 3.17–3.13 (m, 1H), 1.59 (s, 3H), 1.14 (t, J=7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 172.9, 140.1, 136.5, 128.5 (×2), 128.3 (×2), 127.1, 121.8, 72.1, 61.2, 46.3, 46.3, 38.4, 34.4, 22.8, 14.0.

4.6.15. Ethyl (3a*R,6*S**,7*R**,7a*R**)-5-methyl-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (12d).** The major isomer from IMDA reaction of **9d** was obtained as a colorless oil; R_f=0.49 (25% EtOAc in hexane); IR (film) 2980, 1785, 1731, 1229, 1184, 1093, 1002 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (t, J=7.4 Hz, 2H), 7.25 (d, J=6.4 Hz, 1H), 7.21 (d, J=7.6 Hz, 2H), 5.82 (s, 1H), 4.53 (dd, J=7.4, 7.4 Hz, 1H), 4.29–4.13 (m, 2H), 4.03 (s, 1H), 3.91 (dd, J=11.2, 8.0 Hz, 1H), 3.29–3.16 (m, 1H), 3.10 (d, J=2.8 Hz, 1H), 2.46 (dd, J=14.0, 3.2 Hz, 1H), 1.63 (s, 3H), 1.28 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 171.1, 142.2, 137.4, 128.8 (×2), 128.0 (×2), 127.0, 121.1, 70.9, 61.2, 48.9, 45.2, 39.7, 37.2, 22.5, 14.0; MS (+ESI) m/z 623 ($2M+Na^+$, 100), 323 ($M+Na^+$, 8), 301 ($M+H^+$, 10); HRMS (+ESI) calcd for $C_{18}H_{20}O_4Na$ ($M+Na^+$), 323.1254; found, 323.1244.

4.6.16. Ethyl (3a*R,6*S**,7*S**,7a*S**)-5-methyl-6-phenyl-1-oxo-3,3a,6,7,7a-hexahydroisobenzofuran-7-carboxylate (13d).** The minor isomer from IMDA reaction of **9d** was obtained as colorless needles; mp 111–112 °C (EtOAc–hexane); R_f=0.23 (25% EtOAc in hexane); IR (KBr) 2924, 1772, 1731, 1193, 1179, 1111 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.20 (m, 5H), 5.67 (s, 1H), 4.44 (dd, J=8.2, 8.2 Hz, 1H), 4.31 (d, J=8.8 Hz, 1H), 4.25–4.10 (m, 2H), 3.91 (d, J=4.8 Hz, 1H), 3.43 (dd, J=9.6, 5.2 Hz, 1H), 3.36–3.27 (m, 1H), 3.29 (dd, J=9.6, 4.8 Hz, 1H), 1.65 (s, 3H), 1.23 (td, J=7.0, 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 170.4, 138.1, 137.2, 130.5 (×2), 127.4 (×2), 127.1, 122.9, 71.2, 60.7, 44.8, 43.9, 36.4, 36.1, 22.8, 13.9; MS (+ESI) m/z 623 ($2M+Na^+$, 98), 323 ($M+Na^+$, 60), 301 ($M+H^+$, 100). Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.78; H, 6.67.

4.6.17. (3a*R,6*S**,7*R**,7a*S**)-7-Benzoyl-6-methyl-3,3a,6,7,7a-hexahydroisobenzofuran-1-one (10e).** The major isomer from IMDA reaction of **8e** was obtained as a white crystalline solid; mp 197–198 °C (EtOAc–hexane); R_f=0.30 (25% EtOAc in hexane); IR (KBr) 2955, 2925, 1765, 1683, 1176, 1095, 984 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J=7.6 Hz, 1H), 7.59 (t, J=7.2 Hz, 1H), 7.49 (t, J=7.6 Hz, 2H), 5.82 (d, J=9.6 Hz, 1H), 5.71 (br d, J=10.0 Hz, 1H), 4.48 (dd, J=7.2, 7.2 Hz, 1H), 3.98 (dd, J=10.4, 5.2 Hz, 1H), 3.97 (dd, J=10.4, 4.4 Hz, 1H), 3.03–2.69 (m, 3H), 0.83 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.4, 174.5, 136.4, 135.8, 133.4, 128.8 (×2), 128.2 (×2), 122.2, 70.3, 44.5, 41.0, 40.4, 33.5, 17.6; MS (+ESI) m/z 535 ($2M+Na^+$, 18), 279 ($M+Na^+$, 100). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.73; H, 6.27.

4.6.18. (3a*R,6*S**,7*S**,7a*R**)-7-Benzoyl-6-methyl-3,3a,6,7,7a-hexahydroisobenzofuran-1-one (11e).** The minor isomer from IMDA reaction of **8e** was obtained as colorless needles; mp 99–100 (EtOAc–hexane); R_f=0.45 (25% EtOAc in hexane); IR (KBr) 2964, 2905, 1765, 1678, 1269, 1207, 1157, 1120, 986 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J=7.6 Hz, 2H), 7.60 (t, J=7.4 Hz, 1H), 7.50 (t, J=7.6 Hz, 2H), 5.76 (ddd, J=10.4, 4.0, 2.0 Hz, 1H), 5.62 (ddd, J=10.0, 2.4, 2.4 Hz, 1H), 4.48 (dd, J=8.4, 7.2 Hz, 1H), 4.15 (dd, J=9.2, 3.6 Hz, 1H), 3.94 (dd, J=3.8, 3.8 Hz, 1H), 3.45–3.36 (m, 1H), 3.18 (dd, J=8.4, 4.4 Hz, 1H), 2.70–2.50 (m, 1H), 1.21 (d, J=7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.2, 178.1, 136.0, 133.4, 133.2, 128.9 (×2), 128.4 (×2), 124.1, 71.9, 44.9, 39.0, 34.0, 31.0, 21.3; MS (+ESI) m/z 535 ($2M+Na^+$, 78), 279 ($M+Na^+$, 46), 257 ($M+H^+$, 100). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.72; H, 6.27.

4.6.19. (3a*R,6*S**,7*R**,7a*R**)-7-Benzoyl-6-methyl-3,3a,6,7,7a-hexahydroisobenzofuran-1-one (12e).** The *minor isomer* from IMDA reaction of **9e** was obtained as colorless needles; mp 144–145 °C (EtOAc–hexane); R_f=0.39 (25% EtOAc in hexane); IR (KBr) 2993, 2962, 1770, 1669, 1184, 1093, 982 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, J=7.5 Hz, 1H), 7.61–7.57 (m, 1H), 7.49 (t, J=7.5 Hz, 2H), 5.85 (ddd, J=9.5, 1.8, 1.8 Hz, 1H), 5.62 (ddd, J=10.0, 3.3, 3.3 Hz, 1H), 4.59 (dd, J=7.5, 7.5 Hz, 1H), 3.90 (dd, J=12.0, 8.0 Hz, 1H), 3.81 (d, J=4.0 Hz, 1H), 3.72–3.69 (m, 1H), 2.76–2.70 (m, 1H), 2.49 (dd, J=13.5, 4.0 Hz, 1H), 1.36 (d, J=7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.9, 175.3, 136.3, 134.0, 133.3, 128.7 (×2), 128.4 (×2), 123.6, 71.1, 44.3, 42.0, 36.1, 34.4, 22.5; MS (+ESI) m/z 535 ($2M+Na^+$, 49), 279 ($M+Na^+$, 100), 257 ($M+H^+$, 98). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.80; H, 6.28.

4.6.20. (3a*R,6*S**,7*S**,7a*S**)-7-Benzoyl-6-methyl-3,3a,6,7,7a-hexahydroisobenzofuran-1-one (13e).** The *major isomer* from IMDA reaction of **9e** was obtained as a colorless oil; R_f=0.23 (25% EtOAc in hexane); IR (film) 2967, 1767, 1678, 1226, 1177, 1017 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88–7.84 (m, 2H), 7.56–7.52 (m, 1H), 7.47–7.42 (m, 2H), 5.74 (ddd, J=10.0, 2.6, 2.6 Hz, 1H), 5.64 (ddd, J=10.4, 2.4, 2.4 Hz, 1H), 4.50 (dd, J=8.4, 8.4 Hz, 1H), 4.39 (dd, J=9.6, 6.0 Hz, 1H), 4.19 (dd, J=5.4, 5.4 Hz, 1H), 3.30–3.20 (m, 2H), 2.87–2.78 (m, 1H), 0.95 (d, J=7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.8, 177.6, 138.9, 132.7, 131.9, 128.5 (×2), 128.0 (×2), 125.3, 71.5, 44.2, 39.6, 34.6, 31.9, 18.6; MS (+ESI) m/z 535 ($2M+Na^+$, 100), 257 ($M+H^+$, 40); HRMS (+ESI) calcd for $C_{16}H_{16}O_3Na$ ($M+Na^+$), 279.0992; found, 279.0985.

4.7. General procedure for microwave-assisted tandem Wittig–IMDA cycloadditions

To a 10-mL pressurized process vial was added one of the 2,4-pentadienyl α-bromoacetates **14**–**16** (0.30 mmol), PPh_3 (0.36 mmol), 2,6-lutidine (0.39 mmol), and one of the α-oxo carbonyl compounds **17**–**19** in MeCN (4 mL). The loaded vial was then sealed with a cap containing a silicon septum, and put into the microwave cavity and heated at 180 °C for 30 min. The reaction mixture was diluted with EtOAc (10 mL) and washed with 6% aqueous HCl and brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by chromatography on silica gel with EtOAc and petroleum ether (60–90 °C) to afford the adducts. The structures and yields are given in Scheme 3 and Table 3.

4.8. X-ray crystallographic structural determination of 12e, 10c, and 13d

The X-ray crystal structures of **12e**, **10c**, and **13d** are given in Figures 1–3 and the crystal data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 604672, CCDC 604671, and CCDC 604673, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.039.

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Self-organization and photoreactivity of anthryl dendron having perfluoroalkyl chains at the terminals

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Abstract—A newly designed anthryl dendron having perfluoroalkyl chains at terminals showed thermotropic liquid crystallinity, which was characterized using polarizing optical microscopy, differential scanning calorimetry, and X-ray diffraction. The anthryl dendron forms S_B phase at room temperature below 53 °C, at which temperature a phase transition to Col_{rd} takes place. In a fluorous solvent, regioselective photodimerization of the anthryl dendron occurred to give head-to-head photodimers, although photodimerization in the S_B phase and chloroform gave both head-to-head and head-to-tail photodimers.

1. Introduction

Dendritic macromolecules (dendrimers) have received considerable attention during the past three decades.[1] In particular, dendrimers possessing fluorine atoms in some part of their structure[2] offer the potential for applications such as surfactants,[3] electronics,[4b] and liquid crystalline devices.[4] For example, DeSimone and co-workers reported the use of fluorinated dendrimer as a surfactant in biphasic systems (water/supercritical CO_2).[3a] Percec and co-workers reported that fluorinated dendrons with conducting organic donor or acceptor groups exhibit high charge mobilities in their supramolecular liquid crystalline phases.[4b] Although a few examples of fluorinated dendrimer that are functionalized with chromophore have been reported,[4b] much less is known about the photoreactivity of fluorinated dendrimers. We have studied on the synthesis and photodimerization of anthracenes bearing a poly(amidoamine) dendritic substituent (anthryl dendrons).[5] Furthermore, we have recently reported that thermotropic liquid crystallinity can be induced for an anthryl dendron.[5d] In this liquid crystalline phase (S_E), the photodimerization reaction of the anthracene moiety proceeded quantitatively and regioselectively to afford only *anti*-photodimer. During our studies on the synthesis and photoreactivity of anthryl dendrons, we found that an anthryl dendron having perfluoroalkyl end groups shows mesomorphic properties and a micellar formation of the dendron was a key to control regioselectivity of the photodimerization reaction. This paper describes the synthesis, characterization, and photoreactivity of the fluorinated anthryl dendron, along with its mesomorphic properties. To our knowledge, these are the first examples of photoreactions of fluorinated dendrimer in mesomorphic state and micellar aggregate.

2. Results and discussion

The anthryl dendron **1** having perfluoroalkyl chains at the terminals was prepared from a first-generation poly(amidoamine) dendron **2** as shown in Scheme 1. Perfluoroalkyl chains were introduced by the Michael addition reaction of 2-(perfluorooctyl)ethyl acrylate with the terminal NH_2 groups of the dendron **2** to afford dendron **1**, which was purified by HPLC (LC918; Japan Analytical Industry Co. Ltd) on gel permeation column (Jaigel 1H+2H; Japan Analytical Industry Co. Ltd) with chloroform as eluent, in 70% yield (Scheme 1). The structure of the dendron **1** was confirmed by ^1H, ^{13}C, and ^{19}F NMR spectroscopies, elemental analysis, and MALDI-TOF-Mass spectrometry. The ^{19}F NMR spectrum showed eight signals, which are assignable with the terminated perfluoroalkyl groups. The MALDI-TOF-Mass spectrum showed clearly the parent peak at *m*/*z* 2566.35, which is consistent with the molecular weight of the dendron **1** ($[MH]^+$, calcd 2566.28) as shown in Figure 1.

Keywords: Dendrimer; Mesophase; Photodimerization; Fluorous chemistry.
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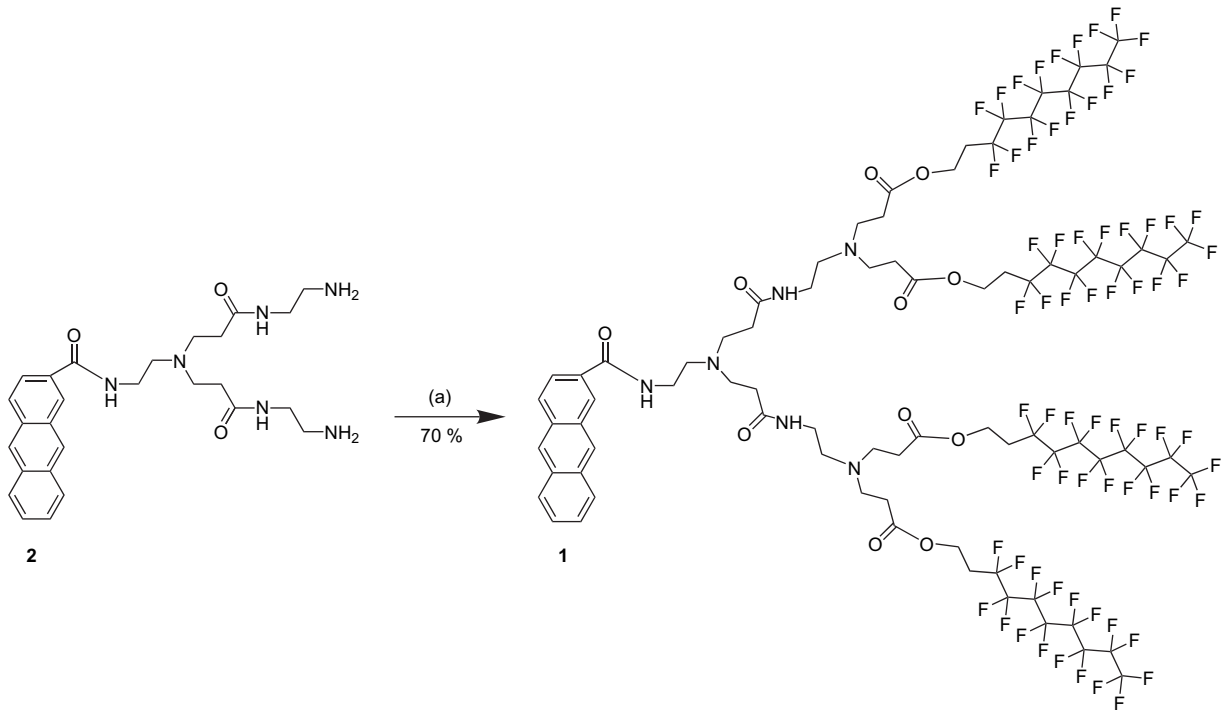


Scheme 1. Reagent and condition: (a) 2-(perfluorooctyl)ethyl acrylate, DMF, 45 °C, 1 week.

In order to evaluate the influence of the fluorophilic end groups for self-organization of dendron **1**, structural analyses of mesophases of dendron **1** were accomplished using a combination of differential scanning calorimetry (DSC), polarized optical microscopy, and X-ray diffraction (XRD). The DSC profile of dendron **1** shows a dominant and endothermic peak at 53 °C (ΔH=43.5 kJ/mol) and smaller one at 87 °C (ΔH=1.82 kJ/mol) as shown in Figure 2. The reversibility of the phase transitions is evidenced by the presence of the corresponding peaks in the cooling curve. A thin sample of dendron **1**, sandwiched between untreated glass plates, was examined between crossed polarizers. Liquid crystallinity of the dendron **1** at room temperature (after annealing at 100 °C) probed by the observation of optical texture showing birefringent as shown in Figure 3a. In agreement with the DSC profile, on heating, a reduction of anisotropy of the polarizability, which should be caused by a change in the molecular arrangement, was observed around 55 °C as shown in Figure 3b. Dendron **1** showed the isotropic phase transition at 87 °C in DSC analysis and POM observation. Identification and unequivocal assignment of mesophases were finally achieved by XRD. Those XRD measurements showed that the mesophases of dendron **1** at room temperature and 60 °C exhibited a smectic phase and a rectangular columnar phase, respectively. The profile for the mesophase of dendron **1** at room temperature shows a sharp reflection at 2Θ=1.98° (44.6 Å), 39.4° (22.4 Å), 5.92° (14.9 Å), 7.88° (11.2 Å), 9.84° (8.98 Å), 11.8° (7.47 Å), and 13.8° (6.40 Å) arising from the smectic layering of molecules (d_{001}, d_{002}, d_{003}, d_{004}, d_{005}, d_{006}, and d_{007}, respectively), and a sharp one at 2Θ=17.6° (5.03 Å) arising

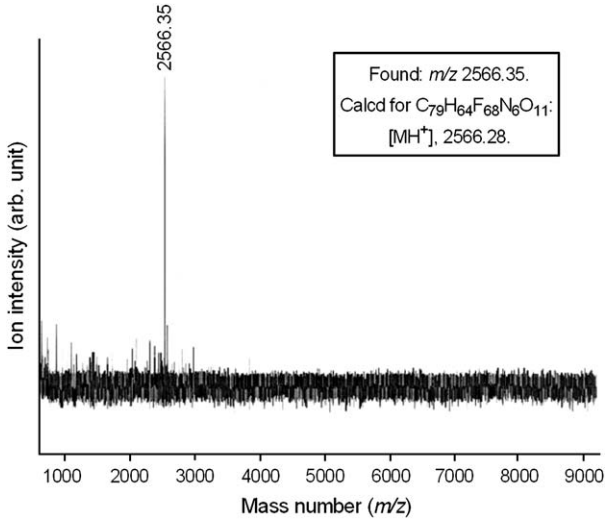


Figure 1. MALDI-TOF spectra of dendron **1**.

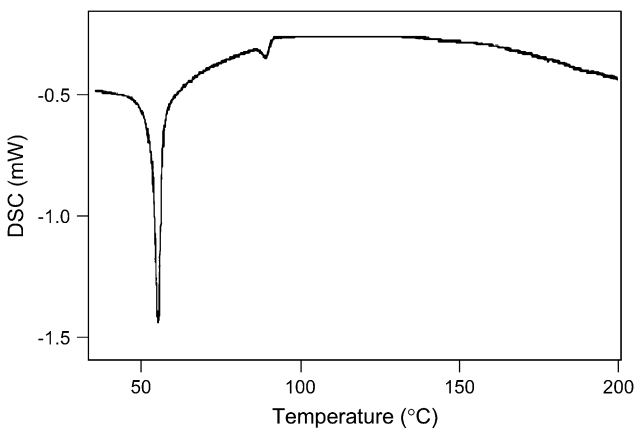


Figure 2. DLC curve for dendron **1**.

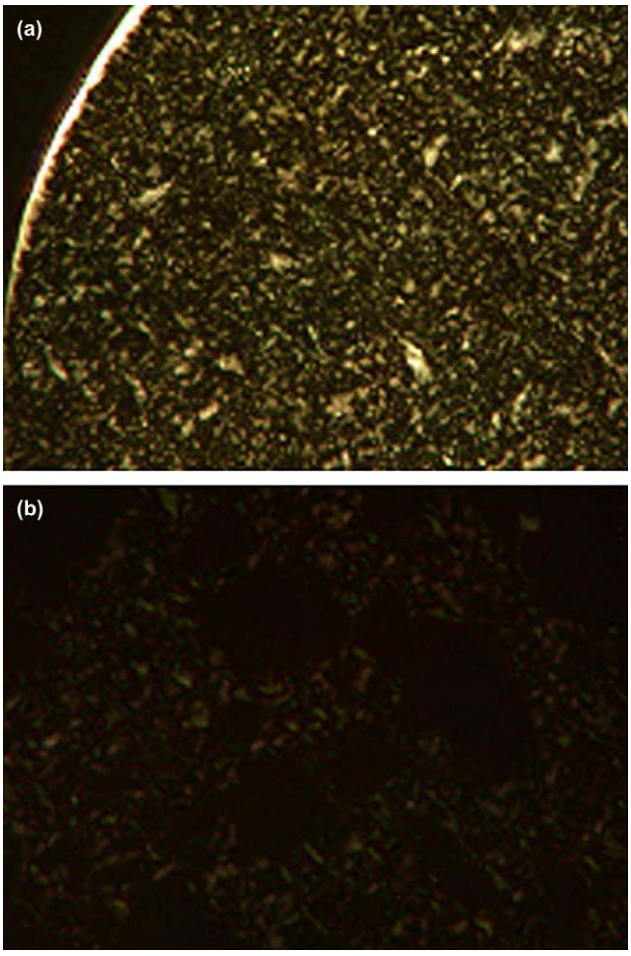


Figure 3. Polarized optical micrographs (×75) of dendron **1** (a) at room temperature and (b) at 60 °C.

Table 1. X-ray diffraction data for dendron **1**

(a) At room temperature		(b) At 60 °C	
2Θ (°)[a]	d_{obs} (Å)[b]	2Θ (°)[a]	d_{obs} (Å)[b]
1.980	44.62	2.316	38.11
3.940	22.41	2.680	32.94
5.920	14.92	3.984	22.16
7.880	11.21	16.66	5.317[c]
9.840	8.981		
11.84	7.468		
13.82	6.402		
17.62	5.029		

[a] Glancing angle.
[b] *d*-Spacing.
[c] Halo of molten alkyl chains.

from the d_{100} direction (Table 1a). This feature suggests that the molecular arrangement of the smectic phase has a hexagonal order within the layer, where the lateral distance of perfluoroalkyl chains is 5.03 Å. From these results, the mesophase is assigned to the smectic B (S_B). This structural analysis could be supposed from adequate consideration of the molecular model shown in Figure 4a. The smectic layer is formed by an anti-parallel arrangement of two molecules, where the perfluoroalkyl chains are interdigitated, although the arrangement might be partially disordered. The profile for the mesophase of dendron **1** at 60 °C shows a set of reflections corresponding to *d*-spacings of 38.1, 32.9, and 22.2 Å (Table 1b). In combination with the modeling, these reflections could be indexed in sequence as (110), (020), and (210) reflections of a rectangular columnar lattice with the lattice parameters of a=46.7 Å and b=65.8 Å as shown in Figure 4b. The phase transition of the S_B into Col_{rd} ($P2_1/a$) might be induced by melting of perfluoroalkyl chain, which is in agreement with the transition enthalpy by DSC, and subsequent liquid–liquid phase separation between the

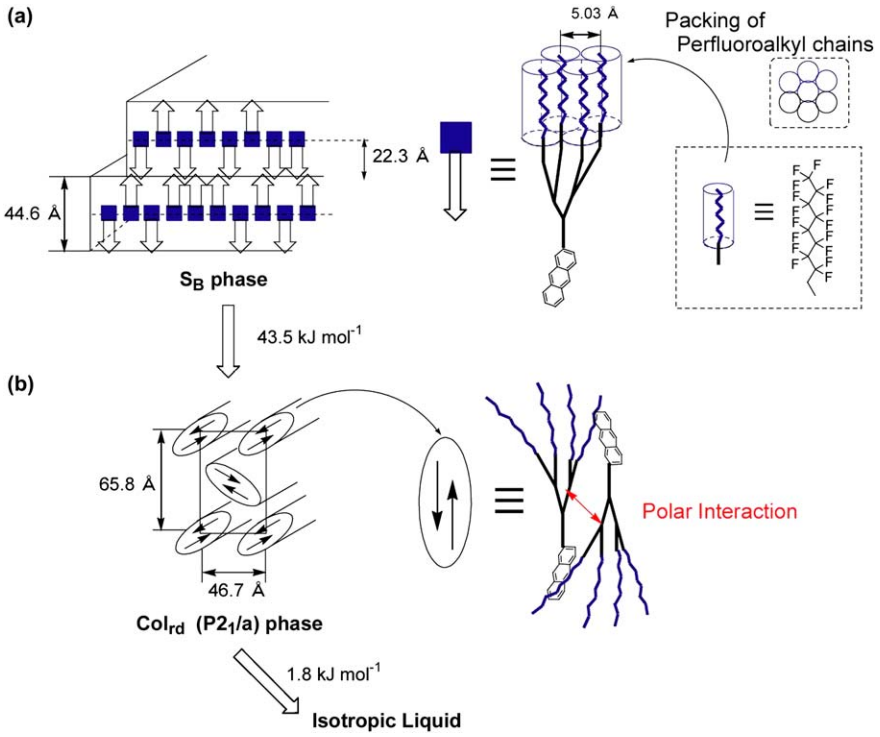
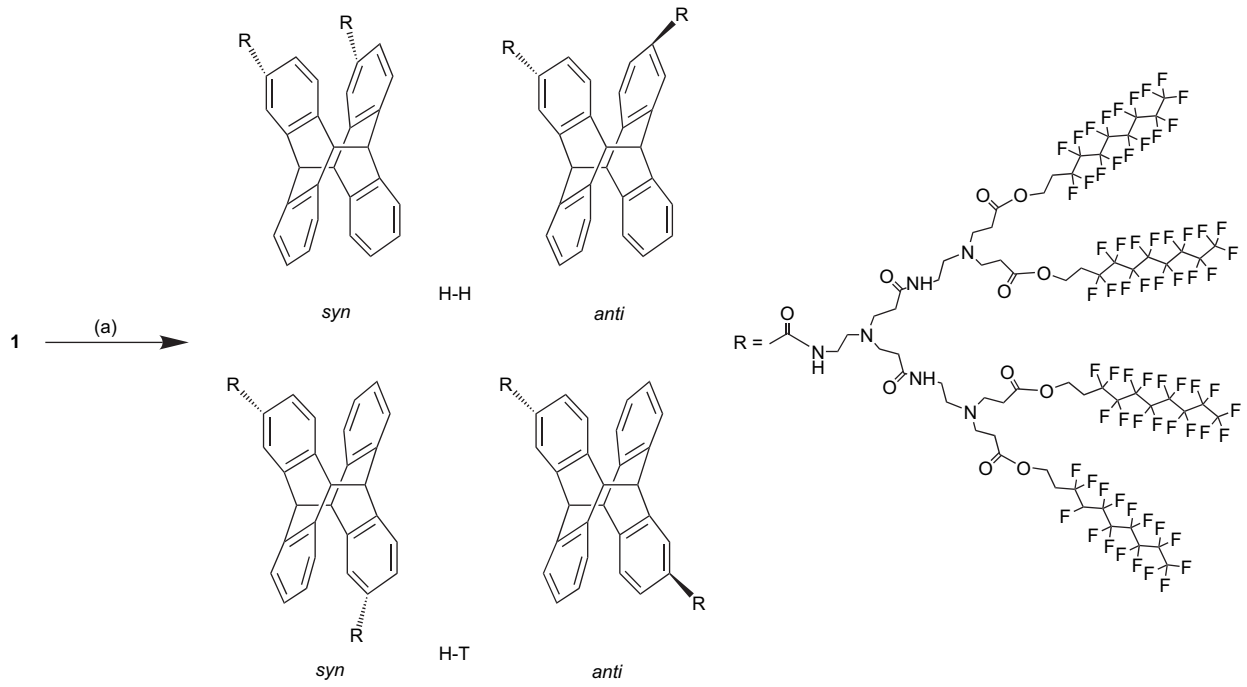


Figure 4. Schematic representation of mesophases of dendron **1** (a) at room temperature and (b) at 60 °C.



Scheme 2. Condition: (a) photoirradiation (>300 nm), rt, 3 h.

molten perfluoroalkyl chains and the polar poly(amido-amine) building blocks.

The photodimerization of 2-substituted anthracene gives four regioisomers (*anti* head-to-tail, *syn* head-to-tail, *anti* head-to-head, and *syn* head-to-head) (Scheme 2).[6] The two head-to-head (H-H) dimers and the two head-to-tail (H-T) dimers arise, respectively, from parallel and anti-parallel pair of the anthracenes. Especially in viscous media, the pairs have insufficient time to reorient themselves during the singlet state of anthryl groups, from which the photodimerization reaction emanates.[7,8] Therefore, the ratio of H-H/H-T should reflect, at least indirectly, the ground state distribution of parallel and anti-parallel pairs of 2-substituted anthracene derivatives.[8] In fact, Weiss and Lin analyzed packing arrangements of cholesteryl 4-(2-anthryloxy)butyrate in its cholesteric liquid crystalline phase, neat isotropic phase, and solution by the distributions of photodimers obtained from each.[8] Therefore, the distributions of photodimers obtained from various phases were analyzed to investigate the packing arrangement of dendron **1**. The mesophase (S_B), chloroform solution, and the perfluorohexane (PFH) solution of dendron **1** were irradiated with a high-pressure mercury lamp at room temperature for 3 h to yield photodimers, which were purified by the use of HPLC. According to the previous report,[8] the ratios of H-H(*syn*:*anti*)/H-T are estimated as shown in Table 2.

Table 2. Distributions of the regioisomer of photodimers

Phase	Dimers	
	H-H (*syn*:*anti*)	H-T
S_B phase	65 (58:42)	35
$CHCl_3$ solution	47 (66:34)	53
PFH solution[a]	100 (50:50)	—

[a] Perfluorohexane solution.

In the cases of the mesophase and chloroform solution, both H-H and H-T regioisomers were obtained, although the photoirradiation of the mesophase gave H-H dimers as slightly rich products. On the other hand, surprisingly, in

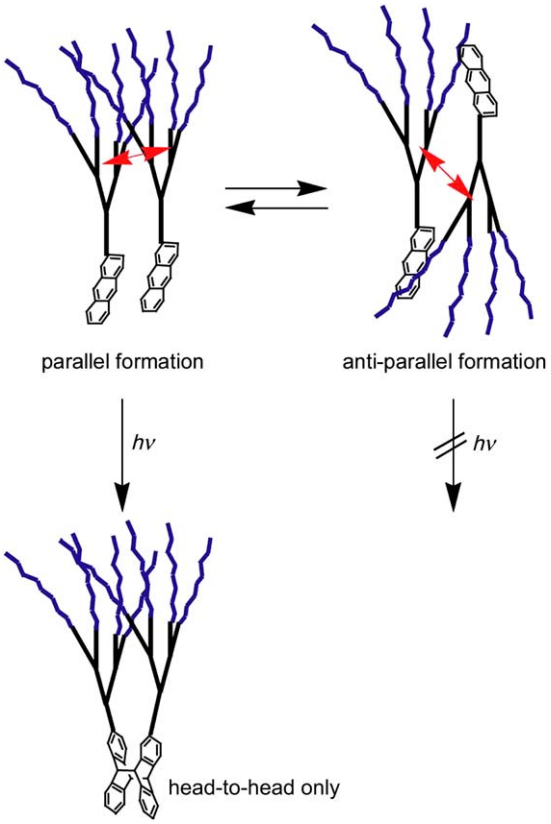


Figure 5. Schematic representation of the photodimerization of dendron **1** in fluorophilic environment.

the case of the PFH solution, only H-H dimers were observed after the photodimerization reaction. Formation of the H-T regioisomers in the S_B phase, but not specific formation of H-H regioisomers, implies that the anthryl group might be interdigitated as in the bilayer, in agreement with the phase structure represented in Figure 4a. The lack of regioselectivity in the case of chloroform solution indicates that dendron **1** can be dissolved molecularly in chloroform. Meanwhile, in the case of the PFH solution, this flourophilic environment induced phase separation of the polar poly(amidoamine) building block of dendron **1** to form micellar aggregates, in which only parallel formation was allowed to form the dimers upon photoirradiation, as shown in Figure 5.

3. Conclusion

In conclusion, we have found the fluorinated anthryl dendron shows the two thermotropic mesophases, of which one is the smectic B phase, which has a lamella structure formed by hexagonal packing of the perfluoroalkyl chains, and another is a rectangular columnar phase, which is induced by the molten perfluoroalkyl chains. Photoirradiation of the mesophase at room temperature gave photodimers with little selectivity. However, photoirradiation of the dendron in perfluorohexane gave only head-to-head regioisomers. This regioselectivity arises from micellar aggregation, which is formed by the phase separation of polar poly(amidoamine) building block in fluorous environment. Further work is in progress to explore the applications and advantages of these mesomorphic properties and micellar formations of fluorinated dendrimers in regioselective syntheses using photoreaction.

4. Experimental

4.1. General

NMR spectra were measured on a Bruker AVANCE400 spectrometer. Matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-Mass) was performed on a Voyager Elite mass spectrometer using dithranol (1,8,9-anthracenetriol) as a matrix. HPLC experiments were performed on a Japan Analytical Industry Co. model LC-918V with Jaigel 1H+2H (eluent: chloroform). Photoirradiation was carried out in a Pyrex reactor. A 500 W high-pressure mercury lamp was used as the light source. The polarized optical microscopic studies were performed on Olympus BHA-751-T microscope equipped with a heating stage (Mettler FP80). Differential scanning calorimetry was performed on a SHIMADZU DSC-60. X-ray diffraction (XRD) patterns were measured with Cu Kα radiation using a Rigaku RAD.

The reagents were obtained from Wako Pure Chemical Industries Ltd, Tokyo Kasei Co. Ltd, Kanto Kagaku Co. Ltd, or Aldrich Chemical Co. *N*,*N*-Dimethylformamide (DMF) used in reactions, were further purified by general methods. Other solvents and reagents were used as received without further purification. According to a previously reported method,[1] dendron **2** was synthesized.

4.2. Synthesis of dendron 1

A mixture of **2** (30 mg, 0.061 mmol), 2-(perfluorooctyl)-ethyl acrylate (360 mg, 0.695 mmol), and *N*,*N*-dimethylformamide (DMF) (0.3 mL) was stirred at 45 °C for 1 week. Chloroform was added to the reaction mixture and the solution was washed with water to remove DMF. After removal of solvent, the residue was purified by HPLC to afford dendron **1** (109 mg, 0.043 mmol), in 70% yield. ^{1}H NMR ($CDCl_3$) δ 2.27–2.47 (m, 24H), 2.58 (t, J=6.4 Hz, 8H), 2.72 (t, J=5.2 Hz, 2H), 2.82 (t, J=6.0 Hz, 4H), 3.17 (q, J=5.6 Hz, 4H), 3.69 (q, J=5.6 Hz, 2H), 4.31 (t, J=6.4 Hz, 8H), 7.45–7.53 (m, 2H), 7.94–8.04 (m, 4H), 8.42 (s, 1H), 8.55 (s, 1H), 8.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 34.7, 36.6, 38.4, 38.5, 49.7, 50.1, 52.0, 52.8, 53.2, 124.9, 126.1, 127.6, 128.9, 129.3, 130.2, 131.7, 132.9, 161.2, 161.4, 174.5, 175.0; ^{19}F NMR ($CDCl_3$) δ −126.7, −124.1, −123.3, −122.5, −122.5, −122.2, −114.2, −81.4; MALDI-TOF-Mass for $C_{79}H_{64}F_{68}N_6O_{11}$ *m*/*z* calcd, 2566.28 [MH^+]; found, 2566.35. Anal. Calcd for $C_{79}H_{64}F_{68}N_6O_{11}$: C, 36.99; H, 2.51; N, 3.28%. Found: C, 36.82; H, 2.44; N, 3.17%.

4.2.1. Photodimerization of dendron 1. *In chloroform*: A solution of dendron **1** (50 mg, 0.019 mmol) in chloroform (250 μL) was irradiated with a high-pressure mercury lamp through a Pyrex filter for 3 h at room temperature. After removal of the solvent, the residue was purified by HPLC with $CHCl_3$ as eluent to isolate the photodimers (38 mg) in 84% yield (after 92% conversion). *In perfluorohexane*: A solution of dendron **1** (50 mg, 0.019 mmol) in perfluorohexane (250 μL) was irradiated with a high-pressure mercury lamp through a Pyrex filter for 3 h at room temperature. After removal of the solvent, the residue was purified by HPLC with $CHCl_3$ as eluent to isolate the photodimers (16 mg) in 44% yield (after 86% conversion). *Without solvent*: The dendron **1** was placed between Pyrex plates and irradiated with the high-pressure mercury lamp (λ>300 nm) at room temperature. The resulting mixture was purified by HPLC with $CHCl_3$ as eluent to isolate the photodimers (21 mg) in 55% yield (after 86% conversion): ^{1}H NMR ($CDCl_3$) δ 2.00–2.82 (m, 76H), 3.00–3.2 (m, 8H), 3.35–3.60 (m, 4H), 4.32 (t, J=6.4 Hz, 16H), 4.55–4.70 (m, 4H), 6.65–7.00 (m, 14H), 7.30–7.60 (m, 6H).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.036.

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Hg^{2+} sensing in aqueous solutions: an intramolecular charge transfer emission quenching fluorescent chemosensors

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Abstract—Compounds **4a** and **4b**, comprising an anthracene moiety as the fluorophore and a pair of dithiocarbamate functionalities as ligating groups, were designed as fluorescent chemosensors for Hg(II). In aqueous solvent systems, upon excitation, in addition to the normal emission bands of locally excited (LE) state of anthracene, both compounds show a prominent pH-independent intramolecular charge transfer (ICT) emissive band, which can be modulated by Hg^{2+} binding. The systems can be exploited to develop a fluorescent sensitive probe for Hg^{2+}.

1. Introduction

Fluoroionophore design based on 'fluorophore–spacer–receptor' motif has been demonstrated to be an effective way of developing both anion and cation chemosensors.[1] A variety of signal transduction mechanisms are established to signify the binding event between a receptor and a guest molecule/ion.[2] Particularly, a number of cation chemosensors operated on modulation of dual fluorescence emission of electron donor–acceptor integrated systems permitting ratiometric measurement are attractive in sensory applications.[2a,3] Chemosensor exhibiting high selectivity and sensitivity toward a target analyte is in great demand due to its low cost, ability on real time monitoring, and high throughput capability. Among all metal analytes detection, mercury chemosensing has been recently attracted considerable interests. Mercury is ubiquitous and its potent neurotoxicity on human warrants the exploration of new methods for monitoring aqueous Hg^{2+} in biological, food, and environmental samples.[4]

To continue our interests in fluorescent sensor development on toxic metal monitoring,[5] we herein report acceptor–spacer–donor systems **4a** and **4b**, in which anthracene (AN) used as an electron acceptor is linked to a pair of dithiocarbamates (DTC) as both metal chelating groups and electron donors via iminophenylmethylene spacer.[6] In aqueous media, both systems display dual fluorescence emissive bands. In which the short wavelength emission bands are ascribed to the locally excited (LE) state of the anthracene moiety, and the long wavelength structureless band could be ascribed to intramolecular charge transfer (ICT) states between the DTC groups and excited AN. Interestingly, the relative intensity of these two emissions was found to be modulated by metal ion binding.

2. Results and discussion

On the outset of the investigation, we envisage that DTC is an effective ligating group for heavy metal ion's complexation and the structural environment of DTC in the system could render the sensors with discriminative binding affinities toward various metal cations. For synthesizing sensors, the requisite precursor **2** obtained from phenyliminodiethanol (**1**) and TsCl in pyridine was treated with ammonium *N*,*N*-diethyldithiocarbamate and morpholinyldithiocarbamate in CH_3CN at room temperature for 2 days, affording the corresponding bis-dithiocarbamate **3a** and **3b** in 95 and 87% yield, respectively. After building up the metal receptive site, incorporation of a fluorescent moiety on the receptor was achieved by Friedel–Crafts alkylation reaction. Thus, to complete the synthesis of sensors **4**, aluminum chloride induced Friedel–Crafts alkylation of **3** with 9-chloromethylanthracene in refluxing chloroform allowed the smooth appendage of the anthracene subunit as the fluorescent signaling handle (Scheme 1).[7]

To probe the best operative conditions for the sensors to exhibit the selective binding of metals, the choice of a proper aqueous solvent system is our first priority. In that connection, the fluorescent titration of **4a** with Hg^{2+} in aqueous acetonitrile (1:1, v/v) was first undertaken. The emission spectra of the corresponding titration curves reveal the

Keywords: Chemosensors; Mercury probe; Dual fluorescence; Anthracene.
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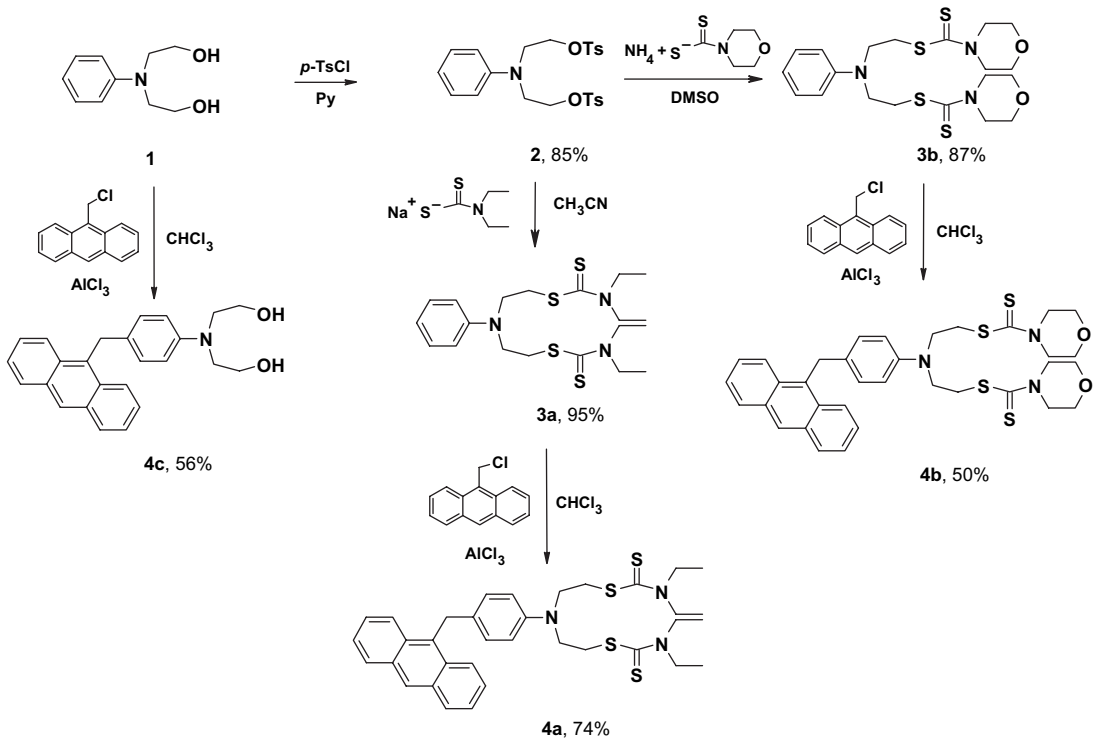

Scheme 1. Synthesis of the chemosensors.

dependence of the fluorescent behavior of the sensor on the metal concentration (Fig. 1A). Increasing the concentration of Hg^{2+} from 5×10^{-8} to 2.5×10^{-4} M, the corresponding titration curves exhibited an increase in the short wavelength emissive peaks with a concomitant decrease in the long wavelength structureless emissive peak at 525 nm. A clear isoemissive point at 455 nm was observed. The long wavelength emissive peak is conceivably arising from ICT fluorescence formed between the excited state of the anthracene moiety and DTC (vide infra). The findings provide the basis for ratiometric fluorescent determination of Hg^{2+}. The limit of detection (LOD) for Hg^{2+} is estimated to be 1×10^{-8} M with the present system. The responsive sensitivity of the sensor can be further increased by changing the solvent system to 1:1 aqueous ethanol. The more polar solvent system adopted further enhances the intensity of ICT emissive band as evidenced in the titration experiments (Fig. 1B). Interestingly, under the same conditions of the titration experiments, in contrast to that of sensor **4a**, sensor **4b** responds quite distinctively to Hg^{2+}. As shown in Figure 2, by increasing the concentration of Hg^{2+} from 5×10^{-8} to 1.0×10^{-3} M, the corresponding titration curves exhibit a decrease in ICT fluorescence with the short wavelength peaks being intact. Conceivably, the two dithiocarbamate moieties bearing morpholine ring present in **4b** could confer a more focused metal binding site for the sensor in such a way that the lone pair of the imino-nitrogen did not take part in the metal complexation. As a result, metal binding in **4b** would not interfere with the photo-induced electron transfer (PET) process. In contrast, the imino-nitrogen present in **4a** could actively participate in the metal binding; the increase of emissive peaks of LE state is attributed to the

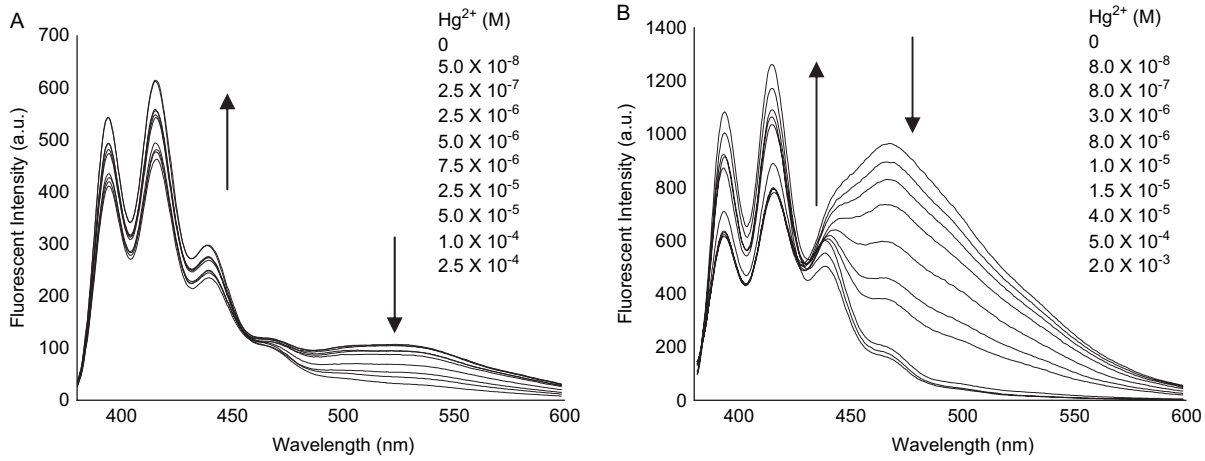

Figure 1. Fluorescence spectra of **4a** as a function of [Hg^{2+}] (A) in CH_3CN/H_2O = 1:1, v/v; (B) in $EtOH/H_2O$ = 1:1, v/v. [**4a**] = 5.0×10^{-6} M.

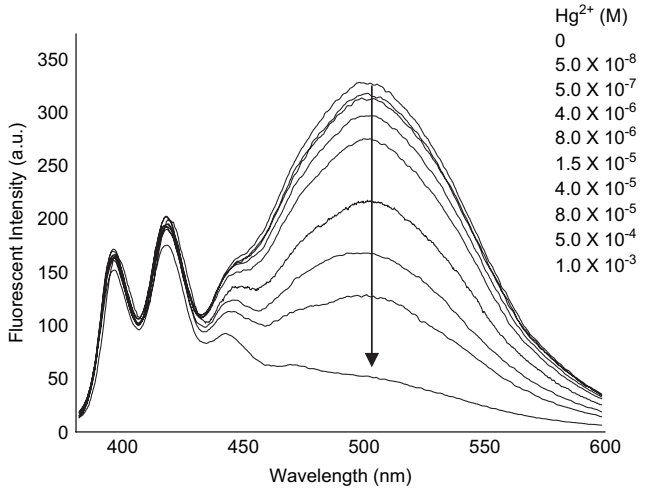

Figure 2. Fluorescence spectra of **4b** as a function of [Hg^{2+}] in EtOH/H_2O = 1:1, v/v. [**4b**] = 1.0×10^{-5} M.

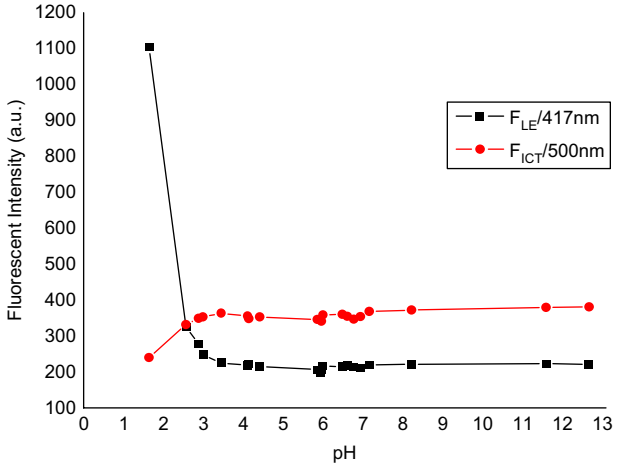

Figure 4. Effect of pH on the emission peaks of LE state (417 nm) and ICT (500 nm) peaks of **4b**.

reduction of the intrinsic PET process as a result of metal binding. Apparently, the fine structural environment of the binding site of sensor can be tuned by a rational design. The selectivity studies of the sensors substantiate that **4b** with more rigid binding site exhibits a better selectivity toward Hg^{2+} among other metal ions. In contrast, sensor **4a** is a less selective sensor as shown in Figure 3. Sensor **4b** in fact does not respond to other common metal cations as well as organo-mercury. Further detailed investigation reveals that sensor **4b** also responded sensitively with Ag^{+}. On the basis of the nonlinear fitting experiments of fluorescent titrations, the binding constant of **4b** with Ag^{+} and Hg^{2+} was determined to be 32.3×10^{4} and 3.9×10^{4} M^{-1}, respectively. The values of binding constants revealed that the sensor forms fairly strong complexes with these metals. On the other hand, the detection sensitivity of both sensors toward Hg^{2+} is excellent with an estimated LOD at 10^{-8} M. Metal ion chemosensors often exhibit pH-dependent emission and could be problematic in practical operation. To our delight, pH fluorescent titration experiments on sensor **4b** indicated that there is no cross response of the sensor to the pH change of the sensor solution. According to Figure 4, emissions from both LE and ICT state remain constant over a wide pH range from 3.5 to 12.5. It is noteworthy that the anthracene emissive peak of the sensor exists at 'off state' when the pH of the solution is greater than 3.2. Considering the fact that Ag^{+} ion is seldom found in biological and environmental samples, thus in terms of high sensitivity and selectivity and wide working pH range, sensor **4b** possesses good potential to be used for monitoring the mercury level of real samples.

To shed light for the response mechanism of the sensory systems, a control molecule **4c** without the DTC receptive groups was synthesized from Friedel–Crafts alkylation of phenyliminodiethanol and 9-chloromethylanthracene. No ICT fluorescence was observed in the emission spectra of **4c** in 1:1 aqueous ethanol solution. As shown in Figure 5, the fluorescent titration curves of **4c** exhibit a typical PET based chemosensor behavior. The fluorescence of the sensor was completely turned off at 10^{-3} M of Hg^{2+}. Thus, the presence of DTC moieties in sensors **4** is essential for the

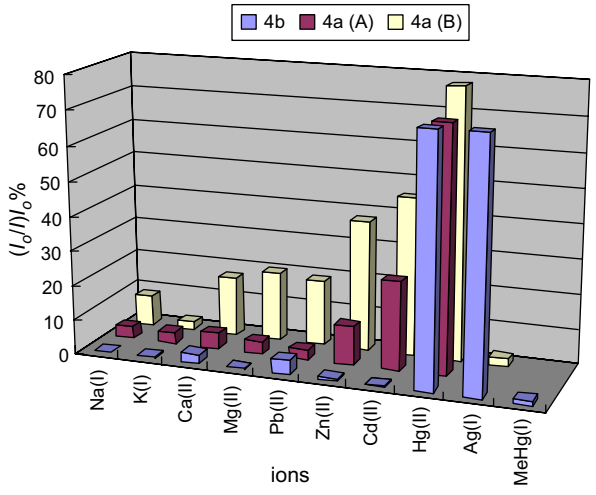

Figure 3. Quenching ratio of **4a** and **4b** with different ions. Compound **4a** (A) EtOH/H_2O = 1:1, v/v; **4a** (B) and **4b** CH_3CN/H_2O = 1:1, v/v; [**4a**] = 5.0×10^{-6} M; [ion] = 2.5×10^{-4} M; [**4b**] = 1.0×10^{-5} M.

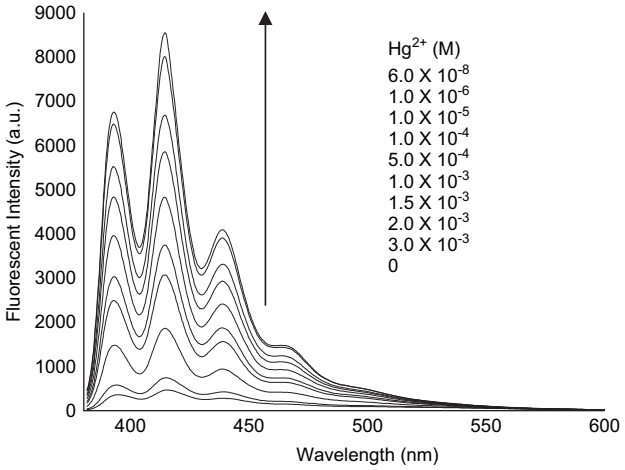

Figure 5. Fluorescence spectra of **4c** as a function of [Hg^{2+}] in EtOH/H_2O = 1:1, v/v. [**4c**] = 2.0×10^{-5} M.

Table 1. Exciplex emissive band of **4a**, **4b**, and **4c** in different solvents, excitation wavelength = 368 nm

	CH_3CN/ H_2O = 1:1 (nm)	EtOH/ H_2O = 1:1 (nm)	DMSO (nm)	DMSO/ H_2O = 0.5:99.5 (nm)
4a	525	467	—	—
4b	—	500	530	465

occurrence of dual fluorescence emission as compared with the control experiment conducted by **4c**. It is noteworthy that the fluorescence maximum of the structureless long wavelength emission of both **4a** and **4b** is highly solvent dependent (Table 1). Such an observation is consistent with the characteristics of ICT fluorescence.[3] The strong binding between Hg^{2+} and the sensors can be rationalized by the combination of electronic and steric effect. On one hand, the four sulfur atoms present in the sensors provide strong affinity to bind heavy metals while on the other hand, the bulkiness of the two morpholine moiety appended on **4b** defines a semi-rigid cavity favoring a selective binding of mercury and silver. Upon binding of metal ions by the DTC groups, the electron density of the receptive site is greatly reduced, the ICT mechanism between the 'donor–receptor' and the excited state of anthracene is retarded.

In summary, we have demonstrated that the dual fluorescence characteristics of a new Hg^{2+}-selective fluoroionophore **4b** could be modulated by its interaction with mercury ion. The excellent selectivity and sensitivity of the sensor toward Hg^{2+} and Ag^{+} are attributed to the combined electronic and steric effect. The fluorescence measurements for analyzing the metal content of the solution can be conducted in un-buffered solutions.

3. Experimental

3.1. General

Melting point was determined with a MEL-TEMPII melting point apparatus (uncorrected). ^1H and ^{13}C NMR spectra were recorded on a JOEL EX 270 spectrometer (at 270 and 67.8 MHz, respectively) in DMSO-d_6 or $CDCl_3$. Chemical shifts were registered relatively to Me_4Si (in ppm). High-resolution mass spectra were recorded on a Bruker Autoflex mass spectrometer (MALDI-TOF) or electrospray ionization high-resolution mass spectra on an API Qstar Pulsari mass spectrometer. Fluorescent emission spectra were collected on a Hitachi F4500 fluorescence spectrophotometer. Unless specified, all fine chemicals were used as received.

3.1.1. Phenyliminodiethanol 1. To a THF solution (15 mL) of phenyliminodiacetic acid diethyl ester[7] (0.5 g, 1.9 mmol) in a round bottomed flask immersed in an ice bath, LAH (0.14 g, 3.6 mmol) was slowly added in two portions. The reaction mixture was allowed to stir for 10 min and then refluxed at 80 °C under N_2 for 2 h. After the reaction mixture was cooled to room temperature, the flask was soaked in the ice bath. H_2O (1 mL), NaOH (1 M, 1 mL), and again H_2O (3 mL) were added successively. White solid appeared, which was filtered off using sinter filter and washed with several portions of ethyl acetate. The filtrate was extracted by CH_2Cl_2 (3×30 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum, affording **1** as a colorless oil (0.34 g, 99% yield). δ_H (270 MHz, $CDCl_3$): 3.33 (s, 2H), 3.58 (t, J=4.86 Hz, 4H), 3.85 (t, J=4.86 Hz, 4H), 6.68–6.77 (m, 3H), 7.24 (t, J=7.83 Hz, 2H).

3.1.2. Phenyliminodi(ethyltosylate) 2. To a pyridine solution (5 mL) of **1** (0.5 g, 2.8 mmol), *p*-toluenesulfonyl chloride (1.6 g, 8.4 mmol) was added. The reaction mixture was stirred in an ice bath under N_2 for 5 h. Water was added (30 mL) and products were extracted by CH_2Cl_2 (3×30 mL). The organic layer was collected and dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum. The crude product was recrystallized in PE to give pure **2** as white crystals (1.15 g, 85% yield). Mp 72.0–75.0 °C; HRMS *m/z* calcd for $C_{24}H_{27}NO_6S_2$ $(M+H)^+$: 490.1358, found: 490.1357; δ_H (270 MHz, $CDCl_3$): 2.42 (s, 6H), 3.55 (t, J=6.75 Hz, 4H), 4.08 (t, J=6.75 Hz, 4H), 6.43 (br d, J=8.10 Hz, 2H), 6.70 (t, J= 8.10 Hz, 1H), 7.13 (br d, J=8.10 Hz, 2H), 7.28 (d, J= 8.10 Hz, 4H), 7.71 (d, J=8.10 Hz, 4H); δ_C (67.8 Hz, $CDCl_3$): 21.72, 50.18, 66.57, 111.89, 117.46, 127.71, 129.34, 129.76, 132.44, 144.85, 145.55.

3.1.3. Ammonium morpholinyldithiocarbamate. Morpholine (5 mL, 57 mmol) was dissolved in CH_3OH (100 mL). CS_2 (5 mL, 83 mmol) and NH_4OH (4 mL, 28%) were added. The reaction mixture was stirred at room temperature for few minutes. Et_2O (100 mL) was added. The product was precipitated and filtered, which was dried under reduced pressure to afford a pale yellow solid (6.67 g, 70% yield). δ_H (270 MHz, DMSO-d_6): 3.48 (t, J=4.05 Hz, 4H), 4.27 (t, J=4.05 Hz, 4H); δ_C (67.8 Hz, DMSO-d_6): 49.74, 66.12.

3.1.4. Phenyliminobis(ethyldiethyldithiocarbamate) 3a. Compound **2** (1 g, 2 mmol) was dissolved in CH_3CN (40 mL). Sodium diethyldithiocarbamate (1.42 g, 6.3 mmol) was added. The reaction mixture was stirred under N_2 at room temperature for 2 days. Water (30 mL) was added and products were extracted with CH_2Cl_2 (3×30 mL). The organic layer was collected and dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum, affording **3a** as white solid (0.9 g, 100% yield). Mp 77–81 °C; HRMS *m/z* calcd for $C_{20}H_{33}N_3S_4Na$ $(M+Na)^+$: 466.1455, found: 466.1459; δ_H (270 MHz, $CDCl_3$): 1.29 (t, J=6.75 Hz, 12H), 3.49–3.54 (m, 4H), 3.67–3.76 (m, 8H), 4.00–4.08 (m, 4H), 6.69 (t, J=8.10 Hz, 1H), 6.97 (br d, J=8.10 Hz, 2H), 7.24 (br d, J=8.10 Hz, 2H); δ_C (67.8 Hz, $CDCl_3$): 11.29, 32.96, 46.46, 49.54, 111.62, 115.91, 128.75, 146.56, 194.07.

3.1.5. Phenyliminobis(ethyldimorpholinyldithiocarbamate) 3b. Compound **2** (0.37 g, 0.8 mmol) was dissolved in DMSO (8 mL). Ammonium morpholinyldithiocarbamate (0.38 g, 2.3 mmol) was added. The reaction mixture was allowed to stir under N_2 at room temperature for 2 days. Water was added and products were extracted with CH_2Cl_2 (3×30 mL). Then, the emulsified layer was combined with satd NH_4Cl. H_2O (4×20 mL) was introduced to wash the organic portion. The organic layer was collected and dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum. The crude product was purified by

column chromatography, eluted with PE:EA (1:1) to give **3b** as white solids (0.31 g, 87% yield). Mp 141–143 °C; HRMS *m/z* calcd for $C_{20}H_{29}N_3O_2S_4$ $(M+Na)^+$: 494.1004, found: 494.0953; δ_H (270 MHz, $CDCl_3$): 3.53–3.59 (m, 4H), 3.68–3.70 (m, 4H), 3.76–3.79 (m, 8H), 3.96–4.00 (m, 4H), 4.30 (m, 4H), 6.73 (t, *J*=7.20 Hz, 1H), 6.92 (br d, *J*=7.20 Hz, 2H), 7.26 (br d, *J*=7.20 Hz, 2H); δ_C (67.8 Hz, $CDCl_3$): 33.37, 49.90, 50.81, 66.22, 112.07, 116.63, 129.33, 146.86, 196.71.

3.1.6. 4-(9-Methylanthracene)phenyliminobis(ethyldiethyldithiocarbamate) 4a. Compound **3a** (0.23 g, 0.5 mmol) and 9-chloromethylanthracene (0.13 g, 0.6 mmol) were added in a 50 mL round bottomed flask. Dry $CHCl_3$ was added (10 mL) and then followed by $AlCl_3$ (0.07 g, 0.5 mmol). The reaction mixture was refluxed at 70 °C under N_2 overnight. At the end of the reaction, NaOH (10 mL, 1 M) was added to the reaction mixture. The combined solution was extracted by CH_2Cl_2 (3×30 mL) and H_2O. The organic layer was collected and dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum. The crude product was purified by column chromatography, eluted with PE:EA (6:1) affording the product **4a** as yellow solids (0.24 g, 74% yield). Mp 92.0–93.0 °C; HRMS *m/z* calcd for $C_{35}H_{43}N_3S_4$ (M^+): 633.2339, found: 633.2369; δ_H (270 MHz, $CDCl_3$): 1.20 (t, *J*=6.75 Hz, 12H), 3.44 (t, *J*=5.40 Hz, 4H), 3.57–3.64 (m, 8H), 3.98 (t, *J*=5.40 Hz, 4H), 4.89 (s, 2H), 6.79 (d, *J*=8.10 Hz, 2H), 6.98 (d, *J*=8.10 Hz, 2H), 7.40–7.44 (m, 4H), 7.99 (br d, *J*=5.40 Hz, 2H), 8.24 (br d, *J*=5.40 Hz, 2H), 8.37 (s, 1H); δ_C (67.8 Hz, $CDCl_3$): 12.48, 33.48, 46.76, 49.52, 49.94, 112.16, 124.69, 124.88, 125.55, 126.00, 128.82, 128.83, 128.85, 130.28, 131.50, 132.64, 145.27, 194.70.

3.1.7. 4-(9-Methylanthracene)phenyliminobis(ethyldimorpholinyldithiocarbamate) 4b. Compound **3b** (0.15 g, 0.3 mmol) and 9-chloromethylanthracene (0.07 g, 0.3 mmol) were mixed in a 50 mL round bottomed flask. Dry $CHCl_3$ was added (11 mL) and then followed by $AlCl_3$ (0.04 g, 0.3 mmol). The reaction mixture was refluxed at 70 °C under N_2 overnight. At the end of the reaction, NaOH (11 mL, 1 M) was added to the reaction mixture. Water (30 mL) was added and CH_2Cl_2 (3×30 mL) was used for extraction. The organic layer was collected and dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum. The crude product was purified by column chromatography, eluted with PE:EA (3:1) affording the product **4b** as yellow solids (0.1 g, 50% yield). Mp: 99.0–99.5 °C; HRMS *m/z* calcd for $C_{35}H_{39}N_3O_2S_4$ $(M+H)^+$: 662.2003, found: 662.2002; δ_H (270 MHz, $CDCl_3$): 3.49 (t, *J*=4.05 Hz, 4H), 3.60 (t, *J*=4.05 Hz, 4H), 3.70–3.73 (m, 8H), 3.91–3.94 (m, 4H), 4.30–4.33 (m, 4H), 4.91 (s, 2H), 6.75 (d, *J*=8.10 Hz, 2H), 7.01 (d, *J*=8.10 Hz, 2H), 7.43–7.47 (m, 4H), 8.02 (br d, *J*=5.40 Hz, 2H), 8.25 (br d, *J*=5.40 Hz, 2H), 8.41 (s, 1H); δ_C (67.8 Hz, $CDCl_3$): 32.46, 33.43, 49.92, 51.11, 62.55, 112.17, 124.76, 124.88, 125.66, 126.15, 128.88, 128.96, 129.37, 130.33, 131.56, 132.53, 145.17, 196.73.

3.1.8. 4-(9-Methylanthracene)phenyliminodiethanol 4c. Compound **1** (0.83 g, 4.6 mmol) and 9-chloromethylanthracene (1.1 g, 4.8 mmol) were mixed in dry $CHCl_3$ (10 mL). $AlCl_3$ (0.6 g, 4.6 mmol) was added. The reaction mixture was refluxed at 70 °C under N_2 overnight. At the end of the reaction, NaOH (10 mL, 1 M) was added to the reaction mixture. Water (30 mL) was added and products were extracted with CH_2Cl_2 (3×30 mL). The organic layer was collected and dried over Na_2SO_4. The solvent was removed by rotary evaporation under vacuum. The crude product was purified by column chromatography, eluted with EA to afford the product **4c** as yellow solids (0.95 g, 56% yield). Mp 46.0–47.5 °C; HRMS *m/z* calcd for $C_{25}H_{25}NO_2$ $(M+H)^+$: 372.1963, found: 372.1958; δ_H (270 MHz, $CDCl_3$): 3.34 (t, *J*=4.73 Hz, 4H), 3.61 (t, *J*=4.73 Hz, 4H), 3.74–3.77 (m, 2H), 4.86 (s, 2H), 6.42 (d, *J*=8.10 Hz, 2H), 6.9l (d, *J*=8.10 Hz, 2H), 7.38–7.42 (m, 4H), 7.95–7.99 (m, 2H), 8.19–8.22 (m, 2H), 8.36 (s, 1H); δ_C (67.8 Hz, $CDCl_3$): 32.37, 55.35, 60.66, 112.60, 114.63, 124.75, 124.79, 125.63, 126.14, 128.76, 128.94, 129.09, 130.29, 131.54, 145.86.

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Facile and catalytic degradation method of DDT using Pd/C–Et_3N system under ambient pressure and temperature

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Abstract—The catalytic degradation method of *p*,*p*′-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and its regioisomer *o*,*p*′-DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane] using the Pd/C–Et_3N system under ambient hydrogen pressure and temperature was established. The presence of Et_3N was necessary for the quick and complete breakdown of DDT. The independent degradation study of two intermediates, *p*,*p*′-DDD [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane] and *p*,*p*′-DDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene] using GC–MS let us to speculate the degradation pathway of *p*,*p*′-DDT. In the initial phase of the reaction, *p*,*p*′-DDT degradation splits into two ways: a dehydrochlorination pathway and a hydrodechlorination pathway. In each pathway, reaction starts from an aliphatic moiety and subsequent hydrodechlorination from the benzene moieties takes place in a stepwise manner. The former pathway leads to the formation of 1,1-diphenylethane and the latter leads to the formation of 1,1-dichloro-2,2-diphenylethane. These diphenylethane analogs, which are less toxic compared with *p*,*p*′-DDT, are terminal degradation products in our system. The distinctive features of our catalytic degradation method of DDTs are reliability, simplicity, efficiency, and inexpensiveness.

1. Introduction

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p*,*p*′-DDT) used to be employed worldwide as a broad-spectrum pesticide to protect crops from insects and to control insect-borne diseases such as malaria. But today the use of *p*,*p*′-DDT is banned in many countries because of its hydrophobic nature and of its persistence based on its chemical stability, leading to bioaccumulation and biomagnification in food chains.[1–3] *p*,*p*′-DDT affects the nervous system to cause tremors and convulsions.[4] Both *o*,*p*′-DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane], a regioisomer of *p*,*p*′-DDT and *p*,*p*′-DDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene], a metabolite of *p*,*p*′-DDT have been recognized as endocrine disruptors as a result of recent studies on DDT families.[5,6] Despite such risks arising from *p*,*p*′-DDT and its related compounds, DDT (technical grade: a mixture of *p*,*p*′-DDT and its regioisomers) is still in use in some countries to combat disease-carrying insects because of the cost-efficiency of DDT. Furthermore, there is a fear of an accidental leak of a major fraction of DDTs, which was packed into drums and stored underground in 1970's especially in Japan due to the lack of suitable degradation methods of DDTs based on the high thermodynamic, chemical, and biological stability. As a global environmental issue, therefore, it is very important to monitor the pollution caused by the persistent insecticide[7,8] and to develop efficient degradation methods of DDT.[9] Numerous studies for the development of DDT degradation methods reported to date include photoremediation,[10–16] bioremediation,[17–19] mechanochemical remediation,[20] hydride reduction,[21] hydrodechlorination,[22–24] reductive dechlorination using metals,[25,26] electrolysis,[27–30] and supercritical water oxidation.[31] Most processes require special and/or expensive equipment and facilities, large amounts of reagents, high heat, high pressure, radiation, and/or strong base conditions, and many of them do not complete degradation of DDT. Recently, Blum and co-workers reported a hydrodechlorination method using a combined Pd–Rh catalyst to achieve the complete removal of five chlorine atoms and saturation of aromatic rings of *p*,*p*′-DDT.[22] Tundo and co-workers also succeeded in the complete dechlorination of *p*,*p*′-DDT by Pd/C or Raney-Ni-catalyzed hydrodechlorination method.[24] These hydrodechlorinations were performed under relatively mild conditions, but still the former method requires heating (80–100 °C) and high pressure (27 atm) and the latter requires heating (50 °C) and continuous bubbling of hydrogen (10 mL/min).

We have reported that addition of a nitrogen-containing base such as NH_3, pyridine, and ammonium acetate to a Pd/C-catalyzed hydrogenation system as a weak catalyst poison chemoselectively inhibited the hydrogenolysis of a benzyl ether with smooth hydrogenation of other reducible functionalities such as olefin, Cbz, benzyl ester, azide, and so on.[32,33] During the course of our further study on the chemoselective hydrogenation using a Pd/C–Et_3N system, the catalytic activity of Pd/C towards the hydrodechlorination

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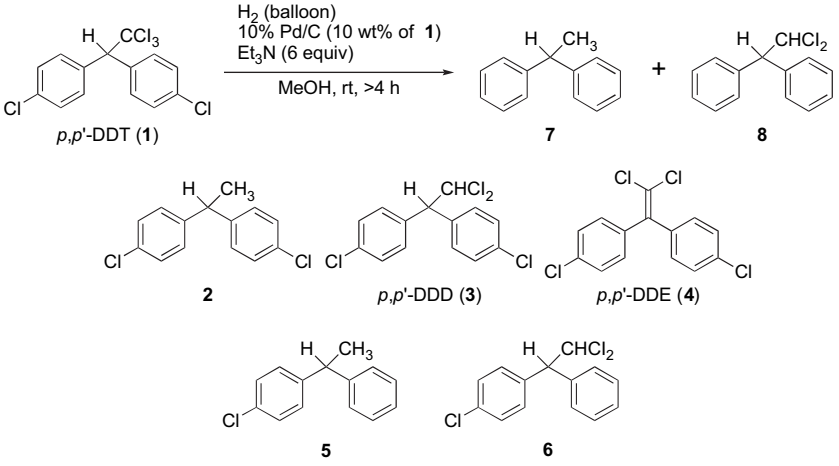
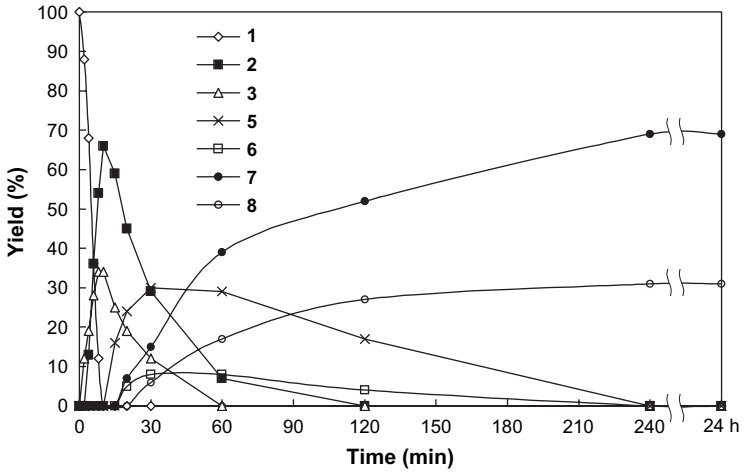
of aromatic chlorides was found to be remarkably and selectively enhanced by the addition of Et_3N[34] and this system efficiently worked for the complete dechlorination of polychlorinated biphenyls (PCBs).[35] This method required only a catalytic amount of Pd/C and an almost stoichiometric amount of Et_3N (1.2 equiv vs chlorine atom) and did not require heating, high pressure of hydrogen, nor special equipment. In this paper, we discuss the application of this mild system to the degradation of *p*,*p*′-DDT and *o*,*p*′-DDT and also propose the dechlorination pathways of *p*,*p*′-DDT in our system.

2. Results and discussion

2.1. Degradation of *p*,*p*′-DDT

In accordance with our previous work for the hydrodechlorination of PCBs,[35] 10% Pd/C was used with 10% of the





Time	Yield (%)							
(min)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0	~100	0	trace	trace	0	0	0	0
2	88	trace	12	trace	0	0	0	0
4	68	13	19	trace	0	0	0	0
6	36	36	28	trace	trace	0	0	0
8	12	54	34	trace	trace	0	0	0
10	trace	66	34	0	trace	0	0	0
15	0	59	25	0	16	trace	trace	0
20	0	45	19	0	24	5	7	trace
30	0	29	12	0	30	8	15	6
60	0	7	trace	0	29	8	39	17
120	0	trace	0	0	17	4	52	27
240	0	0	0	0	trace	0	69	31
24 h	0	0	0	0	0	0	69	31

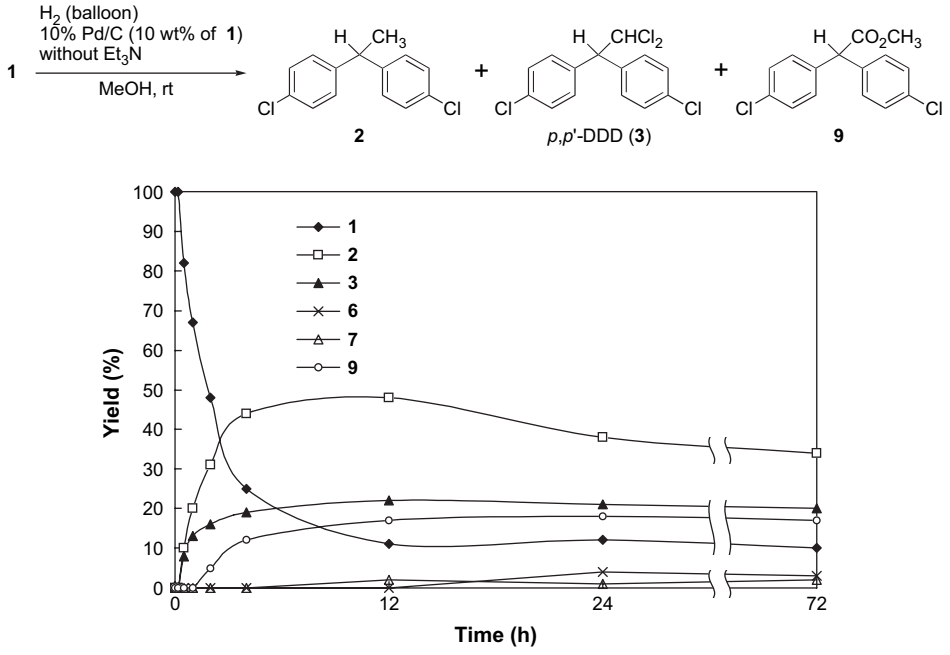
Figure 1. Pd/C catalyzed degradation of *p*,*p*′-DDT (**1**) under ambient hydrogen pressure and temperature with Et_3N (1.2 equiv vs Cl).

substrate weight and 6 equiv of Et_3N (1.2 equiv to each chlorine atom of the substrate) was employed. The hydrodechlorination of *p*,*p*′-DDT **1** (CAS Registry Number 50-29-3) in MeOH using Pd/C–Et_3N system under ambient temperature and pressure is shown in Figure 1. *p*,*p*′-DDT **1** was completely consumed in the first 10 min and 1,1-bis(*p*-chlorophenyl)ethane **2** (CAS Registry Number 3547-04-4),[36] in which structure all aliphatic chlorine atoms of **1** were replaced with hydrogen atoms, and *p*,*p*′-DDD **3** (CAS Registry Number 72-54-8), in which structure an aliphatic chlorine atom of **1** was replaced with a hydrogen atom, were accordingly generated in the ratio of about 2:1. A trace amount of *p*,*p*′-DDE **4** (CAS Registry Number 72-55-9) was also detected during the initial phase (not shown in Fig. 1). After the formation of **2** and **3**, the dechlorination of these compounds took place from their benzene rings step by step to generate mono-chlorophenyl compounds **5** (CAS Registry Number 60617-89-2)[37] and **6** (CAS Registry Number 6952-08-5)[38] and after 4 h the reaction was led to the formation of 1,1-diphenylethane **7** (CAS Registry Number 612-00-0)[37,39] and 1,1-dichloro-2,2-diphenylethane **8** (CAS Registry Number 2387-16-8)[40] in the ratio of ca. 2:1. The reaction was monitored up to 24 h, but further reduction was not observed and the material ratio of **7** and **8** was virtually the same.

On the other hand, the reaction of **1** in the absence of Et_3N did not completely consume **1** even after 72 h of the reaction (Fig. 2). The chlorine atoms on the benzene rings remained almost intact. Both the consumption of **1** and the formation of **2** and **3** were remarkably delayed, suggesting that the presence of Et_3N favorably affects the promotion of the dechlorination of alkyl chlorides as well as aromatic chlorides. Furthermore, methyl bis(*p*-chlorophenyl)acetate **9** (CAS Registry Number 5359-38-6),[41] which is supposed to be formed via methanolysis of **1**, **3**, or **4**, was generated as a by-product, whereas only a trace amount of the ester **9** was detected in the case of the reaction of **1** using Et_3N (not shown in Fig. 1). These results indicate that the presence of Et_3N in the catalytic degradation of *p*,*p*′-DDT **1** is crucial for increasing the efficiency of the reaction.

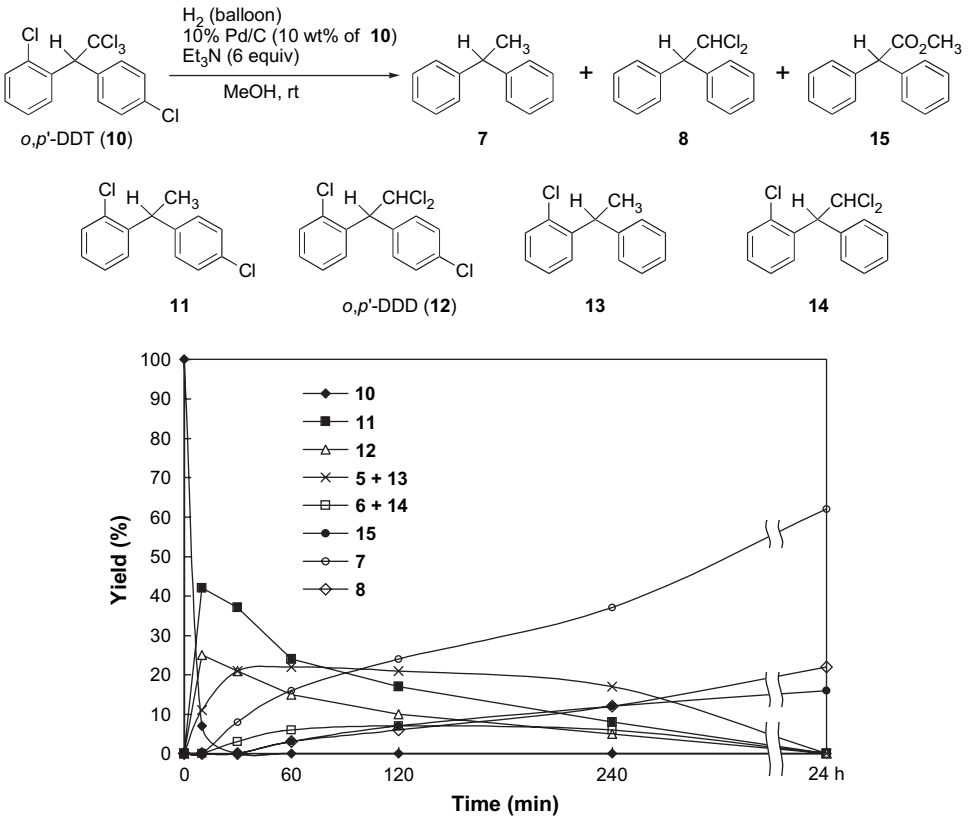
2.2. Degradation of *o*,*p*′-DDT

The application of the hydrodechlorination method using Pd/C and Et_3N was also investigated for the degradation



Time	Yield (%)					
(h)	**1**	**2**	**3**	**6**	**7**	**9**
0	100	0	0	0	0	0
10 min	~100	trace	trace	0	0	trace
0.5	82	10	8	0	0	trace
1	67	20	13	0	0	trace
2	48	31	16	0	0	5
4	25	44	19	trace	trace	12
12	11	48	22	trace	2	17
24	12	38	21	4	1	18
72	10	34	20	3	2	17

Figure 2. Pd/C catalyzed degradation of *p*,*p*′-DDT (**1**) under ambient hydrogen pressure and temperature without Et_3N.



Time	Yield (%)							
(min)	**10**	**11**	**12**	**5 + 13**	**6 + 14**	**15**	**7**	**8**
0	100	0	0	0	0	0	0	0
10	7	42	25	11	0	0	trace	0
30	0	37	21	21	3	trace	8	trace
60	0	24	15	22	6	3	16	3
120	0	17	10	21	7	7	24	6
240	0	8	5	17	6	12	37	12
24 h	0	trace	0	trace	trace	16	62	22

Figure 3. Catalytic degradation of *o*,*p*′-DDT (**10**) using Pd/C–Et_3N system under ambient hydrogen pressure and temperature.

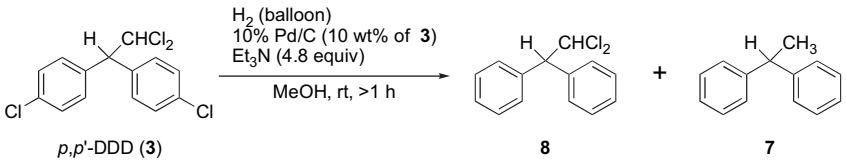
of *o*,*p*′-DDT **10** (CAS Registry Number 789-02-06) (Fig. 3). As we expected, **10** was degraded in a similar way to **1**, although the reaction appeared somewhat more complicated than the reaction of *p*,*p*′-DDT **1** because of the asymmetrical structure of **10**: (i) **10** completely disappeared within 30 min; (ii) the dechlorination started from aliphatic chlorides to generate 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)ethane **11** (CAS Registry Number 77008-62-9)[38,42] and 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane **12** (CAS Registry Number 53-19-0)[38] as the first detectable intermediates; (iii) then mono-hydrodechlorination from the benzene rings proceeded to afford each regioisomeric mixture of monochlorides [**5** and **13** (CAS Registry Number 76690-79-4)[43]] and trichlorides [**6** and **14** (CAS Registry Number 61693-87-6)[38]]; (iv) finally the reaction resulted in the generation of **7** and **8**, which are the same products from *p*,*p*′-DDT **1**, with methyl diphenylacetate **15** (CAS Registry Number 3469-00-9),[44] which was structurally dechlorinated from **9**.

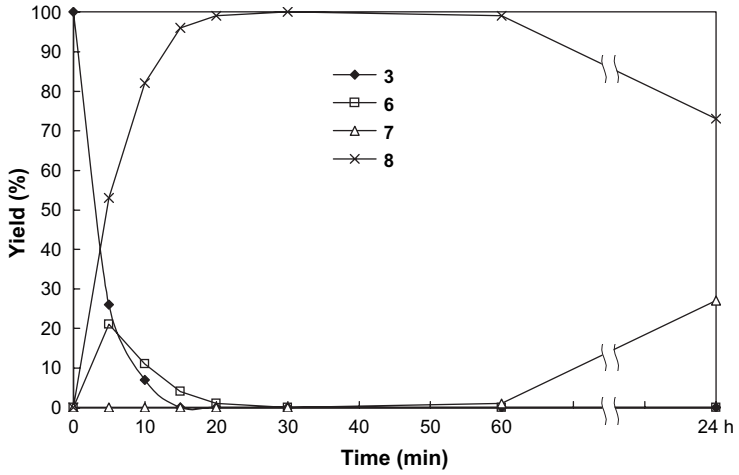
2.3. Degradation of *p*,*p*′-DDD

As we mentioned above, in the early stage of the degradation of *p*,*p*′-DDT **1**, a trace of *p*,*p*′-DDE **4** was detected with **2** and *p*,*p*′-DDD **3**. We anticipated that both **3** and **4** were the first intermediates of the dechlorination of **1**. To investigate the degradation pathway of *p*,*p*′-DDT by the hydrodechlorination using the Pd/C–Et_3N system, the reactions of *p*,*p*′-DDD **3** and *p*,*p*′-DDE **4** were independently monitored. As shown in Figure 4, **3** disappeared within 15 min and the chlorine atoms attached on the benzene rings were completely replaced with hydrogen atoms to generate **8** within 30 min. The reaction showed that the aliphatic chlorines of **3** were quite stable, but further dechlorination of **8** slowly proceeded to give **7** in about 30% yield after 24 h.

2.4. Degradation of *p*,*p*′-DDE

p,*p*′-DDE **4** was dechlorinated easily from both the aliphatic and aromatic portions (Fig. 5): **4** was completely consumed





Time	Yield (%)			
(min)	**3**	**6**	**7**	**8**
0	100	0	0	0
5	26	21	0	53
10	7	11	0	82
15	trace	4	0	96
20	0	trace	0	~100
30	0	0	0	100
60	0	0	trace	~100
24 h	0	0	27	73

Figure 4. Catalytic degradation of *p*,*p*′-DDD (**3**) using Pd/C–Et_3N system under ambient hydrogen pressure and temperature.

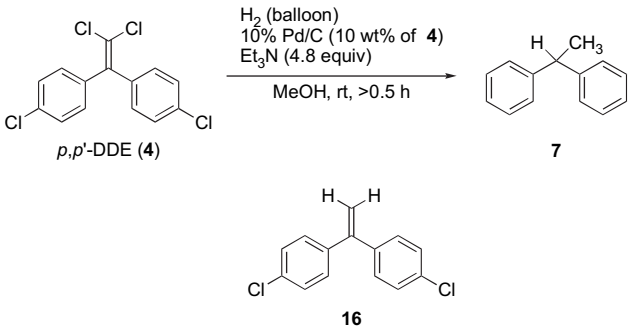
in 10 min; **2** and **5** were detected as intermediates; **7** was obtained as sole product after 30 min of the reaction; further reaction did not occur afterwards. If **4** had undergone the hydrogenation of its double bond before the dechlorination of its aliphatic chlorides, *p*,*p*′-DDD **3** would have been generated as an intermediate, leading to the formation of **8** as a final product as was expected from Figure 4. However, neither **3** nor **8** was detected at all, i.e., **4** was not a precursor of **3**. It is, therefore, reasonable to think that the aliphatic chlorine atoms of **4** underwent the hydrodechlorination first to afford 1,1-di-*p*-chlorophenylethylene **16** (CAS Registry Number 2642-81-1),[36] subsequent quick hydrogenation of the ethylene moiety gave **2**, and stepwise dechlorination from the benzene rings of **2** produced **7**.

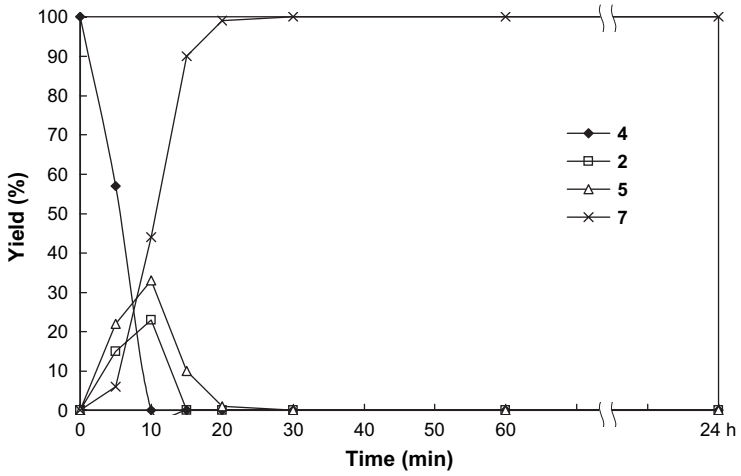
2.5. Reductive degradation pathway of *p*,*p*′-DDT using Pd/C–Et_3N–H_2 system

Considering the degradation reactions of *p*,*p*′-DDD **3** and *p*,*p*′-DDE **4**, the degradation pathway of *p*,*p*′-DDT **1** was proposed in Figure 6. In the initial stage of the reaction, the aliphatic moiety of **1** was subjected, in two different manners, to a dehydrochlorination to afford *p*,*p*′-DDE **4** and to a hydrodechlorination to afford *p*,*p*′-DDD **3** in the ratio of about 2:1, respectively. Two chlorine atoms attached with the alkene moiety of **4** were dechlorinated to generate **16** and subsequent hydrogenation of the alkene afforded **2**. Intermediates **2** and **3** underwent the successive dechlorination of their aromatic moieties to produce **7** and **8**, respectively. As we discussed above, **4** was not transformed to **3**. The dechlorination from the aliphatic moiety of **3** and **6** was so sluggish that no conversion from **3** to **2** nor from **6** to **5** was observed. After the complete dechlorination of the aromatic moieties of **3** and **6**, however, the resulting **8** could be transformed to **7** as shown in Figure 4, while the transformation of **8** to **7** were hardly observed in the reaction mixture starting from *p*,*p*′-DDT **1** (Fig. 1).

2.6. Dechlorination mechanism of trichloromethyl group of *p*,*p*′-DDT using Pd/C–Et_3N–H_2 system

The hydrogenolysis of *p*,*p*′-DDT **1** over Pd/C without Et_3N (Fig. 2) gave **2** and **3** in the ratio of about 2:1, respectively, in which the ratio of **2** and **3** was almost same as the one in the reaction of **1** with Et_3N (Fig. 1). These results indicate that *p*,*p*′-DDE **4**, which is a precursor of **2**, was not formed via a simple base (Et_3N) promoted dehydrochlorination of **1**.[45,46] The dechlorination of the trichloromethyl group of





Time	Yield (%)			
(min)	**4**	**2**	**5**	**7**
0	100	0	0	0
5	57	15	22	6
10	trace	23	33	44
15	0	trace	10	90
20	0	0	trace	~100
30	0	0	0	100
60	0	0	0	100
24 h	0	0	0	100

Figure 5. Catalytic degradation of *p*,*p*′-DDE (**4**) using Pd/C–Et_3N system under ambient hydrogen pressure and temperature.

1 in our system would involve a single electron transfer (SET) process (Fig. 7), as we previously proposed in the hydrodechlorination of aromatic chlorides using Pd/C–Et_3N system, where Et_3N worked as an electron donor as well as an HCl scavenger.

According to the reported molecular orbital (CNDO/2) study, 98% of the electron density in the LUMO of *p*,*p*′-DDT is localized in aliphatic carbon–chlorine σ antibonding orbital.[47] Initial single electron transfer from Pd(0) to this orbital of **1** affords a chloride anion and an alkyl radical **A** since the reductive cleavage of aliphatic halides does not afford anion radicals as discrete intermediates.[48] In the hydrodechlorination pathway, **A** abstracts hydrogen to form *p*,*p*′-DDD **3**, whereas in the dehydrodechlorination pathway, **A** undergoes an abstraction of the hydrogen at the benzylic position by the chloride radical to form *p*,*p*′-DDE **4**, which undergoes successive hydrodechlorinations and subsequent hydrogenation of the double bond to afford **2**.

In a polarographic study reported by Rosenthal et al., the half-wave potential ($E_{1/2}$) on the first reduction wave of **1** (for the reaction from **1** to **3**) and the potential $E_{1/2}$ on the first reduction wave of **3** [for the reaction from **3** to 2,2-bis(*p*-chlorophenyl)-1-chloroethane] were −0.93 V and −2.31 V (vs a saturated calomel electrode), respectively.[49] This study suggests that **3** cannot readily accept an electron compared with **1** and it is rationale to think that in our system the SET initiated dechlorination of aliphatic moiety of **3** did not take place until the dechlorinations of aromatic moiety completed.

3. Conclusion

In summary, the hydrodechlorination method using the Pd/C–Et_3N system accomplished the complete degradation of DDTs with the use of a catalytic amount of Pd/C under ambient pressure and temperature, generating much less

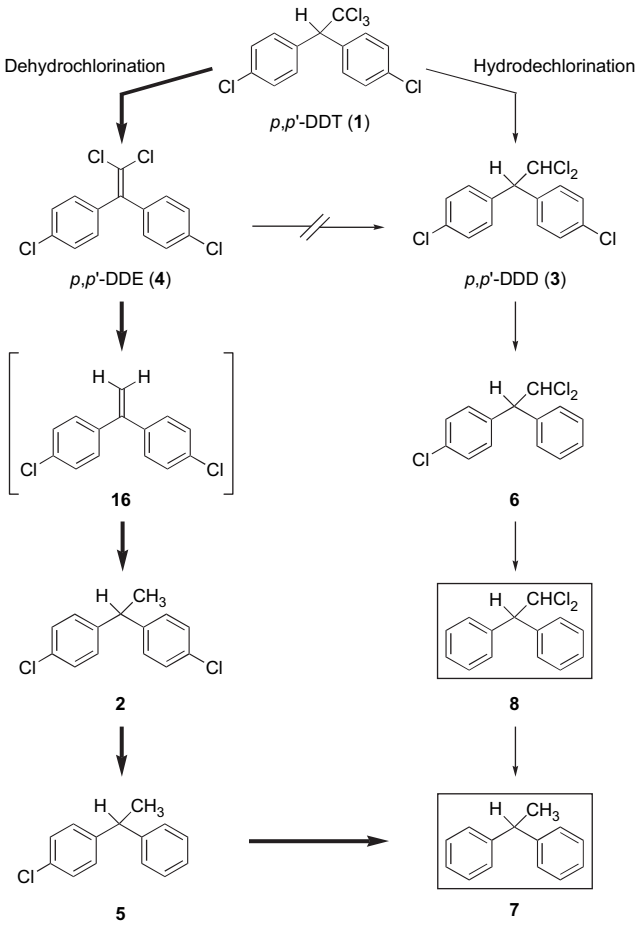


Figure 6. Proposed degradation pathway of *p*,*p*′-DDT under H_2 atmosphere using Pd/C–Et_3N system.

toxic 1,1-diphenylethane, 1,1-dichloro-2,2-diphenylethane, and triethylammonium chloride. The addition of Et_3N accelerated the dechlorination from the alkyl moiety as well as the phenyl moiety. All reagents and solvents used for the degradation reaction are commercially available and could be recovered and reused. The method is very simple, efficient, and does not require any expensive facilities. Further study to achieve the complete dechlorination of DDT under mild conditions is now ongoing in our laboratory.

4. Experimental

4.1. Chemicals

p,*p*′-DDT, *p*,*p*′-DDD [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane], *p*,*p*′-DDE, and *o*,*p*′-DDT were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan) and used without any purification prior to use. Pd/C (10%) and Et_3N were purchased from Sigma–Aldrich (St. Louis, MO) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. MeOH (analytical grade) was purchased from Kanto Chemical Co. Inc. and used without any purification prior to use.

4.2. General procedure

After two vaccum/H_2 cycles to remove air from a round-bottom flask, a suspension of *p*,*p*′-DDT (50 mg, 0.14 mmol), 10% Pd/C (5.0 mg), and Et_3N (86 mg, 0.85 mmol) in MeOH (10 mL) was vigorously stirred using a stir bar under hydrogen atmosphere (balloon) at ambient temperature (ca. 20 °C). At a given time point, the reaction mixture (1 mL) was sampled using a syringe, filtered through a 0.2 μL Millipore membrane filter (Millipore, Billerica, MA), and concentrated in vacuo. The residue

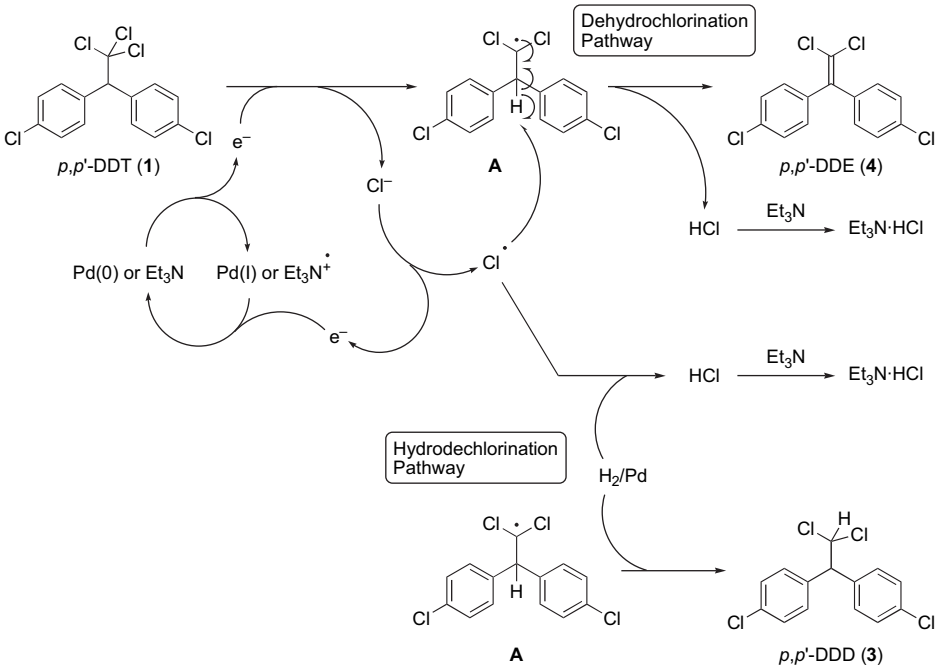


Figure 7. Tentative dechlorination mechanism of aliphatic moiety of *p*,*p*′-DDT.

was partitioned between Et_2O (10 mL) and H_2O (10 mL) and the organic layer was washed with brine (10 mL), dried ($MgSO_4$), and filtered. An aliquot (2 mL) was taken from the filtrate, diluted with Et_2O (18 mL), and analyzed by a Hewlett Packard 5891 series II gas chromatograph equipped with a Hewlett Packard 5972 mass-selective detector (Hewlett Packard, Palo Alto, CA) and a Neutrabond-5 capillary column (30 m×0.25 m, 0.4 μm film thickness; GL Science, Tokyo, Japan). Helium was employed as carrier gas with a flow rate of 1.0 mL/min. Injector and detector temperatures were 230 and 250 °C, respectively. The column temperature was programed to ramp from 150 °C (5 min hold) to 250 °C (3 min hold) at a rate of 5 °C/min. Retention time and molecular ion peak and/or fragment peak of each compound are as follows: **1**, 24.08 min, *m/z* 352 (M+, 0.6%), 235 (M−CCl_3, 100%); **2**, 16.10 min, *m/z* 250 (M+, 35%), 235 (M−CH_3, 100%); **3**, 22.75 min, *m/z* 318 (M+, 1.4%), 235 (M−$CHCl_2$, 100%); **4**, 21.19 min, *m/z* 316 (M+, 57%), 246 (M−Cl_2, 100%); **5**, 11.39 min, *m/z* 216 (M+, 39%), 201 (M−CH_3, 100%); **6**, 18.86 min, *m/z* 201 (M−$CHCl_2$, 100%); **7**, 6.88 min, *m/z* 182 (M+, 35%), 167 (M−CH_3, 100%); **8**, 14.68 min, *m/z* 178 (M−H_2Cl_2, 11%), 167 (M−CH_2Cl_2, 100%); **9**, 20.40 min, *m/z* 294 (M+, 12%), 235 (M−CO_2CH_3, 100%); **10**, 22.81 min, *m/z* 352 (M+, 0.9%), 235 (M−CCl_3, 100%); **11**, 14.98 min, *m/z* 250 (M+, 40%), 235 (M−CH_3, 100%); **12**, 21.45 min, *m/z* 318 (M+, 0.8%), 235 (M−$CHCl_2$, 100%); **13**, 10.45 min, *m/z* 216 (M+, 42%), 201 (M−CH_3, 100%); **14**, 17.62 min, *m/z* 201 (M−CH_2Cl_2, 100%); **15** 12.49 min, *m/z* 226 (M+, 11%), 167 (M−CO_2CH_3, 100%).

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An efficient synthesis of chiral terminal 1,2-diamines using an enantiomerically pure [1-(1′*R*)-methylbenzyl]aziridine-2-yl]methanol

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The authors dedicate this article to Professor Peter Beak on the occasion of his 70th birthday

Abstract—Enantiomerically pure terminal 1,2-diamines, which can serve as precursors for the synthesis of many biologically important compounds, were synthesized efficiently from a commercially available chiral [1-(1′*R*)-methylbenzyl]aziridine-2-yl]methanol. Various enantiomerically pure 2-vinylaziridines were prepared by Wittig reactions from aziridine-2-carboxaldehyde and the corresponding phosphonium salts. The C(2)–N bond of the vinyl substituted aziridine ring was regioselectively cleaved by azidotrimethylsilane ($TMSN_3$). The azido group and the double bond were reduced successively to give the target compounds in high yields.

1. Introduction

Compounds incorporating the terminal chiral 1,2-diamine functionality attract attentions from a variety of scientific areas. Previous research shows that some terminal 1,2-diamines and their derivatives have biological activities. For example, 1,2-diaminoplatinum complexes are considered as antitumor agents, biotins[1] have been used as protein immobilization agent in biosensor, and emeriamines[2] are used as inhibitors in fatty acid oxidation. These compounds also play important roles in organic synthesis and they are used as intermediates in the synthesis of heterocycles[3] or nitrogen containing macrocycles,[4] chiral ligands, and auxiliaries in catalytic asymmetric transformations.[5]

Although there has been a variety of applications, only a few preparative methods are available for the chiral terminal 1,2-diamines: from chiral alcohols,[6] alkylimines,[7] simple heterocycles,[8–10] 2-(sufonyloxy)nitriles,[11] nitrones,[12] aziridinium ion,[13] and ephedrine or pseudoephedrine.[14,15] However, each of the above methods has limited scope due to the lack of stereoselectivity and availability of enantiomerically pure starting materials. In addition, we need to consider different factors to establish generalized procedure for each application.[16] The requirement of more efficient preparative pathways to enantiomerically pure terminal 1,2-diamines prompted us to develop a simple and highly efficient new synthetic route.

In this report we described an efficient preparative route to enantiomerically pure terminal 1,2-diamines from commercially available enantiomerically pure 2-hydroxymethylaziridines.

2. Results and discussion

We previously reported the preparation and the application of enantiomerically pure aziridine-2-carboxaldehydes from commercially available aziridine-2-carboxylates.[17] Starting from the aldehydes (2*R*)-**1a–g**, a variety of 2-alkenyl aziridines (2*S*)-**2a–g** were prepared efficiently as a cis/trans mixture by Wittig reaction with the corresponding phosphonium salts in high yields. We also prepared the diastereomeric 2-alkenyl aziridines (2*R*)-**2h–l** using the same reaction conditions from (2*S*)-**1h–l** (Table 1; Scheme 1).

The aziridine ring C(2)–N bond is regioselectively cleaved by treating the 2-alkenyl aziridines ((2*S*)-**2a–g** and (2*R*)-**2h–l**) with 3 equiv of $TMSN_3$ in CH_2Cl_2 to provide 1-amino-2-azido-3-alkenes. Based on our previous results,

Keywords: 1,2-Diamine; 2-Vinylaziridine; Wittig reaction; Ring opening; Azidotrimethylsilane.
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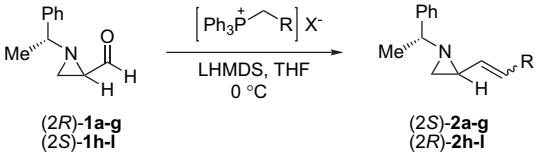
Table 1. Preparation of (2*S*)- and (2*R*)-2-alkenyl aziridines ((2*S*)-**2a–g** and (2*R*)-**2h–l**) from enantiomerically pure (2*R*)- and (2*S*)-aziridine-2-carboxaldehydes ((2*R*)-**1a–g** and (2*S*)-**1h–l**)

Entry	R	Yield (%)
2a	Phenyl	91
2b	2-Cl-Phenyl	94
2c	4-Biphenyl	89
2d	Propyl	92
2e	Nonyl	89
2f	1-Naphthyl	86
2g	Penta-F-phenyl	95
2h	Phenyl	89
2i	1-Naphthyl	87
2j	4-Cl-Phenyl	89
2k	Propyl	96
2l	Benzyl	90



Scheme 1.

the regiochemistry of Lewis acid or protic acid catalyzed nucleophilic ring opening reaction of *N*-α-methylbenzyl-2-alkyl aziridine is determined by steric requirement of the aziridine ring carbon and the ring opening reaction takes place at the less sterically hindered C-3.[18] However, in case of acyl or vinyl substituted aziridine, the ring opening reaction takes place at C-2 due to the assistance of the activating effect of the substituent (Fig. 1).

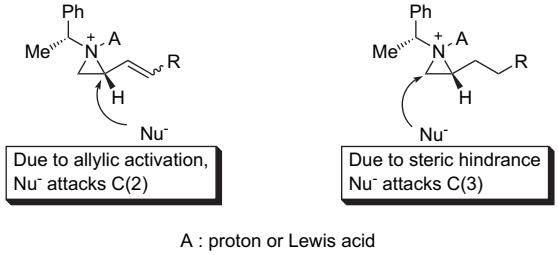
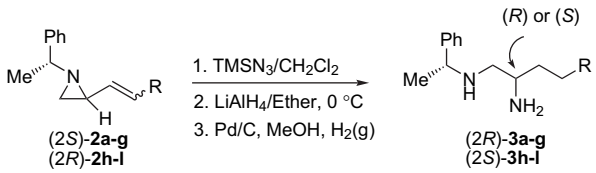


Figure 1. Regiochemistry in aziridine ring opening reactions.

The treatment of 1-amino-2-azido-3-alkenes with LAH in diethyl ether at 0 °C provided the corresponding 1,2-diamino-3-alkenes in high yields. It was unnecessary to separate the cis/trans mixture of the 1,2-diamino-3-alkenes since the double bond was saturated by catalytic hydrogenation in the presence of 20 wt % of Pd/C catalyst. The catalytic hydrogenation in MeOH was completed in 1 h at room temperature to provide the corresponding chiral terminal 1,2-diamines ((2*R*)-**3a–g** and (2*S*)-**3h–l**) and the results are summarized in Scheme 2. Therefore, the present



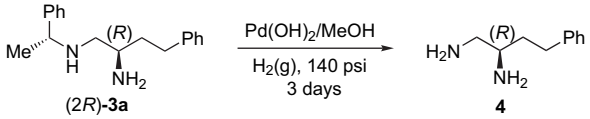
Scheme 2.

transformations show that the absolute configuration at C-2 of the final terminal 1,2-diamines is originated from that of C-2 position of the chiral aziridines and results are summarized in Table 2. The benzyl group on the nitrogen was successfully removed by catalytic hydrogenation in the presence of 20 wt % $Pd(OH)_2$ at 140 psi of H_2(g) to give the terminal 1,2-diamine **4** in high yields (Scheme 3).

Table 2. Preparation of (2*R*)- and (2*S*)-1,2-diaminoalkanes ((2*R*)-**3a–g** and (2*S*)-**3h–l**) from (2*S*)- and (2*R*)-2-alkenyl aziridines ((2*S*)-**2a–g** and (2*R*)-**2h–l**)

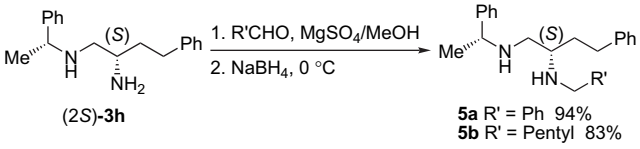
Entry	R	Yield (%)[a]
2a	Phenyl	80
2b	2-Cl-Phenyl	85
2c	4-Biphenyl	83
2d	Propyl	86
2e	Nonyl	80
2f	1-Naphthyl	79[b]
2g	Penta-F-phenyl	75[b]
2h	Phenyl	77
2i	1-Naphthyl	81
2j	4-Cl-Phenyl	80
2k	Propyl	78
2l	Benzyl	81

[a] Isolated yields.
[b] 2HCl salts.

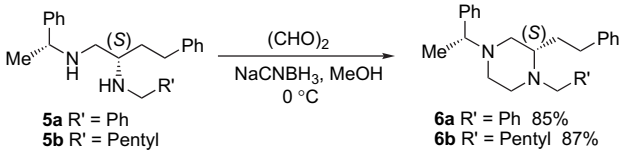


Scheme 3.

We have also applied the enantiomerically pure terminal 1,2-diamines for the preparation of orthogonally protected 2-substituted piperazine. The importance of substituted piperazines can be found in a wide variety of pharmacologically active compounds.[19–23] The reaction of terminal 1,2-diamine (2*S*)-**3h** with RCHO and $MgSO_4$ at room temperature generated the corresponding imine, which was then reduced with $NaBH_4$ at 0 °C to provide the 1-(1′*R*)-phenethyl-2-(*S*)-alkylamino-1,2-diamine **5** in high yields (Scheme 4). Reductive cyclization of **5** with 40% aqueous glyoxal solution in the presence of $NaCNBH_3$ as the reducing agent in MeOH at 0 °C proceeded smoothly and the desired 2-substituted-1,4-piperazine **6** was isolated in high yields (Scheme 5).



Scheme 4.



Scheme 5.

In summary, an operationally simple and high yielding four-step synthesis of 1-(*N*)-protected chiral terminal 1,2-diamine compounds has been developed from commercially available enantiomerically pure aziridine-2-methanols. The process includes regioselective ring opening of 2-vinyl substituted aziridines by azidotrimethylsilane followed by the sequential reduction of the azido group and the double bond. We also developed an efficient new synthetic route for the preparation of enantiomerically pure orthogonally protected 2-substituted-1,4-piperazines using N′-alkylation and intramolecular reductive cyclization.

3. Experimental

3.1. General methods

All reactions were carried out using standard Schlenk technique in an N_2 atmosphere. Solvents were dried by standard methods and distilled under N_2. Flash chromatography was performed with 230–400 mesh silica gel. Melting points were determined on a capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian Gemini 300 and 500 MHz spectrometers. NMR spectra were recorded in parts per million (δ) relative to the peak for tetramethylsilane (δ=0.00) as an internal standard unless stated otherwise and are reported as follows: chemical shift, multiplicity (br=broad, s=singlet, t=triplet, q=quartet, m=multiplet), coupling constant, and integration. Elemental analyses were performed by an elemental analyzer. Optical rotations were obtained on a digital polarimeter. Data are reported as follows: $[\alpha]_D^{24}$ (concentration (g/1000 mL), solvent). Solvents and liquid reagents were transferred using hypodermic syringes. All other reagents and solvents used were reagent grade. All glassware was dried in an oven at 150 °C prior to use. Small- and medium-scale purifications were performed using flash chromatography.

3.1.1. Preparation of the 4-phenyl-*N*-[(*R*)-(+)-α-methylbenzyl]butane-1,2(*R*)-diamine ((2*R*)-3a). To a solution of 1,2-diamino-3-alkene (120 mg, 0.43 mmol)[18a] in 1.42 mL of MeOH was added Pd/C (24 mg, 20 wt %). The reaction mixture was stirred at room temperature with 1 atm of H_2(g) for 1 h and then the catalyst was filtered and concentrated in vacuo. Purification by silica gel flash chromatography (CH_2Cl_2/MeOH 50:50) provided 109 mg (90%) of the product (2*R*)-**3a** as a yellow oil. $[\alpha]_D^{24}$ +123.5 (*c* 3.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32–7.16 (m, 10H), 3.73 (q, *J*=6.6 Hz, 1H), 2.81 (m, 1H), 2.68 (m, 1H), 2.55 (m, 2H), 2.19 (dd, *J*=11.5, 8.6 Hz, 1H), 1.66 (m, 1H), 1.53 (m, 1H), 1.34 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 146.2, 142.4, 128.7, 128.6, 128.5, 127.1, 126.8, 126.0, 59.0, 54.9, 51.3, 38.2, 32.8, 24.6; Anal. Calcd for $C_{18}H_{24}N_2$: C, 80.55; H, 9.01; N, 10.44. Found: C, 80.47; H, 9.12; N, 10.45.

Compound (2*R*)-**3b**: liquid, $[\alpha]_D^{24}$ +27.3 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.05 (m, 9H), 3.71 (q, *J*=6.5 Hz, 1H), 2.76–2.45 (m, 4H), 2.31 (td, *J*=8.4, 3.0 Hz, 1H), 1.69 (m, 1H), 1.55 (m, 1H), 1.34 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.2, 140.8, 129.9, 128.7, 128.6, 127.1, 126.8, 126.0, 59.0, 55.0, 51.2, 38.1, 32.1, 24.6; Anal. Calcd for $C_{18}H_{23}ClN_2$: C, 71.39; H, 7.66; N, 9.25. Found: C, 71.44; H, 7.75; N, 9.11.

Compound (2*R*)-**3c**: liquid, $[\alpha]_D^{24}$ +59.5 (*c* 2.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39–7.19 (m, 14H), 3.62 (q, *J*=6.5 Hz, 1H), 2.71–2.50 (m, 3H), 2.31 (dd, *J*=9.8, 5.9 Hz, 1H), 2.13 (m, 1H), 1.52 (m, 1H), 1.35 (m, 1H), 1.29 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 146.0, 141.9, 139.7, 130.2, 129.4, 129.3, 128.5, 128.3, 127.6, 127.0, 126.9, 126.7, 125.9, 58.8, 54.4, 51.1, 38.0, 29.7, 24.5; Anal. Calcd for $C_{24}H_{28}N_2$: C, 83.68; H, 8.19; N, 8.13. Found: C, 83.61; H, 8.06; N, 8.22.

Compound (2*R*)-**3d**: liquid, $[\alpha]_D^{24}$ +127.1 (*c* 3.1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.23 (m, 5H), 3.74 (q, *J*=6.5 Hz, 1H), 2.74 (m, 1H), 2.51 (qd, *J*=11.6, 3.4 Hz, 1H), 2.21 (qd, *J*=11.4, 2.9 Hz, 1H), 1.35 (d, *J*=6.5 Hz, 3H), 1.31–1.24 (m, 8H), 0.86 (t, *J*=6.3 Hz, 3H); ^{13}C NMR (755 MHz, $CDCl_3$) 146.2, 128.6, 127.1, 126.8, 126.9, 58.9, 54.8, 51.7, 36.5, 32.2, 26.0, 24.7, 22.8, 14.2; Anal. Calcd for $C_{15}H_{26}N_2$: C, 76.87; H, 11.18; N, 11.95. Found: C, 76.80; H, 11.10; N, 12.05.

Compound (2*R*)-**3e**: liquid, $[\alpha]_D^{24}$ −69.2 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.19 (m, 5H), 3.72 (q, *J*=6.5 Hz, 1H), 2.72 (m, 1H), 2.50 (qd, *J*=11.6, 3.5 Hz, 1H), 2.20 (qd, *J*=11.6, 2.5 Hz, 1H), 1.35 (d, *J*=6.5 Hz, 3H), 1.29–1.23 (m, 20H), 0.88 (t, *J*=6.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 146.2, 128.6, 127.0, 126.7, 58.9, 58.4, 55.0, 51.6, 36.7, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 26.3, 24.6, 22.9, 14.4; Anal. Calcd for $C_{21}H_{38}N_2$: C, 79.18; H, 12.02; N, 8.79. Found: C, 79.22; H, 12.32; N, 8.65.

Compound (2*R*)-**3h**: liquid, $[\alpha]_D^{24}$ +109.8 (*c* 1.3, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.19 (m, 10H), 3.72 (q, *J*=6.5 Hz, 1H), 2.78–2.47 (m, 4H), 2.30 (qd, *J*=11.6, 8.4 Hz, 1H), 1.74–1.46 (m, 1H), 1.34 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.3, 142.4, 128.7, 128.6, 128.5, 127.1, 126.8, 126.0, 59.0, 55.0, 51.3, 38.2, 32.8, 24.6; Anal. Calcd for $C_{18}H_{24}N_2$: C, 80.55; H, 9.01; N, 10.44. Found: C, 80.60; H, 9.13; N, 10.41.

Compound (2*R*)-**3i**: liquid, $[\alpha]_D^{24}$ +64.6 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.13 (m, 12H), 3.70 (q, *J*=6.5 Hz, 1H), 2.90–2.69 (m, 3H), 2.56 (qd, *J*=11.6, 3.5 Hz, 1H), 2.31 (qd, *J*=11.6, 8.4 Hz, 1H), 1.84–1.56 (m, 2H), 1.34 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 145.8, 139.8, 133.8, 132.1, 128.6, 128.1, 127.7, 127.5, 127.4, 127.0, 126.7, 126.4, 126.0, 125.3, 58.3, 54.2, 51.0, 37.9, 32.8, 24.7; HRMS (EI) calcd for $C_{22}H_{26}N_2$: 318.2096, found: 318.2099.

Compound (2*R*)-**3j**: liquid, $[\alpha]_D^{24}$ +53.0 (*c* 1.9, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.05 (m, 9H), 3.73 (q, *J*=6.5 Hz, 1H), 2.80 (m, 1H), 2.76–2.49 (m, 3H), 2.20 (qd, *J*=8.7, 3.7 Hz, 1H), 1.76–1.43 (m, 2H), 1.34 (d, *J*=6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 145.9, 140.8, 129.9, 128.7, 128.6, 127.2, 126.8, 126.0, 58.4, 54.3, 51.0, 38.0, 32.1, 24.8; Anal. Calcd for $C_{18}H_{23}ClN_2$: C, 71.39; H, 7.66; N, 9.25. Found: C, 71.46; H, 7.65; N, 9.14.

Compound (2*R*)-**3k**: liquid, $[\alpha]_D^{24}$ +387.0 (*c* 5.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.22 (m, 5H), 3.74

(q, J=6.5 Hz, 1H), 2.74 (m, 1H), 2.54 (dd, J=11.6, 3.4 Hz, 1H), 2.14 (dd, J=11.4, 8.8 Hz, 1H), 1.35 (d, J=6.5 Hz, 3H), 1.25–1.06 (m, 8H), 0.87 (t, J=5.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 145.8, 128.6, 127.0, 126.8, 58.5, 54.7, 51.6, 36.7, 32.2, 26.1, 24.9, 22.8, 14.3; Anal. Calcd for $C_{15}H_{26}N_2$: C, 76.87; H, 11.18; N, 11.95. Found: C, 76.90; H, 11.16; N, 12.01.

Compound (2*R*)-**3l**: liquid, $[\alpha]_D^{24}$ +54.5 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.22 (m, 10H), 3.72 (q, J=6.6 Hz, 1H), 2.78 (m, 1H), 2.59–2.42 (m, 3H), 2.14 (dd, J=11.6, 8.7 Hz, 1H), 1.72–1.40 (m, 2H), 1.33 (d, J= 6.6 Hz, 3H), 1.29–1.17 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.0, 142.6, 128.7, 128.6, 128.5, 127.1, 126.8, 126.0, 58.5, 54.4, 51.5, 36.2, 29.4, 28.3, 24.9; Anal. Calcd for $C_{15}H_{26}N_2$: C, 80.80; H, 9.28; N, 9.92. Found: C, 80.92; H, 9.51; N, 10.11.

3.1.2. Preparation of the 2HCl salt of 4-naphthyl-*N*-[(*R*)-(+)-α-methylbenzyl]butane-1,2(*R*)-diamine ((2*R*)-3f*). To a solution of 4-naphthyl-*N*-[(*R*)-(+)-α-methylbenzyl]-butane-1,2(*R*)-diamine (2*R*)-**3f** (90 mg, 0.28 mmol) in 1.40 mL of THF under nitrogen atmosphere was added concd HCl at room temperature. The mixture was stirred for 2 h at room temperature. After evaporation, Et_2O was added and the product was filtered and recrystallized from Et_2O to give 102 mg (92%) of (2*R*)-**3f*** as a white solid; mp 294–295 °C; $[\alpha]_D^{24}$ +14.8 (*c* 1.5, DMSO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88–7.82 (m, 3H), 7.71 (s, 1H), 7.64 (d, J=6.6 Hz, 1H), 7.51–7.38 (m, 6H), 4.41 (q, J=6.3 Hz, 1H), 3.59 (m, 1H), 3.37 (m, 1H), 3.02 (d, J=12.1 Hz, 1H), 2.80 (t, J=7.8 Hz, 1H), 2.01 (m, 1H), 1.64 (d, J=6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 138.1, 137.2, 133.1, 131.7, 129.0, 128.9, 127.9, 127.8, 127.5, 127.3, 127.2, 126.2, 126.1, 125.4, 58.3, 48.1, 47.0, 32.2, 30.6, 19.2; Anal. Calcd for $C_{22}H_{23}Cl_2N_2$: C, 67.51; H, 7.21; N, 7.16. Found: C, 67.54; H, 7.24; N, 7.08.

Compound (2*R*)-**3g***: mp 264–265 °C; $[\alpha]_D^{24}$ +28.8 (*c* 1.8, DMSO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.43 (m, 5H), 4.59 (q, J=6.6 Hz, 1H), 3.74 (m, 1H), 3.52–3.41 (m, 2H), 3.21 (d, J=4.3 Hz, 1H), 2.94 (t, J=8.1 Hz, 1H), 2.16–2.04 (m, 2H), 1.89 (d, J=6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 137.3, 131.1, 131.0, 130.7, 130.6, 129.2, 129.1, 127.8, 61.5, 50.3, 48.1, 32.7, 31.5, 19.2; Anal. Calcd for $C_{18}H_{21}F_5Cl_2N_2$: C, 50.13; H, 4.91; N, 6.50. Found: C, 50.20; H, 5.02; N, 6.37.

3.1.3. Preparation of the 4-phenyl-butane-1,2(*R*)-diamine (4). To a solution of 4-phenyl-*N*-[1(*R*)-(+)-α-methylbenzyl]butane-1,2(*R*)-diamine (2*R*)-**3a** (80 mg, 0.30 mmol) in 1.49 mL of MeOH under H_2(g) was added $Pd(OH)_2$ at room temperature. The mixture was stirred for 70 h under 120 psi of H_2(g) at room temperature, then the catalyst was filtered and washed with MeOH. The solvent was evaporated to give the product as yellow oil which was purified by silica gel flash chromatography with 50% CH_2Cl_2/MeOH to give 46 mg (93%) of **4**; $[\alpha]_D^{24}$ −4.2 (*c* 0.2, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.16 (m, 5H), 2.81–2.61 (m, 4H), 2.49 (dd, J=12.2, 7.4 Hz, 1H), 1.74 (m, 1H), 1.59 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 142.4, 128.7, 128.6, 126.1, 53.5, 49.0, 37.7, 32.8; Anal. Calcd for $C_{10}H_{16}N_2$: C, 73.13; H, 9.82; N, 17.06. Found: C, 73.22; H, 9.96; N, 16.98.

3.1.4. Preparation of the 2-*N*-benzyl-4-phenyl-*N*-(1(*R*)-(+)-α-methylbenzyl)butane-1,2(*S*)-diamine (5a). To a solution of 4-phenyl-*N*-[1(*R*)-α-methylbenzyl]butane-1,2(*S*)-diamine **3h** (100 mg, 0.37 mmol) in 1.86 mL of MeOH under nitrogen atmosphere was added benzaldehyde (0.08 mL, 0.75 mmol) at room temperature. To the mixture was added $MgSO_4$ (90 mg, 0.75 mmol) and stirred for 6 h. To the mixture was slowly added $NaBH_4$ (21 mg, 0.56 mmol) at 0 °C. The mixture was stirred for 30 min at 0 °C. The reaction was quenched with water at room temperature. The aqueous layer was extracted with CH_2Cl_2. The combined extract was dried over $MgSO_4$, and the solvent was evaporated to give the crude product, which was purified by silica gel flash chromatography with 30% EtOAc/hexane to give 125 mg (94%) of the product **5a** as a colorless oil; $[\alpha]_D^{24}$ +14.5 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.10 (m, 15H), 3.70 (q, J=13.1 Hz, 2H), 3.61 (q, J=6.5 Hz, 1H), 2.69–2.45 (m, 4H), 2.34 (dd, J=11.4, 7.4 Hz, 1H), 1.84–1.60 (m, 2H), 1.32 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 146.0, 142.6, 141.1, 128.7, 128.6, 128.5, 128.4, 128.3, 127.0, 126.9, 126.8, 125.9, 58.2, 56.2, 51.0, 50.4, 34.6, 32.4, 24.9; HRMS (EI) calcd for $C_{25}H_{30}N_2$: 358.2409, found: 358.2414.

Compound **5b**: liquid, $[\alpha]_D^{24}$ +6.9 (*c* 0.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31–7.12 (m, 10H), 3.72 (q, J= 6.5 Hz, 1H), 2.63–2.45 (m, 6H), 2.31 (dd, J=12.4, 8.5 Hz, 1H), 1.79–1.10 (m, 13H), 0.89 (t, J=6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 146.3, 142.8, 128.7, 128.6, 128.5, 127.0, 126.9, 125.9, 58.6, 57.5, 50.8, 47.1, 34.8, 32.7, 32.1, 30.8, 27.4, 24.9, 22.9, 14.3; Anal. Calcd for $C_{24}H_{36}N_2$: C, 81.76; H, 10.29; N, 7.95. Found: C, 81.90; H, 10.21; N, 8.05.

3.1.5. Preparation of the 1-*N*-benzyl-2(*R*)-phenethyl-*N*-(4(*R*)-(+)-α-methylbenzyl)piperazine (6a). To a solution of 2-*N*-benzyl-4-phenyl-*N*-(1(*R*)-(+)-α-methylbenzyl)-butane-1,2(*S*)-diamine **5a** (120 mg, 0.33 mmol) in MeOH (0.01 M, 33.5 mL) under nitrogen atmosphere was added glyoxal (0.05 mL, 0.47 mmol) and $NaCNBH_3$ (43.0 mg, 0.67 mmol) at 0 °C. The mixture was stirred for 14 h. The reaction was quenched with water at room temperature. The solvent was evaporated and washed with $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined extract was dried over $MgSO_4$, and the solvent was evaporated to give the crude product, which was purified by silica gel flash chromatography with 30% EtOAc/hexane to give 110 mg (85%) of the product **6a** as a yellow oil; $[\alpha]_D^{24}$ +14.5 (*c* 0.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.05 (m, 15H), 3.94 (d, J=13.3 Hz, 1H), 3.32 (q, J=6.6 Hz, 1H), 3.26 (d, J=13.2 Hz, 1H), 2.74–2.59 (m, 3H), 2.52–2.43 (m, 3H), 2.39–2.23 (m, 3H), 2.03–1.87 (m, 2H), 1.35 (q, J=6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 144.4, 142.8, 139.2, 129.1, 128.6, 128.5, 128.4, 128.3, 127.8, 127.0, 126.9, 125.9, 65.2, 59.4, 58.0, 54.8, 51.1, 50.4, 34.7, 32.3, 20.2; Anal. Calcd for $C_{27}H_{32}N_2$: C, 84.33; H, 8.39; N, 7.28. Found: C, 84.29; H, 8.24; N, 7.32.

Compound **6b**: liquid, $[\alpha]_D^{24}$ +39.6 (*c* 0.6, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.14 (m, 10H), 3.32 (q, J=6.5 Hz, 1H), 2.80–2.20 (m, 12H), 1.84 (d, J=6.8 Hz, 1H), 1.36 (d, J=6.6 Hz, 3H), 1.31–1.19 (m, 8H), 0.87 (q, J=6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 144.6, 142.9,

128.6, 128.5, 128.4, 127.9, 127.1, 126.0, 65.2, 64.9, 59.0, 54.9, 53.8, 50.7, 32.7, 32.1, 27.6, 26.4, 22.9, 20.3, 15.3, 14.3; Anal. Calcd for $C_{26}H_{38}N_2$: C, 82.48; H, 10.12; N, 7.40. Found: C, 82.29; H, 9.97; N, 7.34.

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Facile and selective synthesis of chloronicotinaldehydes by the Vilsmeier reaction

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Abstract—Eleven enamides were prepared by adopting different procedures. The various enamides prepared were subjected to Vilsmeier reaction using (i) $POCl_3$/DMF; (ii) diphosgene/DMF; (iii) triphosgene/DMF leading to the formation of various multisubstituted chloronicotinaldehydes. Studies carried out indicate that Vilsmeier reagent concentration and the replacement of $POCl_3$ by diphosgene or triphosgene, provides excellent selectivity and higher yields. Under modified reaction conditions one can get only chloronicotinaldehydes and not the chloropyridines as products. The various advantages in using diphosgene and triphosgene are illustrated. The mechanism of formation of chloronicotinaldehyde was discussed.

1. Introduction

Among heterocyclic compounds, pyridine and its derivatives are important compounds and are present in many biological systems.[1,2] Among the various applications of these pyridine derivatives, the pharmaceutical and agrochemical[3] applications are more important. Extensive studies have been carried out on the synthesis of pyridine compounds owing to their wide importance as drugs, biologically active natural products, and for other various applications. Introducing a formyl group into the aromatic ring system of pyridine is particularly significant, considering the lack of reactivity of pyridines toward electrophilic substitution reactions compared to benzenoids. The formyl group present on the pyridine ring opens up the possibility of carrying out a diverse range of functional group transformations. Furthermore, chloronicotinaldehydes are very good precursors for annulation of a wide variety of heterocyclic ring systems.

Vilsmeier reaction was initially used for the formylation of activated aromatic substrates and carbonyl compounds;[4] it is now used as a powerful synthetic tool for the construction of many heterocyclic compounds[5–10] such as quinolines, indoles, quinozolines, and pyridines. The synthesis of various substituted chloronicotinaldehydes using Vilsmeier reaction have been much less reported in the literature.[11,12] Meth-Cohn and Westwood reported the synthesis of 2-chloropyridines, pyridones, and quinolines using enamides under Vilsmeier reaction conditions.[12] This led us to conduct a systematic investigation on the feasibility of cyclization of enamides under Vilsmeier conditions to synthesize chloronicotinaldehydes. These chloronicotinaldehydes are very good precursors for the synthesis of arachidonic acid metabolite heterocyclic analogues 8-HETE.[13,14] Our continuing interest in the synthesis of heterocycles for biological activity[15] led us to report a facile and an efficient method for the synthesis of various substituted chloronicotinaldehydes (pyridine-3-carboxaldehyde) from enamides. Studies carried out on the formation of chloronicotinaldehydes in Vilsmeier reactions dramatically improved the selectivity and yield by using diphosgene/triphosgene compared to classical method of using $POCl_3$ in the formation of Vilsmeier reagent.

2. Results and discussion

Various enamides (**1**–**11**; Scheme 1) were prepared for the purpose of synthesizing various chloronicotinaldehydes (**12**–**22**; Scheme 1). Enamides **1**–**5** were synthesized by condensing appropriate aldehyde with benzylamine[16] to form initially a Schiff base and followed by acetylation[17] using acetic anhydride and triethylamine. Aldol condensation of benzaldehyde/*p*-methoxybenzaldehyde with acetone provided an α,β-unsaturated ketones, which were converted into the corresponding oximes and subsequent treatment using PCl_5[11] afforded enamides **6** and **7**. Enamide **8** was prepared by treating propiophenone oxime with Fe powder in the presence of acetic anhydride and acetic acid.[18] β-Keto ester was first converted into an enamine derivative,

Keywords: Enamides; Vilsmeier reaction; Diphosgene/triphosgene; Chloronicotinaldehydes; Selectivity; Mechanism.
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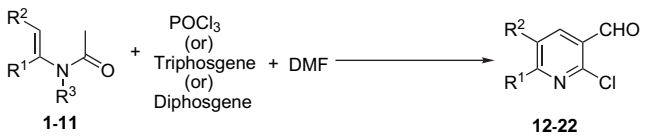
which was acetylated to give enamide[19] **9**. Serine and threonine methyl ester hydrochlorides[20] were prepared and then converted into the corresponding enamides[21,22] **10** and **11**.



Enamide

1: R^1 = H; R^2 = Me; R^3 = CH_2-Ph
2: R^1 = H; R^2 = Et; R^3 = CH_2-Ph
3: R^1 = H; R^2 = n-Pr; R^3 = CH_2-Ph
4: R^1 = H; R^2 = i-Pr; R^3 = CH_2-Ph
5: R^1 = H; R^2 = Pent; R^3 = CH_2-Ph
6: R^1 = H; R^2 = Ph; R^3 = H
7: R^1 = H; R^2 = p-$(OCH_3)C_6H_4$; R^3 = H
8: R^1 = Ph; R^2 = Me; R^3 = H
9: R^1 = Ph; R^2 = CO_2Et; R^3 = H
10: R^1 = CO_2Me; R^2 = H; R^3 = H
11: R^1 = CO_2Me; R^2 = CH_3; R^3 = H

Chloronicotinaldehyde

12: R^1 = H; R^2 = Me
13: R^1 = H; R^2 = Et
14: R^1 = H; R^2 = n-Pr
15: R^1 = H; R^2 = i-Pr
16: R^1 = H; R^2 = Pent
17: R^1 = H; R^2 = Ph
18: R^1 = H; R^2 = p-$(OCH_3)C_6H_4$
19: R^1 = Ph; R^2 = CH_3
20: R^1 = Ph; R^2 = CO_2Et
21: R^1 = CO_2Me; R^2 = H
22: R^1 = CO_2Me; R^2 = CH_3

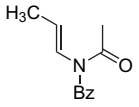
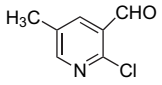
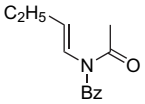
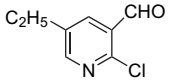
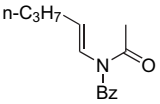
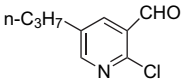
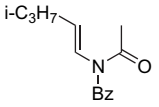
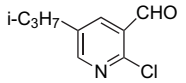
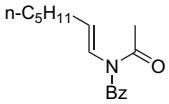
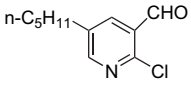
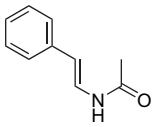
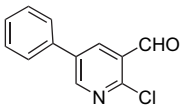
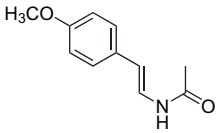
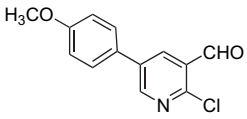
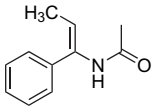
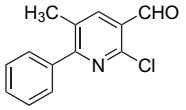
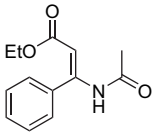
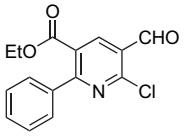
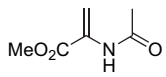
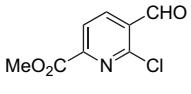
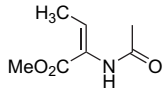
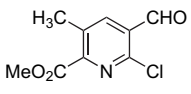
Scheme 1.

Scheme 1 illustrates the synthesis of various chloronicotinaldehydes (Table 1). A DMF solution of enamide was added to the excess Vilsmeier reagent (7 equiv) initially prepared from DMF and $POCl_3$/diphosgene/triphosgene. The reaction mixture was held at room temperature and then refluxed at ~75 °C. The crude product was extracted and purified by column chromatography (Table 1). Various chloronicotinaldehydes **12**–**22** were synthesized with very good yields by using enamides **1**–**11** that are listed in Table 1.

The results arranged in Table 2 show that the selectivity toward the formation of chloronicotinaldehyde improved upon increasing Vilsmeier reagent concentration. Enamide **1** was used for the studies as a representative example to understand selectivity and yield improvements. Upon using 2.5 equiv of Vilsmeier reagent (entry 1; Table 2) the yield of chloronicotinaldehyde **12** was less and 2-chloro-5-methylpyridine **23** was the major product (Scheme 2). Using excess of Vilsmeier reagent (7 equiv entry 5; Table 2), chloronicotinaldehyde **12** was obtained as the major product with a trace amount of 2-chloro-5-methylpyridine **23** (Scheme 2). Replacing $POCl_3$ by triphosgene (entries 9 and 10; Table 2) provided the same selectivity in the formation of chloronicotinaldehyde but with higher yields (Scheme 2). The diphosgene (liquid) or triphosgene (solid) are employed because they are safe substitutes for the toxic phosgene gas, offer mild reaction conditions, provide excellent yields, and avoid the formation of inorganic phosphorus salts. The same procedure was extended to other enamides **2**–**11** and the corresponding chloronicotinaldehydes **13**–**22** were synthesized in higher yields (Table 1).

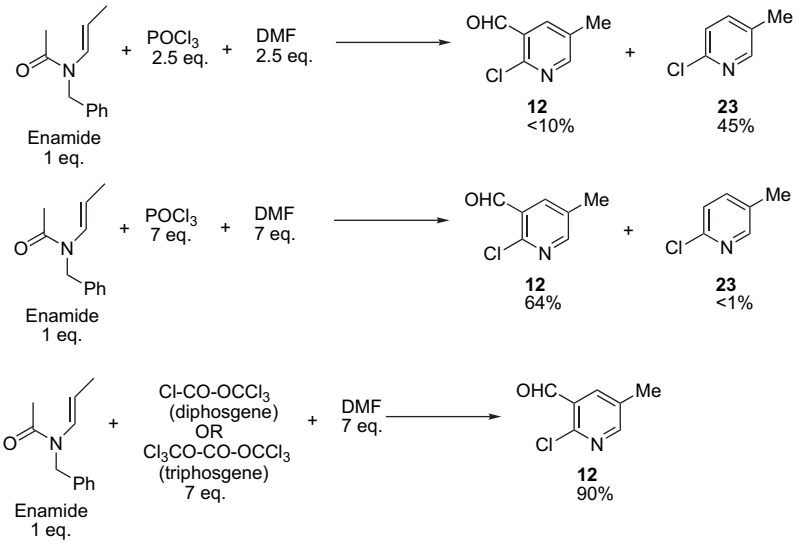
A mechanism is proposed to explain the formation of chloronicotinaldehydes with selectivity (Scheme 3). The enamide initially reacts with Vilsmeier reagent to form bis-enamine having a chloro group (Scheme 3). The net result is the formation of a more nucleophilic bis-enamine. The bis-enamine undergoes formylation to produce two possible mono-formylated bis-enamines. The mono-formylated bis-enamine can undergo cyclization to give 2-chloro-5-methyl pyridine[23] (**23**) or the mono-formylated bis-enamine can undergo second formylation before undergoing cyclization. The second formylation process is quite possible at higher concentrations

Table 1. Synthesis of substituted chloronicotinaldehydes

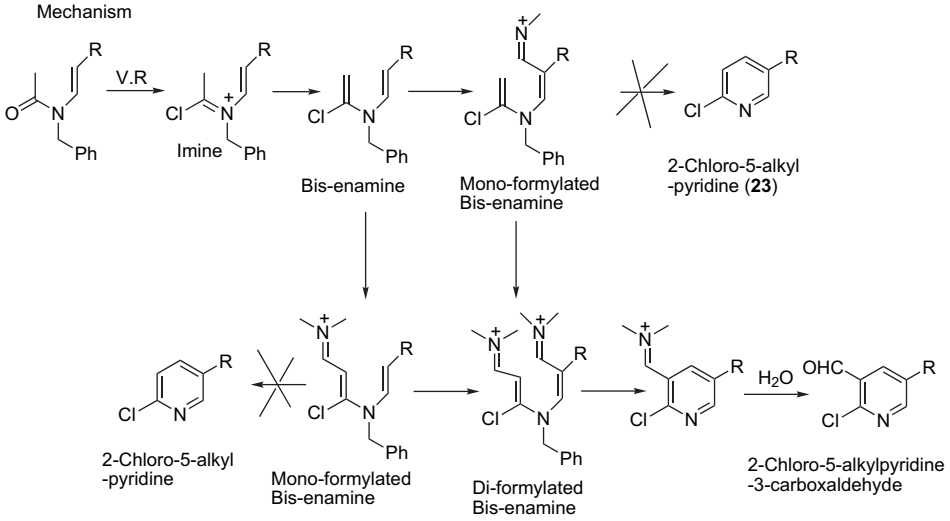
S.No.	Enamides (**1**–**11**)	Chloronicotinaldehydes (**12**–**22**)	Yields[a] (%)
1			94
2			92
3			92
4			91
5			90
6			92
7			92
8			90
9			88
10			90
11			90

[a] Isolated yields using diphosgene/triphosgene.

of Vilsmeier reagent and the same was observed (Table 2; Scheme 2). The di-formylated bis-enamine undergoes cyclization to give the substituted chloronicotinaldehyde (pyridine-3-carboxaldehyde), which is the main product. The products isolated were substituted chloronicotinaldehyde and benzyl chloride (in the case of enamides **1**–**5**). The dimethylamine remains in aqueous phase as amine hydrochloride after work up. These observations clearly indicate that under modified Vilsmeier reaction conditions (i.e., at higher Vilsmeier reagent concentrations) the enamide undergoes



Scheme 2.



Scheme 3.

Table 2. Studies to improve the formation of chloronicotinaldehydes

No.	DMF (mol equiv)	$POCl_3$/DP/TP (mol equiv)	Temperature (°C)	Time (h)	Yield (%)	
					12	**23**
1	2.5	2.5	75	5	<10	45
2	4.0	4.0	75	5	24	25
3	5.0	5.0	75	5	36	12
4	6.0	6.0	75	5	54	6
5	7.0	7.0	75	5	64	<1
6	8.0	8.0	75	5	64	<1
7	8.0	8.0	90	5	64	<1
8	8.0	8.0	90	8	64	<1
9	2.5	2.5	75	5	10	45
10	7.0	7.0	75	5	90	<1

DP = diphosgene; TP = triphosgene. Enamide used is **1**; isolated yields; addition of enamide to Vilsmeier reagent (DMF–$POCl_3$/DP/TP) was carried out at ~0 °C; mol equiv with respect to enamide.
For entries 1–8 $POCl_3$ was used and for 9 and 10 diphosgene/triphosgene was used.

di-formylation before undergoing cyclization to give selectively chloronicotinaldehyde.

3. Conclusions

Various enamides were prepared to synthesize substituted chloronicotinaldehydes. The selectivity toward the formation of chloronicotinaldehydes was achieved at higher Vilsmeier reagent concentration. The replacement of $POCl_3$ by diphosgene/triphosgene provides higher yields and avoids formation of inorganic phosphorus salts. A mechanism is proposed to explain the selectivity.

4. Experimental

4.1. General procedure for the preparation of aldimines

In the synthesis of enamides **1–5**, the aldimines were prepared by condensation of appropriate aldehyde and benzylamine.[16]

Benzylamine (5 g, 46 mmol) was added to a 50 mL round bottom flask fitted with magnetic stirrer and dropping funnel. Propionaldehyde (2.71 g, 46 mmol) was added gradually over a period of 1 h at 0 °C, with constant stirring. Potassium hydroxide flakes (1.3 g, 23 mmol) were added to the reaction mixture at 0 °C and the mixture was allowed to stand until separation into two layers appeared complete at 0 °C. The organic layer was then removed and allowed to stand over potassium hydroxide pellets in the refrigerator for over night. The same procedure was adopted for making other aldimines (Schiff bases).

4.2. Preparation of various enamides

4.2.1. General procedure for the preparation of *N*-alkenyl-*N*-benzyl acetamides (1–5).[17] Acetic anhydride (4.163 g, 40 mmol) was added to a stirred solution of *N*-benzyl propionaldimine (6 g, 40 mmol) and triethylamine (4.12 g, 40 mmol) at 0–5 °C for about 30 min. The reaction mixture was allowed to reach room temperature. Distillation under reduced pressure yields *N*-benzyl-*N*-[(1*E*)-prop-1-en-1-yl]-acetamide (**1**) in 88% yield. The same procedure was adopted in making other enamides (**2–5**).

4.2.2. Preparation of *N*1-[(*E*)-2-phenyl-1-ethenyl]acetamide (6) and *N*1-[(*E*)-2-(4-methoxyphenyl)-1-ethenyl]-acetamide (7).[11] Phosphorus pentachloride (3.1 g, 15 mmol) was added to 4-phenyl-3-butene-2-one-oxime (5.0 g, 30 mmol) in dry THF (50 mL) at 0 °C, and the mixture was shaken for 30 min. The mixture was then poured slowly into a vigorously stirred mixture of crushed ice (50 g) and 40% aqueous potassium carbonate (50 mL), the pH being maintained at 7 (pH paper) by the addition of solid potassium carbonate. The THF layer was then removed and the resultant solid was collected by filtration, and washed twice with light petroleum. TLC (7:3 light petroleum–ethyl acetate) showed the presence of *E* (R_f 0.40) and *Z* (R_f 0.52) isomers of *N*1-[2-phenyl-1-ethenyl]acetamide (**6**) and these two compounds were characterized by ^{1}H NMR.

The same is carried out for the synthesis of *N*1-[2-(4-methoxyphenyl)-1-ethenyl]acetamide (**7**) by using 4-[(*p*-methoxy)-phenyl]-3-butene-2-one-oxime.

4.2.3. Preparation of *N*1-[(*E*)-1-phenyl-1-propenyl]acetamide (8).[18] 1-Phenyl-1-propanone oxime (5 g, 33 mmol) was heated in acetic anhydride (3.3 g, 33 mmol) and acetic acid (10 mL, 3 mol equiv) in the presence of iron powder (1 g) for 4 h at 100 °C. The mixture was then filtered, extracted with dichloromethane, and the organic layer was washed with water and aqueous K_2CO_3, dried, and evaporated.

4.2.4. Preparation of ethyl (*E*)-3-(acetylamino)-3-phenyl-2-propenoate (9).[19] A solution of β-keto ester, ethyl benzoylacetate (2.3 g, 12 mmol), and NH_4OAc (4.6 g, 60 mmol) in MeOH (15 mL) was stirred at room temperature for 3 days. After the solvent was evaporated under reduced pressure, the residue was diluted with $CHCl_3$ (30 mL). The resulting solid was filtered off and washed with $CHCl_3$ (2×30 mL). The combined filtrate was washed with water and brine, and dried over sodium sulfate. Evaporation of the solvent gave 3-amino-2-alkenoate, which was used for the next step without purification.

To a solution of 3-amino-2-alkenoate in THF (12 mL) were added pyridine (2 mL) and acetic anhydride (6 mL). The reaction mixture was then heated under reflux for 24 h. Reaction mixture was rotary evaporated to remove solvent and triethylamine. The residue was dissolved in EtOAc (20 mL) and the solution was washed with water (10 mL), 1 N HCl (10 mL), 1 M KH_2PO_4 (10 mL), $NaHCO_3$ (saturated, 10 mL), and brine (15 mL). After the solution was dried over sodium sulfate, the solvent was evaporated under reduced pressure. Chromatography of the residue on silica gel with a solvent gradient of EtOAc in hexane (15–70%) as eluant gave compound **9**.

4.2.5. Preparation of methyl 2-(acetylamino)acrylate (10) and methyl (*E*)-2-(acetylamino)-2-butenoate (11).[20,21] Triethylamine (30 mL) was added to methyl ester of L-serine hydrochloride (5 g, 27.9 mmol) at 0 °C. After 10 min stirring acetic anhydride (7.0 g, 70 mmol) was added drop wise. The reaction mixture was allowed to stir at room temperature over night. The reaction mixture was washed with 10% sodium bicarbonate solution and extracted with dichloromethane solvent. The obtained methyl 2-(acetylamino)acrylate (**10**) was characterized with ^{1}H NMR and Mass spectroscopies. The same procedure using methyl ester of L-threonine afforded methyl (*E*)-2-(acetylamino)-2-butenoate (**11**).

4.3. Synthesis of substituted chloronicotinaldehydes

4.3.1. General procedure for the synthesis of substituted 2-chloronicotinaldehydes (substituted 2-chloro-pyridine-3-carboxaldehydes) using $POCl_3$ (12–22). *N*,*N*-Dimethylformamide (13.5 g, 185 mmol) was added to a well stirred and cooled solution of phosphorus oxychloride (28.3 g, 185 mmol) at 0 °C for 30 min and followed by *N*-benzyl-*N*-[(1*E*)-prop-1-en-1-yl]acetamide (**1**) (5 g, 26 mmol) at the same temperature. The ice bath was removed and the mixture was further stirred for 2 h at room temperature. The reaction mixture was heated to 75 °C for 5 h. The orange-yellow colored organic mass was poured into crushed ice (200 g) with stirring. The mass was extracted with methylene chloride (2×200 mL) and the layers were separated. The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. Thus obtained residue was subjected to column chromatography purification on silica gel to give 2-chloro-5-methylnicotinaldehyde (2-chloro-5-methylpyridine-3-carboxaldehyde) (**12**) in 64% yield. The same procedure was adopted in making other chloronicotinaldehydes (**13–22**).

4.3.2. General procedure for the synthesis of substituted 2-chloronicotinaldehydes (substituted 2-chloro-pyridine-3-carboxaldehydes) using triphosgene or diphosgene (12–22). *N*,*N*-Dimethylformamide (13.5 g, 185 mmol) was added to a cooled solution of triphosgene (28.3 g, 185 mmol), in the case of diphosgene (36.6 g, 185 mmol) at 0–10 °C for 30 min and followed by *N*-benzyl-*N*-[(1*E*)-prop-1-en-1-yl]acetamide (**1**) (5 g, 26 mmol) at the same temperature. The ice bath was removed and the mixture was further stirred for 2 h at room temperature. The reaction mixture was heated to 75 °C for 5 h. The orange-yellow colored organic mass was poured into ice cold water (200 g) with stirring. The mass was extracted with methylene chloride

(2×200 mL). The organic layer was dried over sodium sulfate and the solvent was removed under reduced pressure. Thus obtained residue was subjected to column chromatography purification on silica gel to give 2-chloro-5-methylnicotinaldehyde (**12**) in 92% yield. The same procedure was adopted for the preparation of other chloronicotinaldehydes (**13**–**22**).

4.3.2.1. 2-Chloro-5-ethylnicotinaldehyde (13). White solid. Mp 67–69 °C; δ_H (200 MHz, $CDCl_3$) 1.35 (t, 3H, *J* 7.56 Hz, *Me*), 2.75 (m, 2H, CH_2), 8.02 (s, 1H, hetero aromatic), 8.42 (s, 1H, hetero aromatic), 10.4 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 188.6, 153.2, 150.2, 138.8, 136.3, 127.6, 24.6, 14.2; EIMS (*m*/*z*) 169 (M^+) (100), 153 (55), 140 (27), 132 (56), 105 (31), 90 (22), 77 (50), 63 (30), 51 (43); IR (KBr) ν 2362, 1696, 1559, 1426, 1374, 1275, 1220, 1058, 746 cm^{-1}; HRMS (EI^+), exact mass calcd for C_8H_8NOCl: 169.0292. Found: 169.0292.

4.3.2.2. 2-Chloro-5-propylnicotinaldehyde (14). White solid. Mp 34–36 °C; δ_H (200 MHz, $CDCl_3$) 0.9 (t, 3H, *J* 7.56 Hz, *Me*), 1.7 (m, 2H, CH_2), 2.64 (t, 2H, *J* 7.52 Hz, CH_2), 8.0 (s, 1H, hetero aromatic), 8.38 (s, 1H, hetero aromatic), 10.4 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 189.2, 154.0, 150.7, 137.7, 137.2, 127.9, 33.7, 23.6, 13.2; EIMS (*m*/*z*) 183 (M^+) (57), 154 (100), 147 (15), 99 (15), 90 (20), 40 (36); IR (KBr) ν 3376, 2960, 2871, 2364, 1695, 1587, 1432, 1378, 768 cm^{-1}; HRMS (EI^+), exact mass calcd for $C_9H_{10}NOCl$: 183.0450. Found: 183.0467.

4.3.2.3. 2-Chloro-5-isopropylnicotinaldehyde (15). White solid. Mp 38–39 °C; δ_H (200 MHz, $CDCl_3$) 1.2–1.4 (m, 6H, 2*Me*), 3.0 (m, 1H, *CH*), 8.04 (s, 1H, hetero aromatic), 8.44 (s, 1H, hetero aromatic), 10.4 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 189.1, 152.7, 150.7, 143.7, 135.3, 128.1, 30.9, 23.1; EIMS (*m*/*z*) 183 (M^+) (44), 168 (100), 104 (40), 77 (40), 51 (24); IR (KBr) ν 2966, 2873, 2361, 1695, 1586, 1432, 1378, 1281, 1090, 959, 771, 607 cm^{-1}; HRMS (EI^+), exact mass calcd for $C_9H_{10}NOCl$: 183.0450. Found: 183.0448.

4.3.2.4. 2-Chloro-5-pentylnicotinaldehyde (16). White solid. Mp 44–46 °C; δ_H (200 MHz, $CDCl_3$) 0.9 (t, 3H, *J* 7.56 Hz, *Me*), 1.38 (m, 4H, 2CH_2), 1.70 (m, 2H, CH_2), 2.7 (t, 2H, *J* 7.52 Hz, CH_2), 8.0 (s, 1H, hetero aromatic), 8.44 (s, 1H, hetero aromatic), 10.4 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 189.2, 153.9, 150.7, 138.0, 137.2, 128.0, 31.8, 30.9, 30.2, 22.1, 13.7; EIMS (*m*/*z*) 211 (M^+) (40), 168 (75), 155 (100), 141 (27), 126 (8), 119 (15), 103 (21), 91 (47); IR (KBr) ν 2930, 2864, 1696, 1588, 1431, 1337, 1281, 1154, 1071 cm^{-1}; HRMS (EI^+), exact mass calcd for $C_{11}H_{14}NOCl$: 211.0763. Found: 211.0767.

4.3.2.5. 2-Chloro-5-methyl-6-phenylnicotinaldehyde (19). Light yellow solid. Mp 67–69 °C; δ_H (200 MHz, $CDCl_3$) 2.42 (s, 3H, *Me*), 7.39–7.61 (m, 5H), 8.04 (s, 1H, hetero aromatic), 10.42 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 189.2, 163.1, 149.9, 140.1, 137.8, 130.8, 129.1, 128.8, 128.1, 126.6, 19.2; EIMS (*m*/*z*) 231 (M^+) (50), 230 (100), 166 (20), 139 (12), 115 (10), 77 (9); IR (KBr) ν 2442, 1690, 1540, 1420, 1370, 1265, 1224, 1048, 746 cm^{-1}. Anal. Calcd for $C_{13}H_{10}ClNO$: C, 67.39; H, 4.35; N, 6.05. Found: C, 67.46; H, 4.50; N, 6.11.

4.3.2.6. 2-Chloro-5-(ethoxyacetate)-6-phenylnicotinaldehyde (20). Light yellow solid. Mp 72–73 °C; δ_H (200 MHz, $CDCl_3$) 1.12 (t, 3H, *J* 7.26 Hz, *Me*), 4.20 (q, 2H, *J* 7.26 Hz, OCH_2), 7.25–7.35 (m, 5H, aromatic), 9.05 (s, 1H, hetero aromatic), 10.55 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 188, 165, 144, 143, 138, 131, 129, 128, 123, 122, 116, 57, 14; EIMS (*m*/*z*) 289 (M^+) (14), 260 (100), 244 (34), 216 (13), 153 (14), 126 (12), 77 (7); IR (KBr) ν 1736, 1560, 1450, 1369, 1310, 1261, 1189, 735 cm^{-1}. Anal. Calcd for $C_{15}H_{12}ClNO_3$: C, 62.18; H, 4.17; N, 4.83. Found: C, 62.21; H, 4.21; N, 4.88.

4.3.2.7. Methyl 6-chloro-5-formyl-2-pyridincarboxylate (21). White solid. Mp 70–72 °C; δ_H (200 MHz, $CDCl_3$) 4.0 (s, 3H, OCH_3), 8.2 (d, *J* 3.2 Hz, 1H, hetero aromatic), 8.4 (d, *J* 3.2 Hz, 1H, hetero aromatic), 10.5 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 188, 163, 153, 151, 139, 131, 124, 53; EIMS (*m*/*z*) 199 (M^+) (6), 169 (23), 141 (100), 112 (23), 76 (50), 59 (29); IR (KBr) ν 3433, 1730, 1554, 1445, 1359, 1314, 1251, 1199, 1137, 1063, 956, 874, 813, 735 cm^{-1}. Anal. Calcd for $C_8H_6ClNO_3$: C, 48.14; H, 3.02; N, 7.01. Found: C, 48.21; H, 3.21; N, 7.08.

4.3.2.8. Methyl 6-chloro-5-formyl-3-methyl-2-pyridincarboxylate (22). White solid. Mp 43–45 °C; δ_H (200 MHz, $CDCl_3$) 2.60 (s, 3H, *Me*), 4.0 (s, 3H, OCH_3), 8.1 (s, 1H, hetero aromatic), 10.45 (s, 1H, C*H*O); δ_C (50 MHz, $CDCl_3$) 188, 164, 152, 148, 142, 135, 129, 53, 18; EIMS (*m*/*z*) 213 (M^+) (44), 181 (54), 155 (52), 149 (75), 97 (29), 71 (56), 43 (100); IR (KBr) ν 1730, 1559, 1440, 1359, 1319, 1069, 966, 874, 755 cm^{-1}. Anal. Calcd for $C_9H_8ClNO_3$: C, 50.60; H, 3.77; N, 6.55. Found: C, 50.62; H, 3.81; N, 6.58.

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Enantioselective synthesis of (*S*)-2-amino-3-phosphonopropionic acid, (*S*)-AP-3, and (*R*)-2-amino-4-phosphonobutanoic acid, (*R*)-AP-4, via diastereoselective azidation of (4*R*,5*R*)-*trans*-*N*-[(diethoxyphosphoryl)propionyl]- and (4*R*,5*R*)-*trans*-*N*-[(diethoxyphosphoryl)butanoyl]hexahydrobenzoxazolidin-2-one

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Abstract—N-Acylation of readily available, enantiopure oxazolidinone (4*R*,5*R*)-**1b** with (diethoxyphosphoryl)propionyl chloride and (diethoxyphosphoryl)butanoyl chloride affords de title substrates (4*R*,5*R*)-**2** and (4*R*,5*R*)-**7**, respectively, which are azidated with high diastereoselectivity by means of the reaction between their sodium enolates (4*R*,5*R*)-**2**-Na and (4*R*,5*R*)-**7**-Na with trisyl azide. Removal of the chiral auxiliary from azidated products (4*R*,5*R*,2′*S*)-**3** and (4*R*,5*R*,2′*R*)-**8** followed by hydrogenation and hydrolysis of the resulting carboxylic acids (*S*)-**4** and (*R*)-**9** gave the pharmacologically relevant aminophosphonic acids (*S*)-AP-3 and (*R*)-AP-4 in good yield.

1. Introduction

Excitatory amino acids (EAA) are the most common neurotransmitters in the mammalian central nervous system (CNS).[1] Thus, EAA receptors offer an opportunity to develop therapeutic compounds for the treatment of several pathological conditions affecting the brain, such as Parkinson's and Alzheimer's diseases.[2,3] In this context, several studies have shown that phosphonic analogues of the aminodicarboxylic acids, glutamic acid, and their higher homologues, are modulators for the *N*-methyl-D-aspartate (NMDA) receptor site. Indeed, aminophosphonic acids AP-3, AP-4, AP-5, and AP-6 (Fig. 1) have demonstrated to be particularly potent.

The biological activity of these compounds has been shown to depend markedly on their stereochemical configuration. For example, the (*S*)-enantiomer of 2-amino-4-phosphonobutanoic acid AP-4 is 40 times more active than the (*R*)-enantiomer in the suppression of glutamate-mediated neurotransmission.[4] As a consequence, the asymmetric synthesis of aminophosphonic acids has been intensely pursued in recent years.[5]

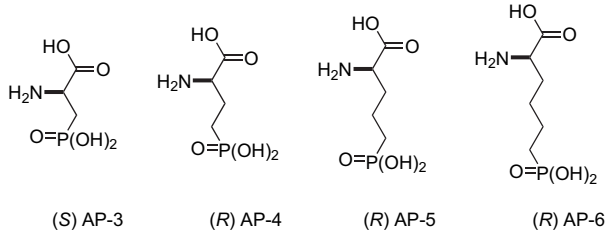

Figure 1.

Some years ago, we reported a convenient procedure for the preparation of both pairs of enantiomeric hexahydrobenzoxazolidin-2-ones **1a–d** from inexpensive cyclohexene oxide and (*S*)-α-phenylethylamine[6,7] (Fig. 2). We have also described the use of **1a–d** as effective chiral auxiliaries for the stereoselective alkylation, acylation, and aldol condensation of propionic and hydrocinnamic acids.[8] More recently, the application of oxazolidinones **1a–d** as chiral sulfinyl transfer reagents was also reported.[9]

Keywords: Chiral oxazolidinones; Aminophosphonic acids; Diastereoselective electrophilic amination; Enantioselective synthesis.
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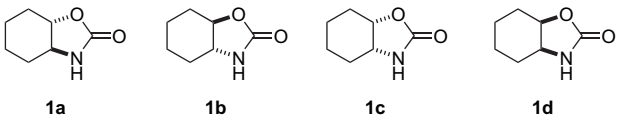


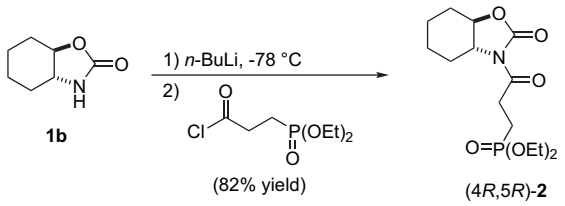
Figure 2.

We would like to communicate the use of *trans*-oxazolidinone (4*R*,5*R*)-**1b** for the enantioselective synthesis of (*S*)-AP-3 and (*R*)-AP-4.[10]

2. Results and discussion

2.1. Preparation of (4*R*,5*R*)-*trans*-*N*-[(diethoxyphosphoryl)propionyl]hexahydrobenzoxazolidin-2-one, (4*R*,5*R*)-2

trans-Hexahydrobenzoxazolidinone **1b** was N-acylated following the established protocol,[11] by treatment with *n*-butyllithium at −78 °C, followed by addition (−78 °C) of (diethoxyphosphoryl)propionyl chloride[12] to generate the *N*-propionyl derivative **2** (82% yield) (Scheme 1).

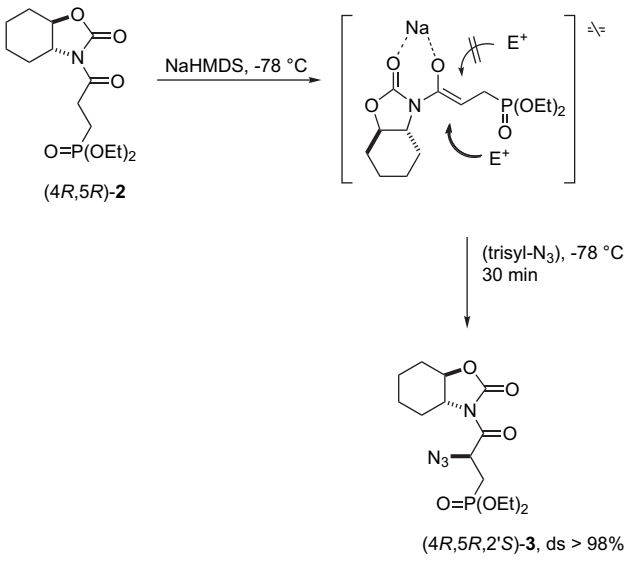


Scheme 1.

2.2. Diastereoselective azidation of (4*R*,5*R*)-2

From the various reagents available for the electrophilic amination of carbanionic substrates,[13] we chose to effect direct azide transfer to the sodium enolate of phosphorylated propionyl substrate (4*R*,5*R*)-**2**. Thus, the sodium enolate derived from oxazolidinone (4*R*,5*R*)-**2**, generated with sodium hexamethyldisilazide (NaHMDS), was treated with 1.1 equiv of 2,4,6-triisopropylbenzylsulfonyl azide (trisyl-N_3) at −78 °C for 30 min[14] (Scheme 2). Most gratifyingly, ^{1}H NMR analysis of the crude azidated product **3** showed a single set of signals, indicating a diastereomeric purity≥98%.

The absolute configuration of the newly created stereogenic center at C(2′) in product **3** was established to be (*S*) by means of chemical correlation with 2-amino-3-phosphonopropionic acid (AP-3) as discussed in Section 2.3. This result is consistent with the intermediacy of a (*Z*)-configured enolate,[15] where the sodium cation is chelated by both carbonyl



Scheme 2.

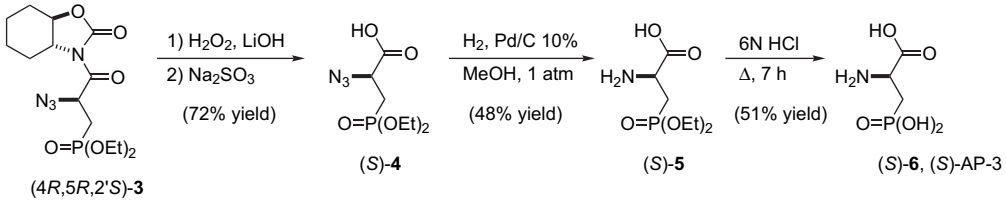
oxygens and the electrophile is incorporated from the less sterically hindered face of the enolate (Scheme 2).

2.3. Removal of the chiral auxiliary and hydrogenation/hydrolysis to give (*S*)-AP-3

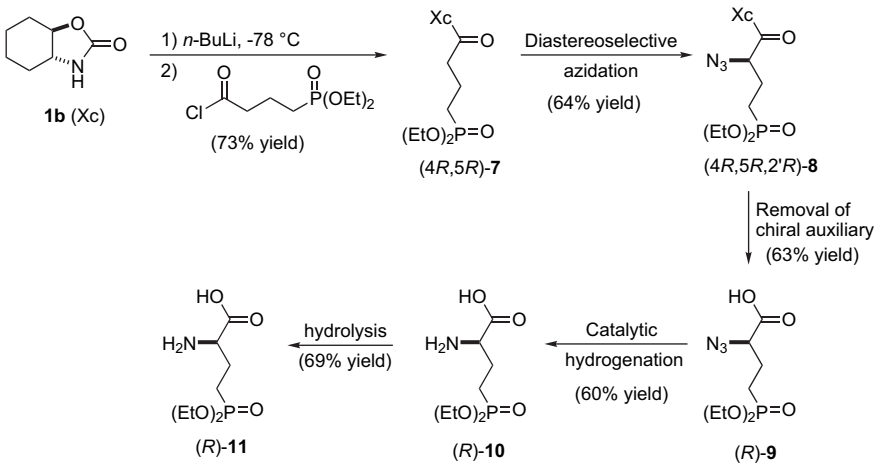
Isolation of the desired aminophosphonic acid (*S*)-AP-3 was accomplished in three steps, as outlined in Scheme 3. Thus, removal of the oxazolidinone chiral auxiliary was achieved by treatment of azide (4*R*,5*R*,2′*S*)-**3** with lithium hydroperoxide, as suggested by Evans and co-workers.[14,15] This reaction proceeded with 72% yield and the resulting azide-acid (*S*)-**4** was reduced by catalytic hydrogenation to afford α-amino acid (*S*)-**5** in 48% yield. Finally, hydrolysis of the diethyl phosphonate group was carried out with 6 N HCl under reflux for 7 h to give the expected aminophosphonic acid (*S*)-AP-3 [(*S*)-**6**, 51% yield], whose physical properties and optical rotation coincided with those reported in the literature.[10,16] The overall yield of the preparation of (*S*)-AP-3 from phosphorylated oxazolidinone (4*R*,5*R*)-**2** was 11.6%.

2.4. Enantioselective synthesis of (*R*)-2-amino-4-phosphonobutanoic acid, (*R*)-AP-4

In a further application of hexahydrobenzoxazolidinone **1b** in the enantioselective preparation of α-amino-ω-phosphonocarboxylic acids, (*R*)-2-amino-4-phosphonobutanoic acid, (*R*)-AP-4, was synthesized via N-acylation of **1b** with (diethoxyphosphoryl)butanoic acid chloride to give derivative (4*R*,5*R*)-**7**, which was then azidated, hydrogenated, and



Scheme 3.



Scheme 4.

hydrolyzed as described above in the case of (4*R*,5*R*)-**2**. (*R*)-2-Amino-4-phosphonobutanoic acid, (*R*)-AP-4, was isolated in 12.2% overall yield (Scheme 4).

3. Summary

The potential of hexahydrobenzoxazolidinones **1a**–**d** as effective chiral auxiliaries in the enantioselective synthesis of α-amino-ω-phosphonocarboxylic acids is demonstrated by the use of phosphoryl derivatives (4*R*,5*R*)-**2** and (4*R*,5*R*)-**7**, which were prepared by N-acylation of **1b**, for the highly stereoselective preparation of (2*S*)-amino-3-phosphonopropanoic acid, (*S*)-AP-3, and (2*R*)-amino-4-phosphonobutanoic acid, (*R*)-AP-4.

4. Experimental

4.1. General

Flasks, stirring bars, and hypodermic needles used for the generation and reactions of organometallic compounds were dried for ca. 12 h at 120 °C and allowed to cool in a desiccator over anhydrous $CaSO_4$. Anhydrous solvents were obtained by distillation from benzophenone/ketyl radical.[18] *n*-Butyllithium was titrated according to the method of Juaristi and co-workers.[19] TLC: Merck DC-F_{254} plates, detection UV light, iodine vapor or ninhydrin spray. Flash chromatography:[20] Merck silica gel (0.040–0.063 mm). Melting points: Melt Temp apparatus, not corrected. ^{1}H NMR spectra: Jeol Eclipse-400 (400 MHz), Bruker Ultra Shield (300 MHz), and Jeol GSX-270 (270 MHz) spectrometers; ^{13}C NMR spectra: Jeol Eclipse-400 (100 MHz) and Bruker Ultra Shield (75 MHz); ^{31}P NMR spectra: Jeol Eclipse-400 (162 MHz) and Bruker Ultra Shield (121 MHz) spectrometers; Chemical shifts δ are given in parts per million relative to Me_4Si as an internal reference, coupling constants are given in *J* (hertz). Mass spectra were obtained in a Hewlett–Packard HP-5986 instrument. High-resolution mass spectra (HRMS) were obtained at the Instituto de Química, UNAM, México on an HPLC 1100 coupled to MSD TOF Agilent Technologies mod. 1969A.

4.2. (4*R*,5*R*)-*trans*-*N*-[3′-(Diethoxyphosphoryl)propionyl]hexahydrobenzoxazolidin-2-one, (4*R*,5*R*)-2

A dry 250-mL two-necked flask provided with a magnetic stirrer, a dropping funnel, and a low-temperature thermometer was charged with a mixture of (4*R*,5*R*)-**1b** (1.8 g, 12.75 mmol) in dry THF (130 mL) under nitrogen. The solution was cooled to −78 °C in dry ice/acetone bath before the dropwise addition of a precooled solution of *n*-BuLi (5.31 mL, 2.4 M in hexane, 12.75 mmol). Stirring was continued for 2 h at −78 °C and then a precooled solution of (diethoxyphosphoryl)propionyl chloride (3.8 g, 16.57 mmol) in THF (50 mmol) was added dropwise. The reaction mixture was stirred at −78 °C for 1 h, and allowed to warm up to −30 °C with continued stirring during 3 h, and then quenched with saturated aqueous solution of NH_4Cl (30 mL). Water (120 mL) was added and the organic material was extracted with EtOAc (3×100 mL), dried with anhydrous Na_2SO_4, filtered, and evaporated. The crude product was purified by silica gel column chromatography (hexane/EtOAc, 80:20 to 50:50) to give 3.49 g (82% yield) of (4*R*,5*R*)-**2** as a colorless oil, $[\alpha]_D^{25}$ −47.0 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, J=7.0 Hz, 6H), 1.35 (m, 3H), 1.58 (m, 1H), 1.79 (m, 1H), 1.86 (m, 1H), 2.03 (m, 2H), 2.17 (m, 1H), 2.74 (m, 1H), 2.97 (m, 1H), 3.22 (m, 1H), 3.48 (ddd, $J^1 \approx J^2$=10.8 Hz, J^3=3.3 Hz, 1H), 3.81 (ddd, $J^1 \approx J^2$=11.5 Hz, J^3=3.6 Hz, 1H), 4.02 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.4 (d, $J_{C/P}$=6.2 Hz), 20.2 (d, $J_{C/P}$=144.5 Hz), 23.5, 23.7, 28.4, 28.8, 30.0 (d, $J_{C/P}$=2.3 Hz), 61.8 (d, $J_{C/P}$=6.9 Hz), 63.2, 81.6, 154.6, 173.5 (d, $J_{C/P}$=17.7 Hz). ^{31}P NMR ($CDCl_3$, 162 MHz) δ 31.7. MS (20 eV) *m*/*z* 334 (M^++1, 24), 236 (16), 193 (99), 165 (100), 137 (89), 96 (45), 81 (17), 55 (9). HRMS (FAB) calcd for $C_{14}H_{25}NO_6P$: 334.1420; found: 334.1425.

4.3. (4*R*,5*R*)-*trans*-*N*-[4′-(Diethoxyphosphoryl)butanoyl]hexahydrobenzoxazolidin-2-one, (4*R*,5*R*)-7

The same procedure described for the preparation of (4*R*,5*R*)-**2** was followed, with 2.0 g (14.2 mmol) of oxazolidinone **1b**, 5.1 mL of *n*-butyllithium (2.77 M in hexane, 14.2 mmol), and 4.12 g (17 mmol) of (diethoxyphosphoryl)-butanoic acid chloride to give 3.6 g (73% yield) of (4*R*,5*R*)-**7**

that was crystallized from CH_2Cl_2–AcOEt, mp 70–71 °C, $[\alpha]_D^{25}$ −52.8 (*c* 1.04, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, J=7.0 Hz, 6H), 1.30 (m, 3H), 1.60 (m, 1H), 1.70–2.00 (m, 6H), 2.17 (m, 1H), 2.70–2.90 (m, 2H), 3.05 (m, 1H), 3.48 (ddd, $J^1 \approx J^2$=10.8 Hz, J^3=3.2 Hz, 1H), 3.82 (ddd, $J^1 \approx J^2$=11.5 Hz, J^3=3.5 Hz, 1H), 4.03 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.5, 16.5, 17.5 (d, $J_{C/P}$=4.6 Hz), 23.5, 23.7, 24.9 (d, $J_{C/P}$=141.3 Hz), 28.4, 28.9, 36.6 (d, $J_{C/P}$=16.3 Hz), 61.5, 61.6, 63.1, 81.4, 154.7, 174.3. ^{31}P NMR ($CDCl_3$, 121.5 MHz) δ 32.4. MS (20 eV) *m*/*z* 349 (M^++2, 1), 348 (M^++1, 6), 347 (M^+, 4), 302 (3), 250 (7), 207 (100), 179 (82), 165 (82), 152 (57), 151 (53), 125 (26), 123 (17), 96 (20). Elem. Anal. Calcd for $C_{15}H_{26}NO_6P$: C, 51.86; H, 7.55; N, 4.03; found: C, 52.00; H, 7.28; N, 4.03.

4.4. (4*R*,5*R*)-*trans*-*N*-[3′-(Diethoxyphosphoryl)-(2′*S*)-azidopropionyl]hexahydrobenzoxazolidin-2-one, (4*R*,5*R*,2′*S*)-3

A dry two-necked flask provided with magnetic stirrer, dropping funnel, and low-temperature thermometer was charged with a mixture of (4*R*,5*R*)-**2** (300 mg, 0.9 mmol) in THF (30 mL) under nitrogen. The solution was cooled to −78 °C in dry ice/acetone bath before the dropwise addition of a precooled solution of NaHMDS (0.9 mL, 1 M in hexane, 0.9 mmol). After 30 min at −78 °C a precooled solution of trisyl azide (306.5 mg, 0.99 mmol) in THF (5 mL) was added dropwise. The reaction mixture was stirred for 30 min before the addition of glacial acetic acid (0.24 mL, 4.14 mmol). The reaction mixture was allowed to warm up to room temperature and stirred for 3 h. Saturated aqueous NH_4Cl (5 mL) was added and the organic material was extracted with EtOAc (3×30 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in a rotary evaporator. The crude product was purified by flash chromatography (hexane/EtOAc, 1:1) to yield 222 mg (66% yield) of azide (4*R*,5*R*,2′*S*)-**3** as a colorless oil, $[\alpha]_D^{25}$ −42.0 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.33 (m, 6H), 1.40 (m, 3H), 1.66 (m, 1H), 1.88 (m, 2H), 2.20 (m, 2H), 2.51 (m, 1H), 2.60 (m, 1H), 3.59 (m, 1H), 3.98 (ddd, $J^1 \approx J^2$=11.3 Hz, J^3=3.3 Hz, 1H), 4.11 (q, J=7.2 Hz, 2H), 4.16 (q, J=7.1 Hz, 2H), 5.22 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.5 (d, $J_{C/P}$=6.2 Hz), 23.5, 23.7, 27.2 (d, $J_{C/P}$=142.7 Hz), 28.5, 28.6, 56.2 (d, $J_{C/P}$=1.5 Hz), 62.3 (d, $J_{C/P}$=4.1 Hz), 62.4 (d, $J_{C/P}$=4.1 Hz), 63.6, 82.1, 154.2, 171.0 (d, $J_{C/P}$=13.8 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ 26.4. MS (20 eV) *m*/*z* 375 (M^++1, 0.8), 346 (1.7), 249 (8.6), 205 (2.3), 178 (61), 150 (62), 122 (100), 97 (22), 81 (17), 58 (5.4). HRMS (FAB) calcd for $C_{14}H_{24}N_4O_6P$: 375.1433; found: 375.1436.

4.5. (4*R*,5*R*)-*trans*-*N*-[4′-(Diethoxyphosphoryl)-(2′*S*)-azidobutanoyl]hexahydrobenzoxazolidin-2-one, (4*R*,5*R*,2′*R*)-8

The same procedure described for the preparation of (4*R*,5*R*,2′*S*)-**3** was followed with 1.0 g (2.9 mmol) of (4*R*,5*R*)-**7**, 3.17 mL of LiHMDS (1 M in hexane, 3.2 mmol), 0.98 g (3.2 mmol) of trisyl azide, and 0.76 mL (13.2 mmol) of glacial acetic acid to give 712 mg (63.7% yield) of (4*R*,5*R*,2′*R*)-**8** as a slightly yellow oil, $[\alpha]_D^{25}$ −39.0 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (t, J=7.1 Hz, 6H), 1.42 (m, 3H), 1.65 (m, 1H), 1.80–2.05 (m, 5H), 2.20 (m, 2H), 2.80 (m, 1H), 3.57 (ddd, $J^1 \approx J^2$=11.0 Hz, J^3=3.3 Hz, 1H), 3.94 (ddd, $J^1 \approx J^2$=11.5 Hz, J^3=3.6 Hz, 1H), 4.08 (m, 4H), 4.79 (dd, J^1=8.5 Hz, J^2=7.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.4, 16.5, 22.2 (d, $J_{C/P}$=142.2 Hz), 23.5, 23.7, 24.1 (d, $J_{C/P}$=3.1 Hz), 28.4, 28.6, 60.9 (d, $J_{C/P}$=17.6 Hz), 61.9 (m), 63.5, 82.2, 154.2, 171.6. ^{31}P NMR ($CDCl_3$, 162 MHz) δ 31.1. MS (20 eV) *m*/*z* 389 (M^++1, 2.1), 220 (6.1), 192 (62.2), 164 (46.2), 136 (100), 109 (16.1). HR-ESI-TOF *m*/*z* [**2a**·Na]$^+$ calcd for $C_{15}H_{25}N_4O_6PNa$: 411.1404; found: 411.1411 (1.73 ppm).

4.6. (2*S*)-Azido-3-(diethoxyphosphoryl)propionic acid, (*S*)-4

In a 100-mL flask provided with magnetic stirrer and nitrogen atmosphere was placed 856 mg (2.28 mmol) of azide (4*R*,5*R*,2′*S*)-**3** in 35 mL of THF and 11.5 mL of water. The resulting solution was cooled to 0 °C before the addition of 1.04 mL (9.12 mmol) of 30% H_2O_2 and 191.8 mg (4.56 mmol) of $LiOH \cdot H_2O$. The reaction mixture was stirred at 0 °C for 3 h and then 634 mg (5.02 mmol) of Na_2SO_3 in 6.0 mL of water was added. Immediately thereafter, 20 mL of 0.5 N solution of $NaHCO_3$ was added and the aqueous solution was extracted with EtOAc (2×50 mL) to remove the oxazolidinone auxiliary. The aqueous phase was acidulated to pH=2.0 with 1 N HCl and extracted with CH_2Cl_2 (3×50 mL). The organic extracts were combined, dried with anhydrous Na_2SO_4, and evaporated. The product (*S*)-**4** was purified by silica gel column chromatography (*i*-PrOH/MeOH/AcOH, 8:1:0.3) to give 417 mg (72% yield) of a colorless oil, $[\alpha]_D^{25}$ +44.1 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 (t, J=7.0 Hz, 6H), 2.19 (ddd, $J^1 \approx J^2$=16.5 Hz, J^3=9.2 Hz, 1H), 2.42 (ddd, J^1=19.1 Hz, J^2=15.4 Hz, J^3=4.4 Hz, 1H), 4.11–4.21 (m, 5H), 9.35 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.3 (d, $J_{C/P}$=23.7 Hz), 27.9 (d, $J_{C/P}$=144.5 Hz), 57.0 (d, $J_{C/P}$=3.8 Hz), 63.0 (d, $J_{C/P}$=6.9 Hz), 63.1 (d, $J_{C/P}$=6.9 Hz), 171.1 (d, $J_{C/P}$=15.3 Hz). ^{31}P NMR ($CDCl_3$, 162 MHz) δ 27.8. MS (20 eV) *m*/*z* 252 (M^++1, 38), 180 (60), 152 (35), 134 (8), 122 (100), 97 (52), 80 (26), 70 (30), 58 (13), 43 (70). HRMS (FAB) calcd for $C_7H_{15}N_3O_5P$: 252.0749; found: 252.0744.

4.7. (2*R*)-Azido-4-(diethoxyphosphoryl)butanoic acid, (*R*)-9

The same procedure described for the preparation of (*S*)-**4** was followed with 1.12 g (2.9 mmol) of (4*R*,5*R*,2′*R*)-**8**, 1.31 mL (11.57 mmol) of H_2O_2, 243 mg (5.8 mmol) of $LiOH \cdot H_2O$, and 802 mg (6.4 mmol) of Na_2SO_3 to give 483 mg (63% yield) of (*R*)-**9** as a pale yellow oil, $[\alpha]_D^{25}$ +32.4 (*c* 1.02, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.30 (t, J=7.0 Hz, 6H), 2.00 (m, 4H), 3.98 (br, 1H), 4.08 (q, J=7.0 Hz, 2H), 4.10 (q, J=7.0 Hz, 2H), 9.36 (br, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.4, 16.5, 21.6 (d, $J_{C/P}$=141.2 Hz), 25.0, 62.3, 62.4, 63.2 (d, $J_{C/P}$=16.4 Hz), 174.5. ^{31}P NMR ($CDCl_3$, 121 MHz) δ 32.6. MS (20 eV) *m*/*z* 26.5 (M^+, 16.6), 236 (10.5), 219 (8.5), 192 (62.7), 164 (43.8), 136 (100), 128 (51.1), 109 (26.1), 100 (45.1), 82 (20.2), 72 (16.2), 54 (41.0), 44 (56.9). HR-ESI-TOF *m*/*z* [**2a**·Na]$^+$ calcd for $C_8H_{16}N_3O_5PNa$: 288.07198; found: 288.07231 (1.15 ppm).

4.8. (2*S*)-Amino-3-(diethoxyphosphoryl)propionic acid, (*S*)-5

In a hydrogenation flask were placed 370 mg (1.47 mmol) of (*S*)-**4**, 30 mL of methanol, and 37 mg of 10% Pd/C. The flask was pressurized with hydrogen (1 atm) and shaken at room temperature for 1 h. The reaction mixture was filtered over Celite and the filtrate was evaporated in a rotary evaporator. The residue was purified by silica gel column chromatography *i*-PrOH/MeOH/NH_4Cl (6:1:0.5) to give 160 mg (48% yield) of (*S*)-**5** as a waxy solid with mp 123–125 °C, $[\alpha]_D^{25}$ +14.7 (*c* 1.02, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.29 (t, J=7.0 Hz, 3H), 1.30 (t, J=7.0 Hz, 3H), 2.42 (ddd, $J^1 \approx J^2$=15.4 Hz, J^3=10.6 Hz, 1H), 2.58 (m, 1H), 3.86 (m, 1H), 4.08 (q, J=7.0 Hz, 2H), 4.12 (q, J=7.0 Hz, 2H), 7.01 (br, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.4, 26.4 (d, $J_{C/P}$=139.2 Hz), 49.8, 62.7, 172.2 (d, $J_{C/P}$=15.3 Hz). ^{31}P NMR ($CDCl_3$, 162 MHz) δ 29.9. MS (20 eV) *m/z* 226 (M^++1, 3), 180 (100), 152 (45), 138 (7), 124 (55), 106 (35), 97 (8), 83 (6), 57 (7), 45 (74). HRMS (FAB) calcd for $C_7H_{17}NO_5P$: 226.0844; found: 226.0841.

4.9. (2*R*)-Amino-4-(diethoxyphosphoryl)butanoic acid, (*R*)-10

The same procedure described for the preparation of (*S*)-**5** was followed with 483 mg (1.8 mmol) of (*R*)-**9**, 25 mL of methanol, and 48.3 mg of 10% Pd/C to give 262 mg (60% yield) of (*R*)-**10** as a white solid, mp 152–154 °C, $[\alpha]_D^{25}$ −8.24 (*c* 3.64, H_2O); lit.[10c] $[\alpha]_D^{25}$ +9.22 (*c* 5.0, H_2O) for the (*S*)-enantiomer. ^1H NMR (D_2O, 300 MHz) δ 1.30 (t, J=7.0 Hz, 6H), 1.80–2.20 (m, 4H), 3.78 (dd, J^1=J^2=5.4 Hz, 1H), 4.12 (q, J=7.2 Hz, 2H), 4.14 (q, J=7.2 Hz, 2H). ^{13}C NMR (D_2O, 75 MHz) δ 15.8, 15.9, 20.3 (d, $J_{C/P}$= 140.0 Hz), 23.7 (d, $J_{C/P}$=3.8 Hz), 54.7 (d, $J_{C/P}$=19.1 Hz), 63.8 (d, $J_{C/P}$=1.3 Hz), 63.9 (d, $J_{C/P}$=1.3 Hz), 173.6. ^{31}P NMR (D_2O, 121 MHz) δ 34.4.

4.10. (2*S*)-Amino-3-phosphonopropionic acid, (*S*)-6

In a 25-mL flask provided with magnetic stirrer were placed 174 mg (0.77 mmol) of (*S*)-**5** and 4.0 mL of 6 N HCl. The reaction mixture was heated to reflux for 7 h, allowed to cool to room temperature, and concentrated in a rotary evaporator. The residue was redissolved in 6.0 mL of ethanol and treated with 2.0 mL of propylene oxide. The resulting suspension was heated to 50 °C for 2 h, and concentrated in a rotary evaporator to give a white solid, which was redissolved in 1.0 mL of water and crystallized upon addition of 3.0 mL of ethanol to give 67 mg (51% yield) of amino acid (*S*)-**6** [(*S*)-AP-3], mp 224–226 °C, $[\alpha]_D^{25}$ −12.0 (*c* 1.0, 1 N NaOH); lit.[10d] $[\alpha]_D^{25}$ +12.6 (*c* 0.75, 1 N NaOH) for the (*R*)-enantiomer.[16] $[\alpha]_{Hg}^{25}$ −54.0 (*c* 1.0, 1 N NaOH); lit.[10d] $[\alpha]_{Hg}^{25}$ +58.9 (*c* 1.0, 1 N NaOH) for the (*R*)-enantiomer.[16] ^1H NMR (D_2O, 270 MHz) δ 2.09 (m, 1H), 2.30 (ddd, $J^1 \approx J^2$=16.5 Hz, J^3=3.9 Hz, 1H), 4.14 (ddd, J^1=15.1 Hz, J^2=9.8 Hz, J^3=3.9 Hz, 1H). ^{13}C NMR (D_2O, 67 MHz) δ 28.1 (d, $J_{C/P}$=130.9 Hz), 49.7 (d, $J_{C/P}$=4.2 Hz), 171.9 (d, $J_{C/P}$=12.5 Hz). ^{31}P NMR (D_2O, 109 MHz) δ 18.6.

4.11. (2*R*)-Amino-4-phosphonopropionic acid, (*R*)-11

The same procedure described for the preparation of (*S*)-**6** was followed with 224 mg (0.94 mmol) of (*R*)-**10** and 5.1 mL of 6 N HCl to give 119 mg (69% yield) of amino acid (*R*)-**11** [(*R*)-AP-4] as a white solid, mp 206–208 °C, $[\alpha]_D^{25}$ −25.0 (*c* 1.0, 6 N HCl); lit.[10c] $[\alpha]_D^{25}$ +27.6 (*c* 3.0, 6 N HCl) for the (*S*)-enantiomer. ^1H NMR (D_2O, 400 MHz) δ 1.71 (m, 2H), 2.13 (m, 2H), 4.03 (dd, J^1=J^2=5.7 Hz, 1H). ^{13}C NMR (D_2O, 100 MHz) δ 23.4 (d, $J_{C/P}$= 133.8 Hz), 24.5, 53.7 (d, $J_{C/P}$=16.0 Hz), 172.3. ^{31}P NMR (D_2O, 162 MHz) δ 25.4.

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Preparation of functionalized tertiary thiols and nitrosothiols

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Abstract—The development and preparation of five series of tertiary thiols and nitrosothiols as nitric oxide releasing molecules functionalized with acid, alcohol, or amine groups for future conjugation are reported.

1. Introduction

S-Nitrosothiols were generally considered unstable until the relatively stable trityl nitrosothiol was reported in 1931[1] and the room-temperature-stable *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP) in 1978.[2] The functional group of *S*-nitrosothiols did not receive much pharmaceutical attention until the discovery of the biological function of nitric oxide, and the role of *S*-nitrosothiols as its physiological carrier. The color of primary and secondary nitrosothiols is typically orange red and that of tertiary is green. The *S*-nitrosothiol group is made up of a sulfur–nitrogen single bond and a nitrogen–oxygen double bond according to X-ray crystallographic analysis.[2] The orientation of the N=O relative to the alkyl group attached to sulfur can be predominantly *syn* for the primary or secondary nitrosothiols and predominantly *anti* for the tertiary ones, which is predicted by theoretical calculation and confirmed by ^{15}N NMR and X-ray data.[3] The ^{15}N NMR signal of $Me_3CS^{15}NO$ appears as a singlet at 20 °C, broadens as the temperature is lowered, and separates into two peaks at −81 °C.[4] *S*-Nitrosothiols are stable under oxidative conditions like treatment with potassium ferricyanide, but decompose rapidly under reductive conditions, e.g., treatment of sodium dithionite. These results are in agreement with the calculation of the bond length of S–N bond of $MeSNO^{\bullet-}$ as the additional electron occupies the antibonding orbital of the S–N bond and elongates the bond length by 0.56 Å.[4]

Nitric oxide is an ubiquitous signaling molecule in mammalian biology and is involved in the regulation of a variety of processes.[5] A number of illnesses are associated with nitric oxide deficiency and thus could potentially be treated with nitric oxide releasing molecules.[6] One class of nitric oxide releasing molecules, which we have focused on is the nitrosothiols. The nitrosothiol functional group is capable of spontaneously releasing nitric oxide without enzymatic preactivation.[7] Due to the inherent lability of the sulfur–nitrogen bond of nitrosothiols, many compounds in this class lack sufficient stability for potential pharmaceutical applications. Thus, our goal in this study was to produce nitrosothiol molecules with appropriate stabilities as well as having at least one other functional group for attachment to therapeutic agents. Generally, tertiary nitrosothiols are more stable than secondary, primary, or aryl nitrosothiols because of the increase in steric interactions associated with the dimerization of the incipient thiyl radical that forms upon homolytic cleavage of the sulfur–nitrogen bond.[8] The stability of *S*-nitrosothiols can also be enhanced by building space protection groups around the *S*-nitrosothiol group such as bowl-shaped triarylmethyl group,[9] a dendrimer-like Bpq group[10] or a Bmt group.[11] Accordingly, our chemical design strategy was to synthesize tertiary nitrosothiols bearing an acid, an alcohol, or a primary amine group.

2. Results and discussion

Our experience with nitrosothiols suggested that, in general, shelf stability is enhanced if the compound is a crystalline solid with a melting point above 70 °C. Since adamantane is a highly symmetrical molecule and its derivatives are usually solid, preparation of adamantane based nitrosothiols was explored. 2-Adamantane thione **1** was prepared according to the literature procedure[12] (Scheme 1). Reaction of **1** with *tert*-butyl acetate and lithium diisopropylamide (LDA) provided thiol **2**. Replacing *tert*-butyl acetate with ethyl acetate also produced the corresponding product but the conversion was not clean and the yield was lower. The ester **2** was converted to acid **3** upon treatment with trifluoroacetic acid (TFA). The thiol acid **3** is a highly crystalline compound, and this reaction has been scaled up to provide 10–100 g quantities of **3** by simple trituration of the crude product with dichloromethane and filtration. The thiol group

Keywords: Tertiary nitrosothiols; Tertiary thiols; Nitric oxide.
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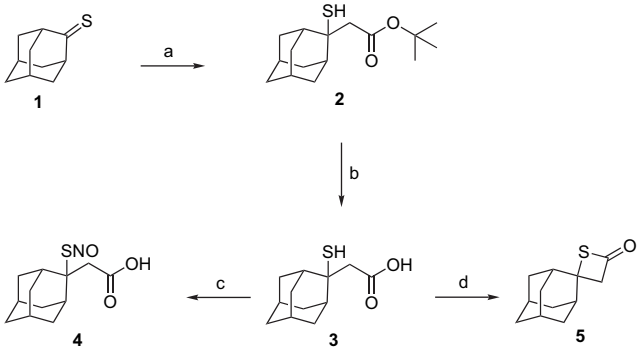
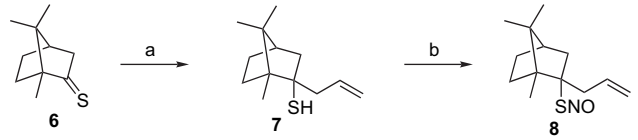
of **3** was converted to the nitrosothiol group by treatment with *tert*-butyl nitrite to afford **4**. Condensation of the acid **4** with alcohol or amine groups from compounds in various therapeutic classes has been done with 1,3-dicyclohexylcarbodiimide (DCC) or 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC). The thiol group of **3**, however, needs protection in order to condense the acid group of **3** with other molecules. To eliminate the need for protection and deprotection steps, the thiol acid **3** has been converted to thiolactone **5** with EDC. The thiolactone has then been reacted with amine or alcohol nucleophiles to prepare the thiol amides or esters, respectively.



a. *t*-butyl acetate, LDA, 93%; b. TFA, 66%; c. *t*-BuONO, 72%; d. EDC, 86%

Scheme 1.

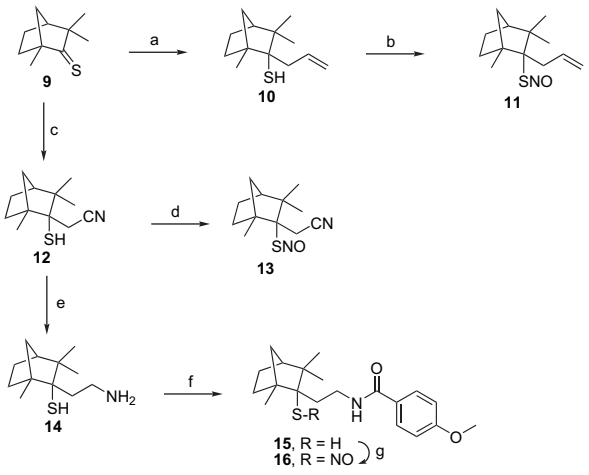
Camphor is an inexpensive high melting point solid (mp 179–181 °C) available in large quantity, and thus it was chosen as the core structure for a second series of nitrosothiols (Scheme 2). The camphor thione **6** was prepared according to the literature procedure[13] and then exposed to the lithium enolate of *tert*-butyl acetate under similar conditions as used for the preparation of **2**. In this reaction, the disappearance of the characteristic orange color of thiones appeared to indicate consumption of **6**, but upon aqueous work up the orange color returned. The recovery of **6** was confirmed by TLC and NMR. Apparently the enolizable proton of **6** was removed by the enolate, thus preventing nucleophilic addition of *tert*-butyl acetate to the thioketone. Preparation of the camphor tertiary thiol **7** was accomplished using allylmagnesium bromide. According to the literature,[14] allyl nucleophile adds to the exposed sulfur of thioketone and subsequent [2,3]-sigmatropic rearrangement produces the allyl thiol **7**. Therefore, allylmagnesium bromide could be used to alkylate a more hindered thioketone as illustrated by this and subsequent examples. The nitrosothiol **8** was then prepared from **7** and *tert*-butyl nitrite.



a. allyl magnesium bromide, 80%; b. *t*-BuONO, 70%

Scheme 2.

Since camphor thione **6** contains two alpha protons and did not react with the enolate of *tert*-butyl acetate, the use of fenchone as the core structure for the third series of



a. allyl magnesium bromide, 68%; b. *t*-BuONO, 66%;
c. *n*-BuLi, CH_3CN, 55%; d. *t*-BuONO, 76%; e. LAH, 41%;
f. 4-methoxybenzoic acid, EDC, 45%; g. *t*-BuONO, 45%

Scheme 3.

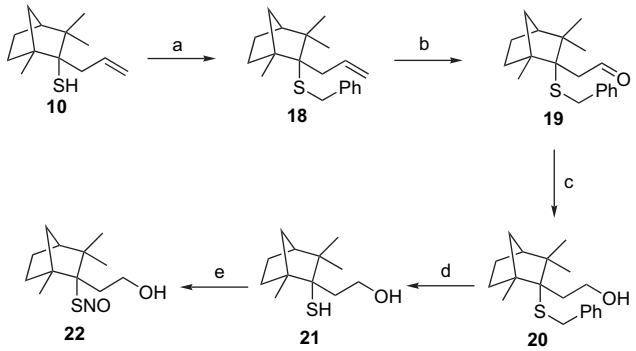
nitrosothiols was examined. Thiofenchone **9** (Scheme 3) was prepared from fenchone according to the literature procedure.[15] However, although, thiofenchone **9** has no alpha protons, it also did not react with the lithium enolate of *tert*-butyl acetate. Since the extreme steric hindrance surrounding the thioketone group of **9** was postulated to be responsible, we again chose allylmagnesium bromide as the nucleophile, which did afford thiol **10**. When the more hindered isoprenylmagnesium bromide was used, the isoprenyl nucleophile did not react with thione **9** to produce the corresponding product. However, with the sterically less encumbered thione **1**, isoprenylmagnesium bromide did react to produce the corresponding thiol **17** (Eq. 1).

(1)

Thus, the steric environment of the thioketone appears to be the primary determining factor governing the ease of nucleophilic addition. Bulky nucleophiles like the enolate of *tert*-butyl acetate or isoprenylmagnesium bromide did not react with thiofenchone **9**. Conversely, the sterically less demanding allyl and acetonitrile anions are able to readily react with the thione group in **9**. The resulting thiols **10** and **12** were readily converted to the nitrosothiols **11** and **13**, respectively, by treatment with *tert*-butyl nitrite. The nitrile group in **12** was converted to an amine **14** by reduction with lithium aluminum hydride (LAH), but attempted nitrosation of **14** with *tert*-butyl nitrite did not yield the desired product. Presuming the basic amine functionality to be the problem, the aminothiol **14** was converted to a model amide by coupling with 4-methoxybenzoic acid. The amide thiol **15** then could be readily nitrosylated to afford the nitrosothiol amide **16**.

Oxidative cleavage of the allyl group to an aldehyde was also studied (Scheme 4). The allyl thiol **10** was treated with osmium tetroxide either at ambient temperature or at 50 °C to give no reaction. Once the thiol **10** was protected

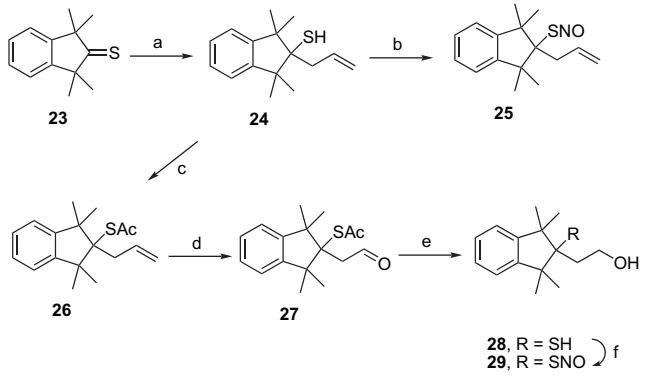
as the benzyl thioether **18**, oxidative cleavage of the allyl group to an aldehyde proceeded as expected. Compound **18** was treated with osmium tetroxide and then with periodic acid to give aldehyde **19**. Reduction of the aldehyde with sodium borohydride gave alcohol **20**. The benzyl group was removed with sodium/ammonia to give thiol alcohol **21**. Using a methanol/dichloromethane mixture as the reaction solvent, the nitrosation of **21** with *tert*-butyl nitrite proceeded smoothly to afford **22** in 90% yield. If this reaction was conducted in just dichloromethane, **22** was obtained only as a minor product with the major product being the bisnitrosylated nitrosothiol nitrite.



a. benzyl bromide, NaH, 71%; b. OsO_4; HIO_4, 51%;
c. $NaBH_4$, 88%; d. Na/NH_3, 88%; e. *t*-BuONO, 90%

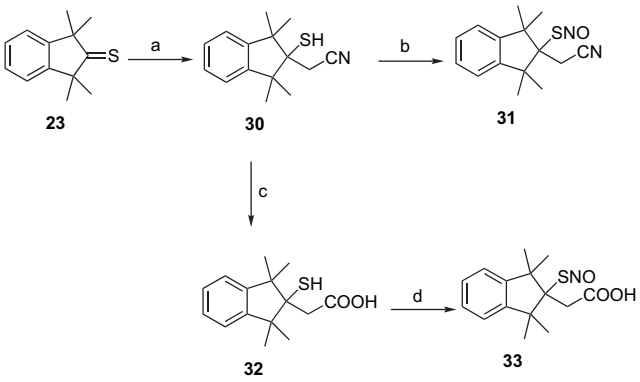
Scheme 4.

The above chemistry was applied to the preparation of other nitric oxide releasing molecules as described below (Schemes 5–7). 1,1,3,3-Tetramethylindane-2-thione **23** was prepared according to the literature procedure[15,16] and reacted with allylmagnesium bromide to give thiol **24** (Scheme 5). Thiol **24** was converted to nitrosothiol **25** using *tert*-butyl nitrite. Alternatively the thiol group of **24** was protected with an acetyl group, instead of a benzyl group, to save one-step in the deprotection, to afford **26**. Compound **26** was treated with osmium tetroxide and then periodic acid to give **27**. The conversion of the aldehyde to an alcohol and the removal of the acetyl group were completed in one reaction with LAH to produce thiol alcohol **28**, which was then converted to nitrosothiol **29** with *tert*-butyl nitrite using methanol/dichloromethane as the reaction solvent.



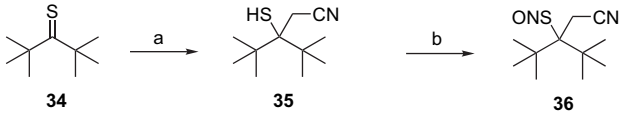
a. allyl magnesium bromide, 83%; b. *t*-BuONO, 51%;
c. Ac_2O, 77%; d. OsO_4; HIO_4, 25%; e. LAH, 58%; f. *t*-BuONO, 88%

Scheme 5.



a. CH_3CN, *n*-BuLi, 69%; b. *t*-BuONO, 45%; c. Conc. HCl, HOAc, 66%;
d. *t*-BuONO, 57%

Scheme 6.



a. CH_3CN, *n*-BuLi, 84%; b. *t*-BuONO, 92%;

Scheme 7.

To evaluate a different approach for the preparation of acid functionalized tertiary thiols and nitrosothiols, the indanethione **23** was reacted with acetonitrile and *n*-butyl lithium to give thiol nitrile **30** (Scheme 6). The nitrile group was hydrolyzed with concentrated hydrochloric acid in acetic acid to give thiol acid **32**. Both thiols **30** and **32** were converted to nitrosothiols **31** and **33**, respectively, with *tert*-butyl nitrite in dichloromethane.

One more series of nitric oxide releasing molecules were explored as shown in Scheme 7. Di-*tert*-butyl thione **34**[17] was reacted with acetonitrile and *n*-butyl lithium to give thiol nitrile **35**. Nitrosation with *tert*-butyl nitrite afforded nitrosothiol **36**.

3. Conclusions

The success of the addition of nucleophiles to thioketones is determined by the overall steric interaction between these two molecules. The carbanion of *tert*-butyl acetate reacts with 2-adamantane thione **1** but not with the more sterically hindered thiofenchone **9**. The linear allylmagnesium bromide reacts with thiofenchone **9**, but the bulkier isoprenylmagnesium bromide did not. With the less sterically hindered 2-adamantane thione **1**, isoprenylmagnesium bromide reacts successfully. In the design of tertiary thiols, the steric interaction between nucleophiles and thioketones must be considered.

Many *S*-nitrosothiols have been prepared under biphasic conditions using nitrous acid, but we enjoyed the convenience of using *tert*-butyl nitrite as the nitrosation agent in dry organic solvents. Alcohol solvents are usually not recommended for the nitrosation reaction using *tert*-butyl

nitrite because of the transfer of NO group to the alcohol solvent. We did find an advantage of using methanol as a co-solvent to suppress the over nitrosation in the preparation of nitrosothiol alcohol **22** and **29**.

In this study, nitric oxide releasing nitrosothiols functionalized with an acid, an alcohol, or an amine group for future conjugation were prepared. These functional groups along with the intermediate aldehydes and 1,2-diols prepared from the allyl double bond offer a broad choice of potential chemical linkages that one might envision utilizing between these nitric oxide releasing molecules and the therapeutic agents of interest.

4. Experimental

4.1. General

All reagents and solvents were obtained from commercial sources and used without further purification. Flash chromatography was performed on silica gel (Merck, 230–400 mesh). ^{1}H and ^{13}C NMR were recorded on a Brucker AMX-300 instrument. Chemical shifts are referenced to TMS and reported in parts per million. Low-resolution mass spectra were recorded on a Perkin–Elmer API-150EX spectrometer with atmospheric pressure turbo ion spray. Elemental analyses were done at Robertson Microlit Laboratories, Madison, NJ. The consumption of thiols in the preparation of nitrosothiol was usually monitored by TLC staining with Phosphomolybdic acid (PMA). Thiol compounds are usually very sensitive to PMA and gave a blue spot instantly at room temperature and the nitrosothiols usually required heating before giving a blue spot.

4.1.1. *tert*-Butyl (2-mercaptoadamantan-2-yl)acetate (2). To butyl acetate (25 mL, 21.6 g, 186 mmol) in THF (400 mL) at −78 °C was added lithium diisopropylamide monotetrahydrofuran (1.5 M solution in cyclohexane, 100 mL, 150 mmol). The solution was stirred at −78 °C for 40 min and 2-adamantane thione **1**[12] (21.9 g, 131.6 mmol) in THF (400 mL) was added. The reaction mixture was stirred at room temperature for 2 h, and then diluted with dichloromethane and HCl (2 N, 75 mL). The organic phase was removed, washed with brine, dried over magnesium sulfate, filtered, and evaporated. The residue was purified by column chromatography (ethyl acetate/hexane 1:19) to give **2** (34.7 g, 93%). Mp 56–62 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.87 (s, 2H), 2.47 (m, 2H), 2.38 (s, 1H), 2.11 (m, 2H), 1.98 (s, 2H), 1.96 (m, 2H), 1.96–1.84 (m, 6H), 1.47 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 80.7, 54.0, 47.2, 38.9, 38.1, 33.9, 33.23, 28.1, 27.4, 26.8. LRMS (APIMS) *m*/*z* 283 (MH^+). Anal. Calcd for $C_{16}H_{26}O_2S$: C, 68.04; H, 9.28. Found: C, 68.14; H, 9.30.

4.1.2. (2-Mercaptoadamantan-2-yl)acetic acid (3). Trifluoroacetic acid (15 mL) was added to **2** (10.76 g, 38.10 mmol) in dichloromethane (15 mL). The reaction mixture was stirred at room temperature for 40 min and concentrated to dryness. The resultant solid was treated with dichloromethane (40 mL) and concentrated to dryness three times. The resultant solid was triturated with dichloromethane (20 mL). The solid was collected by filtration and washed with a minimum amount of dichloromethane to give **3** (5.6447 g, 66%). Mp 178–180 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.5 (br s, 1H), 3.04 (s, 2H), 2.49 (m, 2H), 2.25 (s, 1H), 2.1–2.0 (m, 4H), 1.9 (m, 2H), 1.7–1.6 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.7, 53.4, 46.3, 38.9, 37.8, 33.8, 33.2, 27.4, 26.8. LRMS (APIMS, −ve scan) *m*/*z* 225 ($M-H^-$). Anal. Calcd for $C_{12}H_{18}O_2S$: C, 63.68; H, 8.02. Found: C, 63.40; H, 7.90.

4.1.3. [2-(Nitrosothio)adamantane-2-yl]acetic acid (4). Compound **3** (773.1 mg, 3.416 mmol) was dissolved in hot methylene chloride (40 mL). The methylene chloride solution was cooled to room temperature and *tert*-butyl nitrite (420 μL, 370 mg, 3.59 mmol) was added. The reaction mixture was stirred at room temperature for 30 min and then concentrated. This crude product was purified by column chromatography (silica gel, ethyl acetate/hexane 1:3) to give **4** (628.2 mg, 2.46 mmol, 72%). Mp 72–73 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.8 (br, 1H), 3.77 (s, 2H), 2.78 (s, 2H), 2.4 (m, 2H), 2.1–1.7 (m, 10H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.7, 66.7, 42.4, 38.3, 35.1, 33.4, 32.5, 26.71, 26.66. APIMS (IS, NH_4OAc) *m*/*e* 254 ($M-H^-$).

4.1.4. 4*H*-Spiro[thiethane-2,2′-tricyclo[3.3.1.1^{3,7}]decan]-4-one (5). A mixture of **3** (516 mg, 2.28 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (445 mg, 2.32 mmol) in dichloromethane (10 mL) was stirred at room temperature for 1 h, diluted with dichloromethane, and washed with 0.1 M HCl and brine. The organic phase was dried over magnesium sulfate, filtered, evaporated, and chromatographed (ethyl acetate/hexane 1:3, then 1:1) to give **5** (0.41 g, 86%). Mp 77–78 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.61 (s, 2H), 2.20 (m, 2H), 1.95–1.78 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 63.4, 54.9, 39.9, 36.5, 35.6, 33.7, 26.6, 25.8. LRMS (APIMS) *m*/*z* 209 ($M+H^+$), 226 (MNH_4^+).

4.1.5. 2-Allyl-1,7,7-trimethylbicyclo[2.2.1]heptane-2-thiol (7). A solution of (1*R*)-(−)-thiocamphor **6**[13] (0.5 g, 2.97 mmol) in ether (10 mL) cooled to 0 °C was treated with allylmagnesium bromide (1 M in ether, 4.5 mL, 4.5 mmol) and the reaction mixture was stirred at 0 °C for 30 min. Excess cold 2 N HCl was added carefully, and the solution was extracted with ether. The organic phase was washed with water, brine, dried over magnesium sulfate, filtered, and evaporated. The residue was purified by column chromatography (neat hexane) to give **7** (0.5 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05–5.91 (m, 1H), 5.17–5.10 (m, 2H), 2.54–2.46 (m, 2H), 2.30–2.18 (m, 1H), 2.10 (s, 1H), 1.75–1.68 (m, 3H), 1.58–1.46 (m, 3H), 1.16 (s, 3H), 0.99 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.2, 117.9, 55.4, 52.7, 50.7, 49.7, 47.9, 45.7, 31.3, 27.1, 22.1, 21.4, 14.3.

4.1.6. 2-Allyl-1,7,7-trimethyl-2-(nitrosothio)bicyclo[2.2.1]-heptane (8). A solution of **7** (100 mg, 0.48 mmol) in hexane (5 mL) was treated dropwise with *tert*-butyl nitrite (113 μL, 0.95 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was evaporated, and the residue was chromatographed (neat hexane) to give **8** (80 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.83–5.74 (m, 1H), 5.06–4.99 (m, 2H), 3.26–3.18 (m, 2H), 2.64 (m, 1H), 2.15–2.02 (m, 2H), 1.96–1.82 (m, 2H), 1.75–1.62

(m, 1H), 1.47–1.37 (m, 1H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.6, 117.7, 68.8, 54.7, 50.8, 46.5, 45.8, 45.5, 31.6, 27.1, 21.5, 21.3, 13.5.

4.1.7. 2-Allyl-1,3,3-trimethylbicyclo[2.2.1]heptane-2-thiol (10). (1*R*)-(−)-Fenchone was converted to hydrazone with hydrazine in acetic acid, and the resultant hydrazone was converted to the thioketone **9** with sulfur monochloride and triethylamine according to the literature procedure.[16] A solution of **9** (10.9 g, 65 mmol) in ether (150 mL) was treated with allylmagnesium bromide (1 M in ether, 100 mL, 100 mmol) dropwise at room temperature. After the addition was complete, the reaction mixture was stirred at room temperature for 1 h, cooled in an ice bath, and quenched carefully with 1 N HCl. The organic phase was washed with water, brine, and dried over sodium sulfate. After filtration and evaporation, the residue was purified by column chromatography (neat hexane) to give **10** (9.3 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13–6.04 (m, 1H), 5.10–5.03 (m, 2H), 2.72–2.62 (m, 1H), 2.40–2.30 (m, 1H), 2.27–2.15 (m, 1H), 1.90–1.80 (m, 1H), 1.79–1.67 (m, 2H), 1.47–1.31 (m, 1H), 1.20 (s, 1H), 1.15 (s, 3H), 1.13 (s, 3H), 1.08 (s, 3H), 1.22–1.05 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.3, 116.9, 63.5, 54.1, 50.8, 45.2, 44.6, 40.6, 35.0, 28.3, 27.2, 24.8, 18.2.

4.1.8. 2-Allyl-1,3,3-trimethyl-2-(nitrosothio)bicyclo[2.2.1]-heptane (11). A solution of **10** (80 mg, 0.38 mmol) in hexane (2 mL) was added to a solution of *tert*-butyl nitrite (68 μL, 0.57 mmol) in hexane (2 mL). The reaction mixture was stirred at room temperature in the dark for 30 min, and then additional *tert*-butyl nitrite (20 μL) was added. The reaction mixture was stirred for an additional 1 h at room temperature in the dark. The solvent was evaporated, and the residue was purified by column chromatography (neat hexane) to give **11** (60 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90–5.81 (m, 1H), 4.93–4.84 (m, 2H), 3.43–3.25 (m, 2H), 2.14 (d, J=10.5 Hz, 1H), 1.82–1.61 (m, 3H), 1.60–1.50 (m, 1H), 1.40 (s, 3H), 1.24 (s, 3H), 1.38–1.20 (m, 2H), 0.94 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.6, 117.7, 68.8, 54.7, 50.8, 46.5, 45.8, 45.5, 31.6, 27.1, 21.5, 21.3, 13.5.

4.1.9. (2-Mercapto-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl)-acetonitrile (12). A solution of *n*-butyl lithium (2.5 M in hexane, 29.7 mL, 74.3 mmol) was cooled to −78 °C and then treated with a solution of acetonitrile (3.9 mL, 74.3 mmol) in THF (98 mL). The solution was stirred at −78 °C for 1 h and then treated with a solution of **9**[15] (5 g, 29.7 mmol) in THF (50 mL). The reaction mixture was stirred at −78 °C for 1 h and then warmed to room temperature over 1 h. Water (50 mL) was added carefully and then THF was removed by evaporation. The residue was diluted with more water and extracted with ether. The combined organic phase was washed with water, brine, and dried over sodium sulfate. The residue after filtration and evaporation was purified by column chromatography (ethyl acetate/hexane 1:9) to give **12** (3.41 g, 55%). Mp 170–171 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.79 (d, J=16.6 Hz, 1H), 2.67 (d, J=16.6 Hz, 1H), 2.25–2.13 (m, 1H), 1.78–1.67 (m, 3H), 1.67 (s, 1H), 1.50–1.37 (m, 1H), 1.26 (s, 3H), 1.21 (s, 3H), 1.30–1.19 (m, 2H), 1.10 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 119.9, 60.5, 53.7, 50.1, 45.1, 40.6, 34.3, 30.9, 26.8, 26.3, 24.8, 17.8. LRMS (APIMS) m/z 227 (MNH_4^+).

4.1.10. [1,3,3-Trimethyl-2-(nitrosothio)bicyclo[2.2.1]-hept-2-yl]acetonitrile (13). To a solution of **12** (70 mg, 0.33 mmol) in dichloromethane (5 mL) was added *tert*-butyl nitrite (130 μL, 1 mmol). The reaction mixture was stirred at room temperature in the dark for 2 h. Additional *tert*-butyl nitrite (40 μL, 0.31 mmol) was added and the solution was stirred for an additional 30 min in the dark. The solvent was evaporated, and the residue was purified by column chromatography on a preparative plate (ethyl acetate/hexane 1:4) to give **13** (60 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (d, J=17.0 Hz, 1H), 3.57 (d, J=17.0 Hz, 1H), 2.20–2.10 (m, 1H), 1.95 (br s, 1H), 1.75–1.53 (m, 3H), 1.50 (s, 3H), 1.29 (s, 3H), 1.40–1.21 (m, 2H), 1.01 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 118.8, 70.1, 55.0, 50.2, 48.0, 41.6, 33.6, 27.2, 25.8, 25.5, 25.0, 18.6. LRMS (APIMS) m/z 256 (MNH_4^+).

4.1.11. 2-(2-Aminoethyl)-1,3,3-trimethylbicyclo[2.2.1]-heptane-2-thiol (14). To a solution of **12** (2.9 g, 13.7 mmol) in THF (20 mL) was added a solution of LAH (1 M in THF, 21 mL, 21 mmol). The reaction mixture was refluxed for 1.5 h. The solution was cooled to 0 °C and sodium sulfate decahydrate was added to decompose excess reducing agent. The solid was removed by filtration and washed with dichloromethane/methanol (100 mL, 4:1). The combined filtrate was dried over sodium sulfate, filtered, and evaporated. The residue was purified by column chromatography (hexane/ether 1:19), and the solid was recrystallized from ether/hexane (1:1) to give **14** (1.2 g, 41%). Mp 42–43 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.06–2.95 (m, 1H), 2.92–2.82 (m, 1H), 2.35–2.22 (m, 1H), 2.02–1.91 (m, 1H), 1.80–1.70 (m, 1H), 1.69–1.57 (m, 3H), 1.48–1.30 (m, 4H), 1.10 (s, 6H), 1.20–1.02 (m, 2H), 1.02 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.0, 54.4, 50.7, 44.8, 43.7, 41.2, 40.5, 34.6, 28.0, 26.4, 24.7, 18.2. LRMS (APIMS) m/z 214 (MH^+).

4.1.12. *N*-[2-(2-Mercapto-1,3,3-trimethylbicyclo[2.2.1]-hept-2-yl)ethyl]-4-methoxybenzamide (15). A solution of 4-dimethylaminopyridine (5 mg, 47 μmol), compound **14** (0.1 g, 0.47 mmol), and 4-methoxybenzoic acid (78 mg, 0.52 mmol) in DMF (1 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (99 mg, 0.52 mmol). The reaction mixture was stirred at room temperature overnight, diluted with ethyl acetate, washed with water, brine, and then dried over sodium sulfate. The residue, after filtration and evaporation, was purified by column chromatography (ethyl acetate/hexane 1:2) to give **15** (73 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J=8.8 Hz, 2H), 6.98 (d, J=8.8 Hz, 2H), 6.55 (t, J=6.3 Hz, 1H), 3.83 (s, 3H), 3.85–3.72 (m, 1H), 3.62–3.51 (m, 1H), 2.38–2.14 (m, 2H), 1.80–1.60 (m, 4H), 1.46–1.31 (m, 1H), 1.12 (s, 3H), 1.11 (s, 3H), 1.20–1.10 (m, 3H), 1.01 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7, 162.0, 128.6, 127.0, 113.6, 64.3, 55.5, 54.5, 50.8, 44.8, 41.3, 40.6, 39.5, 34.8, 28.2, 26.3, 24.7, 18.2.

4.1.13. 4-Methoxy-*N*-{2-[1,3,3-trimethyl-2-(nitrosothio)-bicyclo[2.2.1]hept-2-yl]ethyl}benzamide (16). To a solution of *tert*-butyl nitrite (89 μL, 68 mg, 0.66 mmol) in dichloromethane (2 mL) was added dropwise a solution of **15** (66 mg, 0.19 mmol) in dichloromethane (1 mL). The reaction mixture was stirred at room temperature in the dark

for 40 min. The solvent was evaporated and the residue was chromatographed (ethyl acetate/hexane 1:2) to give **16** (32 mg, 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J=8.8 Hz, 2H), 6.90 (d, J=8.8 Hz, 2H), 6.00 (br s, 1H), 3.85 (s, 3H), 3.57–3.35 (m, 2H), 2.99–2.76 (m, 2H), 2.15 (d, 1H), 1.88–1.62 (m, 4H), 1.62–1.45 (m, 1H), 1.45 (s, 3H), 1.31 (s, 3H), 1.4–1.15 (m, 1H), 0.96 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8, 162.1, 128.5, 126.6, 113.7, 74.3, 55.7, 55.3, 50.7, 48.5, 42.1, 39.2, 36.1, 34.0, 28.2, 25.1, 25.0, 19.4. LRMS (APIMS) m/z 377 (MH^+).

4.1.14. 2-(1,1-Dimethylprop-2-en-1-yl)adamantane-2-thiol (17). A solution of **1** (80 mg, 0.48 mmol) in THF (5 mL) was added 3,3-dimethylallylmagnesium bromide (0.12 M in THF, 12 mL, 1.44 mmol) at 0 °C under nitrogen. The reaction mixture was stirred for 15 min and the orange color disappeared gradually. Hydrogen chloride solution (1 M) was added. The organic phase was washed with water and brine, and then dried with magnesium sulfate. The THF solution was concentrated, and the residue was purified by chromatography (neat hexane) to give **17** (80 mg, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (dd, J=17.5 and 10.8 Hz, 1H), 5.03–4.93 (m, 2H), 2.75–2.71 (m, 2H), 2.56–2.52 (m, 2H), 2.18 (m, 2H), 2.02 (s, 2H), 1.86 (m, 2H), 1.72–1.55 (m, 8H), 1.35 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.8, 109.6, 65.9, 46.6, 40.5, 37.5, 36.3, 34.5, 27.4, 27.2, 26.9.

4.1.15. 2-Allyl-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl benzyl sulfide (18). A solution of **10** (10.1 g, 48.1 mmol) in THF (250 mL) was treated in one portion with sodium hydride (1.34 g of 95%, 53 mmol). After 10 min, benzyl bromide (5.8 mL, 48 mmol) was added slowly and the reaction mixture was stirred at room temperature for 3 h. Water (100 mL) was added and then THF was removed by evaporation. The aqueous phase was extracted with ethyl acetate, and the combined organic phase was washed with brine and dried over magnesium sulfate. After filtration and concentration, the residue was purified by column chromatography twice (first chromatography: hexane; second chromatography: hexane followed by dichloromethane) to give **18** (10.2 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.21 (m, 5H), 6.49–6.31 (m, 1H), 5.19–5.08 (m, 2H), 3.70 (d, J=10.5 Hz, 1H), 3.58 (d, J=10.5 Hz, 1H), 2.79–2.62 (m, 2H), 2.51–2.40 (m, 1H), 1.91–1.73 (m, 2H), 1.60–1.38 (m, 3H), 1.25 (s, 3H), 1.20 (s, 3H), 1.17 (s, 3H), 1.15–1.30 (m, 1H).

4.1.16. [2-(Benzylthio)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]acetaldehyde (19). A solution of **18** (10.2 g, 34 mmol) in a mixture of acetone (370 mL) and water (40 mL) was treated with *N*-methylmorpholine oxide (50% in water, 35 mL, 170 mmol) followed by osmium tetroxide (4% in water, 10.3 mL, 1.7 mmol). The reaction mixture was stirred at room temperature for 42 h. Then acetone was removed by evaporation, and the residue was diluted with water and extracted with ethyl acetate. The combined organic phase was washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was dissolved in a mixture of THF (79 mL) and ether (210 mL) and cooled to 0 °C. To this was added slowly a solution of periodic acid in a mixture of THF (30 mL) and ether (90 mL). The solution was stirred at 0 °C for 1 h and at room temperature for 30 min, filtered through Celite, and concentrated. After filtration and concentration, the residue was purified by column chromatography (ethyl acetate/hexane 1:9) to give **19** (5.3 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.08 (t, J=2.4 Hz, 1H), 7.31–7.19 (m, 5H), 3.69 (d, J=10.6 Hz, 1H), 3.62 (d, J=10.6 Hz, 1H), 2.85 (d, J=2.5 Hz, 2H), 2.46–2.34 (m, 1H), 1.86–1.73 (m, 2H), 1.67 (m, 1H), 1.42–1.57 (m, 2H), 1.29 (s, 3H), 1.25 (s, 3H), 1.20–1.30 (m, 1H), 1.12 (s, 3H). LRMS (APIMS) m/z 303 (MH^+).

4.1.17. 2-[2-(Benzylthio)-1,3,3-trimethylbicyclo[2.2.1]-hept-2-yl]ethanol (20). A suspension of **19** (5.3 g, 17.4 mmol) in methanol (70 mL) was treated with sodium borohydride (0.67 g, 17.4 mmol) in one portion. The reaction mixture was stirred at room temperature for 30 min. The solvent was removed by evaporation. The residue was suspended in ethyl acetate, washed with water, brine, and dried over sodium sulfate. After filtration and concentration, the residue was purified by column chromatography (ethyl acetate/hexane 1:4 then 1:3) to give **20** (4.43 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.21 (m, 5H), 4.06–3.95 (m, 1H), 3.91–3.80 (m, 1H), 3.75 (d, J=2.4 Hz, 2H), 2.56–2.43 (m, 1H), 2.32–2.22 (m, 1H), 2.19–2.00 (m, 2H), 1.83–1.72 (m, 2H), 1.53–1.36 (m, 2H), 1.20 (s, 3H), 1.18 (s, 3H), 1.11 (s, 3H), 1.30–1.10 (m, 2H). LRMS (APIMS) m/z 305 (MH^+).

4.1.18. 2-(2-Mercapto-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl)ethanol (21). A solution of **20** (4.4 g, 14.5 mmol) in ether (5 mL) was treated with liquid ammonia followed by the addition of sodium (approx 1 g) until a permanent blue color was obtained. The final reaction mixture was stirred for 45 min, and then ammonium chloride was added to disperse the blue color. Then ammonia was allowed to evaporate. The residue was partitioned between ether and water. The organic phase was washed with water, brine, and dried over sodium sulfate. The residue after filtration and evaporation was purified by column chromatography (ethyl acetate/hexane 1:4) to give **21** (2.8 g, 88%). Mp 55–60 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93–3.80 (m, 2H), 2.39–2.16 (m, 2H), 1.95 (br s, 1H), 1.82–1.50 (m, 4H), 1.34–1.47 (m, 1H), 1.11 (s, 3H), 1.05 (s, 3H), 1.03 (s, 3H), 1.00–1.23 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.01, 62.2, 54.4, 50.6, 44.8, 43.7, 40.6, 34.4, 28.2, 26.2, 24.6, 18.1. LRMS (APIMS) m/z 232 (MNH_4^+).

4.1.19. 2-[1,3,3-Trimethyl-2-(nitrosothio)bicyclo[2.2.1]-hept-2-yl]ethanol (22). A solution of **21** (0.5 g, 2.33 mmol) in a mixture of methanol (5 mL) and dichloromethane (5 mL) was cooled over ice and then treated slowly with *tert*-butyl nitrite (1 mL, 7.5 mmol). The reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 30 min. The solvent was evaporated and the residue was purified by column chromatography (ethyl acetate/hexane 1:4) to give **22** (0.51 g, 90%). Mp 83–84 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71–3.50 (m, 2H), 3.14–2.91 (m, 1H), 1.86–1.74 (m, 1H), 2.19–2.09 (m, 1H), 1.35 (s, 3H), 1.24 (s, 3H), 1.83–1.20 (m, 7H), 0.92 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 73.3, 61.8, 55.6, 50.6, 48.3, 42.0, 38.8, 33.8, 28.1, 25.1, 25.0, 19.3. LRMS (APIMS) m/z 261 (MNH_4^+).

4.1.20. 2-Allyl-1,1,3,3-tetramethylindane-2-thiol (24). 2-Indanone was tetramethylated with methyl iodide and

potassium hydroxide in DMSO, and the resultant 1,1,3,3,-tetramethyl-2-indaneone was treated with hydrazine in acetic acid and then with sulfur monochloride and triethylamine to give **23** following literature procedures.[15,16] A solution of **23** (10 g, 50 mmol) in ether (100 mL) was cooled over ice. To this was added a solution of allylmagnesium bromide (147 mL of 1 M solution in ether, 147 mmol) dropwise. The resultant solution was stirred over ice for 30 min, quenched carefully with excess 2 N HCl. The organic phase was dried over sodium sulfate and filtered. After evaporation, the residue was purified by column chromatography (ether/hexane 1:19) to give **24** (10 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.21 (m, 2H), 7.16–7.12 (m, 2H), 6.2–6.0 (m, 1H), 5.24–5.15 (m, 2H), 2.67–2.65 (m, 2H), 1.55 (s, 1H), 1.50 (s, 6H), 1.41 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0, 135.4, 127.2, 122.2, 118.1, 68.4, 50.5, 40.9, 29.1, 28.6. Anal. Calcd for $C_{16}H_{22}S$: C, 78.00; H, 9.00. Found: C, 77.86; H, 8.97.

4.1.21. 2-Allyl-1,1,3,3-tetramethyl-2-(nitrosothio)indane (25). To a solution of *tert*-butyl nitrite (405 μL, 314 mg, 3 mmol) in dichloromethane (2 mL) was added dropwise a solution of **24** (250 mg, 1 mmol) in dichloromethane (2 mL). The resultant solution was stirred at room temperature in the dark for 45 min. The volatiles were evaporated and the residue was chromatographed (ether/hexane 1:99) to give **25** (150 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.34 (m, 2H), 7.28–7.22 (m, 2H), 6.09–5.95 (m, 1H), 5.31–5.17 (m, 2H), 3.78 (d, J=6.7 Hz, 2H), 1.76 (s, 6H), 1.49 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0, 135.1, 127.6, 122.2, 118.0, 80.7, 51.6, 37.1, 29.2, 28.3. Anal. Calcd for $C_{16}H_{21}NOS$: C, 69.78; H, 7.69; N, 5.09. Found: C, 69.65; H, 7.69; N, 4.82.

4.1.22. *S*-(2-Allyl-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-inden-2-yl)ethanethioate (26). A solution of **24** (9 g, 36.6 mmol) in pyridine (189 mL, 185 g, 2.3 mol) was cooled over ice and treated dropwise with acetic anhydride (110 mL, 119 g, 1.17 mol) and 4-dimethylaminopyridine (0.5 g). The crude reaction mixture was stirred at room temperature for 12 h. The volatile material was evaporated and the residue was chromatographed (ether/hexane 1:19) to give **26** (8.1 g, 77%). Mp 65–67 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19–7.24 (m, 2H), 7.11–7.06 (m, 2H), 6.02–5.85 (m, 1H), 5.17–5.00 (m, 2H), 3.19 (d, J=6.6 Hz, 2H), 2.23 (s, 3H), 1.51 (s, 6H), 1.43 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.5, 149.2, 136.5, 127.7, 122.4, 117.0, 51.7, 34.8, 31.8, 29.3, 28.4. Anal. Calcd for $C_{18}H_{24}OS$: C, 74.95; H, 8.39. Found: C, 74.76; H, 8.38.

4.1.23. *S*-[1,1,3,3-Tetramethyl-2-(2-oxoethyl)-2,3-dihydro-1*H*-inden-2-yl]ethanethioate (27). A mixture of *N*-methylmorpholine *N*-oxide (50% in water, 31 mL, 131 mmol) and **26** (8 g, 26 mmol) in water (100 mL) was treated with acetone to give a homogeneous solution (approx 350 mL). Osmium tetroxide (8 mL of 4% aqueous solution, 1.31 mmol) was introduced and the resulting solution was stirred at room temperature overnight. The volume was reduced by evaporation and the residue was diluted with more water and then extracted with ethyl acetate followed by dichloromethane. The combined organic phases were dried over sodium sulfate, filtered, and concentrated. The residue was dissolved in 240 mL of 3:1 ether/THF and cooled over ice under nitrogen. Periodic acid (9 g, 39 mmol) was added in portions over 20 min. The reaction mixture was stirred over ice for 1 h and at room temperature for 40 min. The solid was removed by filtration through Celite, and the filtrate was washed with water, brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (ethyl acetate/hexane 1:19) to give **27** (2 g, 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.73 (t, J=2.5 Hz, 1H), 7.25–7.19 (m, 2H), 7.11–7.06 (m, 2H), 3.32 (d, J=2.5 Hz, 2H), 2.31 (s, 3H), 1.46 (s, 6H), 1.42 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.6, 196.1, 147.5, 127.7, 122.2, 71.6, 51.4, 45.0, 31.4, 29.3, 27.6. Anal. Calcd for $C_{17}H_{22}O_2S$: C, 70.31; H, 7.64. Found: C, 70.02; H, 7.69. LRMS (APIMS) m/z 291 (MH^+).

4.1.24. 2-(2-Mercapto-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-inden-2-yl)ethanol (28). A solution of **27** (2.07 g, 7.12 mmol) in THF (80 mL) was cooled over ice and a solution of LAH (1 M in THF, 14.2 mL, 14.2 mmol) was added dropwise. The ice bath was removed and the resultant solution was stirred at room temperature for 45 min. Sodium sulfate decahydrate was added to decompose excess reducing agent. The reaction mixture was filtered and the solid was washed with dichloromethane/methanol 4:1. The filtrate was dried over sodium sulfate and filtered. After evaporation the residue was chromatographed (ethyl acetate/hexane 1:4) to give **28** (1.04 g, 58%). Mp 85–87 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.21 (m, 2H), 7.15–7.10 (m, 2H), 4.01 (br s, 2H), 2.20–2.15 (m, 2H), 1.87 (br s, 1H), 1.50 (s, 6H), 1.38 (s, 6H), 1.32 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.5, 127.2, 122.2, 67.9, 60.4, 50.5, 39.0, 29.3, 28.3. LRMS (APIMS) m/z 268 (MNH_4^+).

4.1.25. 2-[1,1,3,3-Tetramethyl-2-(nitrosothio)-2,3-dihydro-1*H*-inden-2-yl]ethanol (29). An ice cooled solution of **28** (1.04 g, 4.15 mmol) in a mixture of dichloromethane/methanol (20 mL, 1:1) was treated dropwise with *tert*-butyl nitrite (2.5 mL, 19 mmol). The reaction mixture was stirred at 0 °C for 15 min and then at room temperature for 30 min. The residue after evaporation was purified by column chromatography (ethyl acetate/hexane 1:4) to give **29** (1.05 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.21 (m, 2H), 7.15–7.10 (m, 2H), 3.86 (t, J=7.5 Hz, 2H), 3.18–3.13 (m, 2H), 1.63 (s, 6H), 1.51 (s, 1H), 1.30 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6, 127.6, 122.2, 80.2, 60.0, 51.3, 35.5, 29.3, 28.1. Anal. Calcd for $C_{15}H_{21}NO_2S$: C, 64.48; H, 7.58; N, 5.01. Found: C, 64.45; H, 7.67; N, 4.67. LRMS (APIMS) m/z 297 (MNH_4^+).

4.1.26. (2-Mercapto-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-inden-2-yl)acetonitrile (30). A solution of *n*-butyl lithium (2.5 M in hexane, 29.4 mL, 73.4 mmol) was cooled to −78 °C, and to it was added dropwise a solution of acetonitrile (3.8 mL, 73.4 mmol) in THF (98 mL). The suspension was stirred at −78 °C for 1 h. A solution of **23**[15,16] (6 g, 29.4 mmol) in THF (49 mL) was added in one portion. The resulting solution was stirred at −78 °C for 1 h, quenched with water, and then THF was evaporated. The residue was treated with ethyl acetate and then with water, and the aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with water and then brine, dried over sodium sulfate, filtered, and evaporated.

The residue was purified by column chromatography twice (ethyl acetate/hexane 1:9 each time) to give **30** (5 g, 69%). Mp 113–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.26 (m, 2H), 7.19–7.14 (m, 2H), 2.83 (m, 2H), 1.85 (s, 1H), 1.55 (s, 6H), 1.44 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.2, 127.8, 122.3, 118.1, 64.0, 50.0, 29.2, 28.1, 27.3. Anal. Calcd for $C_{15}H_{19}NS$: C, 73.42; H, 7.80; N, 5.71. Found: C, 73.18; H, 7.75; N, 5.62. LRMS (APIMS) *m/z* 263 (MNH_4^+).

4.1.27. [1,1,3,3-Tetramethyl-2-(nitrosothio)-2,3-dihydro-1*H*-inden-2-yl]acetonitrile (31). To a solution of *tert*-butyl nitrite (325 μL, 251 mg, 2.4 mmol) in dichloromethane (3 mL) was added **30** (200 mg, 0.82 mmol) dropwise as a solution in dichloromethane (2 mL). The resultant solution was stirred in the dark for 40 min. The solvent was evaporated, and the residue was chromatographed (ethyl acetate/hexane 1:9). The fractions containing the product were pooled and concentrated, and hexane added. After standing overnight at 4 °C, the solid was filtered to give **31** (0.1 g, 45%). Mp 67–69 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.30 (m, 2H), 7.28–7.21 (m, 2H), 3.86 (s, 2H), 1.72 (s, 6H), 1.43 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.1, 128.4, 122.4, 117.6, 73.8, 51.6, 30.1, 27.1, 24.5. LRMS (APIMS) *m/z* 292 (MNH_4^+).

4.1.28. (2-Mercapto-1,1,3,3-tetramethyl-2,3-dihydro-1*H*-inden-2-yl)acetic acid (32). A solution of **30** (0.5 g, 2.1 mmol) in concentrated HCl (10 mL) and acetic acid (10 mL) was refluxed for 52 h. The crude reaction mixture was allowed to cool to room temperature and then extracted with ethyl acetate. The organic phase was washed with water twice and then extracted with saturated sodium bicarbonate. The basic aqueous phase was acidified to pH 2 with concentrated HCl. The resulting solution was then extracted with dichloromethane, and the combined organic phase was dried over sodium sulfate, filtered, and concentrated to give **32** (240 mg). The ethyl acetate phase after basification also contained some product. This ethyl acetate solution was dried with sodium sulfate, filtered, and concentrated and chromatographed (ethyl acetate/hexane 1:1) to give more **32** (120 mg, 360 mg total, 66%). Mp 159–161 °C. ^{1}H NMR ($CDCl_3$) δ 7.28–7.24 (m, 2H), 7.19–7.15 (m, 2H), 2.97 (s, 2H), 2.06 (s, 1H), 1.58 (s, 6H), 1.42 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 177.9, 148.1, 127.4, 122.5, 65.0, 50.9, 41.6, 29.5, 27.5. LRMS (APIMS) *m/z* 282 (MNH_4^+).

4.1.29. [1,1,3,3-Tetramethyl-2-(nitrosothio)-2,3-dihydro-1*H*-inden-2-yl]acetic acid (33). To a solution of *tert*-butyl nitrite (169 μL, 130 mg, 1.27 mmol) in dichloromethane (4 mL) was added **32** (112 mg, 0.42 mmol) in one portion as a solid. The solution was stirred for 45 min in the dark, and the solvent was evaporated. The solid was dissolved in a minimum amount of hot ether, and three volumes of hot hexane were added. The solution was allowed to stand at 4 °C overnight and the solid was collected by filtration to give **33** (75 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.20 (m, 2H), 7.15–7.10 (m, 2H), 3.89 (s, 2H), 1.63 (s, 6H), 1.61 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.1, 127.7, 122.4, 52.0, 37.1, 29.6, 27.7. Anal. Calcd for $C_{15}H_{19}NO_3S$: C, 61.41; H, 6.53; N, 4.77. Found: C, 61.19; H, 6.70; N, 4.50. LRMS (APIMS) *m/z* 294 (MH^+), 311 (MNH_4^+), 292 ($M-H^-$).

4.1.30. 3-Butyl-3-mercapto-4,4-dimethylpentanenitrile (35). A solution of *n*-butyl lithium (2.5 M in hexane, 25.3 mL, 63.2 mmol) was cooled to −78 °C, and to it was added a solution of acetonitrile (3.3 mL, 63.2 mmol) in THF (98 mL). The reaction mixture was stirred at −78 °C for 1 h, and then a solution of **34**, which was prepared following literature procedure,[17] was added in one portion. The reaction mixture was stirred at room temperature for 1 h and quenched carefully with 2 N HCl, and then THF was removed by evaporation. The residue was diluted with water and extracted with ethyl acetate. The combined organic phase was washed with brine and dried over sodium sulfate. After filtration and concentration, the residue was purified by column chromatography (ethyl acetate/hexane 1:9) to give **35** (3.5 g, 84%). Mp 154–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.86 (s, 2H), 1.66 (s, 1H), 1.29 (s, 18H). LRMS (APIMS) *m/z* 217 (MNH_4^+).

4.1.31. 3-Butyl-4,4-dimethyl-3-(nitrosothio)pentanenitrile (36). A solution of **35** (200 mg, 1 mmol) in dichloromethane (5 mL) was treated with *tert*-butyl nitrite (160 μL, 123 mg, 1.2 mmol). The reaction mixture was stirred at room temperature for 30 min. The solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate/hexane 1:9) to give **36** (210 mg, 92%). Mp 92–93 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (s, 2H), 1.36 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 119.5, 73.1, 43.1, 30.3, 24.3. LRMS (APIMS) *m/z* 246 (MNH_4^+).

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.019.

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Synthesis of mollugin

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Abstract—The total synthesis of mollugin, a major constituent of rubiaceous herbs, using a straightforward synthetic approach starting from 1,4-naphthoquinone via a sequence of reactions, including selective prenylation, epoxidation, reduction of the quinone moiety, acid-catalysed ring expansion, bromination, dehydration and methoxycarbonylation is presented.

1. Introduction

The natural product mollugin **1** was isolated first from the rhizome of *Galium mollugo* (Rubiaceae) and identified as methyl 2,2-dimethyl-6-hydroxy-2*H*-naphtho[1,2-*b*]pyran-5-carboxylate.[1] Later, mollugin **1** together with several structurally related compounds such as 3-hydroxymollugin **2**, *cis*-3,4-dihydro-3,4-dihydroxymollugin **3a** and *trans*-3,4-dihydro-3,4-dihydroxymollugin **3b**[2] and also methyl 2,3-epoxy-3-prenyl-1,4-dioxonaphthalene-2-carboxylate **4**, a precursor in the biosynthesis of mollugin via the shikimate biosynthetic pathway,[3] have been reported as major constituents in many rubiaceous herbs including *Putoria calabrica*,[4] *Rubia cordifolia*,[5] *Rubia oncotricha*,[6] *Pentas longiflora*,[2b] *Rubia lanceolata*[7] and *Rubia tinctorum*.[8]

Mollugin **1** has been shown to possess antitumor activity,[5a] antimutagenic activity[8,9] and antiviral activity[10] against the hepatitis B virus (Fig. 1). Strong inhibition of arachidonic acid (AA)-induced and collagen-induced platelet aggregation has been shown for mollugin **1**.[11] As a result, many rubiaceous herbs are of great importance in the Chinese folk medicine for their blood circulation promoting, expectorant, cough-healing and antitumor properties.[5c,6,8] Particularly, in view of the antitumor activities of mollugin **1**, there is a renewed interest in the synthesis of 2*H*-naphtho[1,2-*b*]pyran-5-carboxylate derivatives related to mollugin **1**. Mollugin was synthesised previously according to the synthetic plan in Scheme 1. Condensation of diethyl 3,6-dihydroxyphthalate **5** and diethyl succinate **6** followed by acid-catalysed decarboxylation afforded 1,4-dihydroxynaphthalene-2-carboxylic acid **8**, which was further elaborated to mollugin **1** by reaction with 3-chloro-3-methyl-1-butyne in the presence of aluminium(III) chloride and subsequent esterification of the intermediate molluginic acid **9** with diazomethane.[12] This synthesis, although basically only four steps long, suffers from the disadvantage of a low overall yield, originating mainly from the variable yields of the double Claisen condensation (often as low as 5% and mounting to 48%, pointing to a tricky reaction).

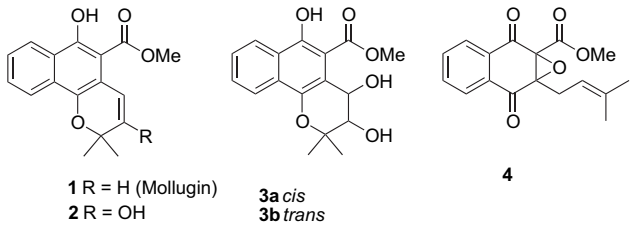

Figure 1.

Mollugin has also been synthesised starting from 1,4-dihydroxynaphthalene-2-carboxylic acid **8**. 3,4-Dihydromollugin **11** as a key step intermediate was obtained after electrophilic aromatic substitution of 2-methyl-3-buten-2-ol onto methyl 1,4-dihydroxynaphthalene-2-carboxylate **10**. Subsequent pyran ring closure in the presence of $BF_3 \cdot OEt_2$ resulted in 3,4-dihydromollugin **11** in 54% yield. Reflux of 3,4-dihydromollugin **11** in dioxane in the presence of DDQ yielded mollugin **1** in 72%.[13] Very recently, mollugin has been synthesised by treating methyl 3-(3-methyl-but-2-enyl)-1,4-dioxo-1,4-dihydro-naphthalene-2-carboxylate with triethylamine, which gave rise to an oxa-6π pericyclic reaction and subsequent formation of mollugin **1**.[14]

In this paper, the total synthesis of mollugin **1** is presented using a straightforward synthetic approach starting from

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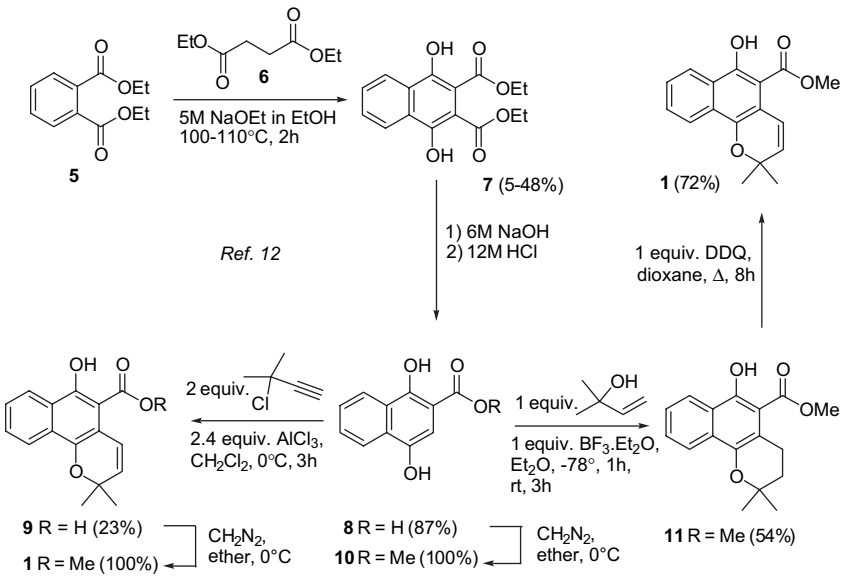


Scheme 1. Previous synthetic approach.

1,4-naphthoquinone **12** via reductive acid-catalysed intramolecular cycylisation of 2-prenyl-1,4-naphthoquinone **13** as a key step for the construction of the 2*H*-naphtho[1,2-*b*]-pyran skeleton.

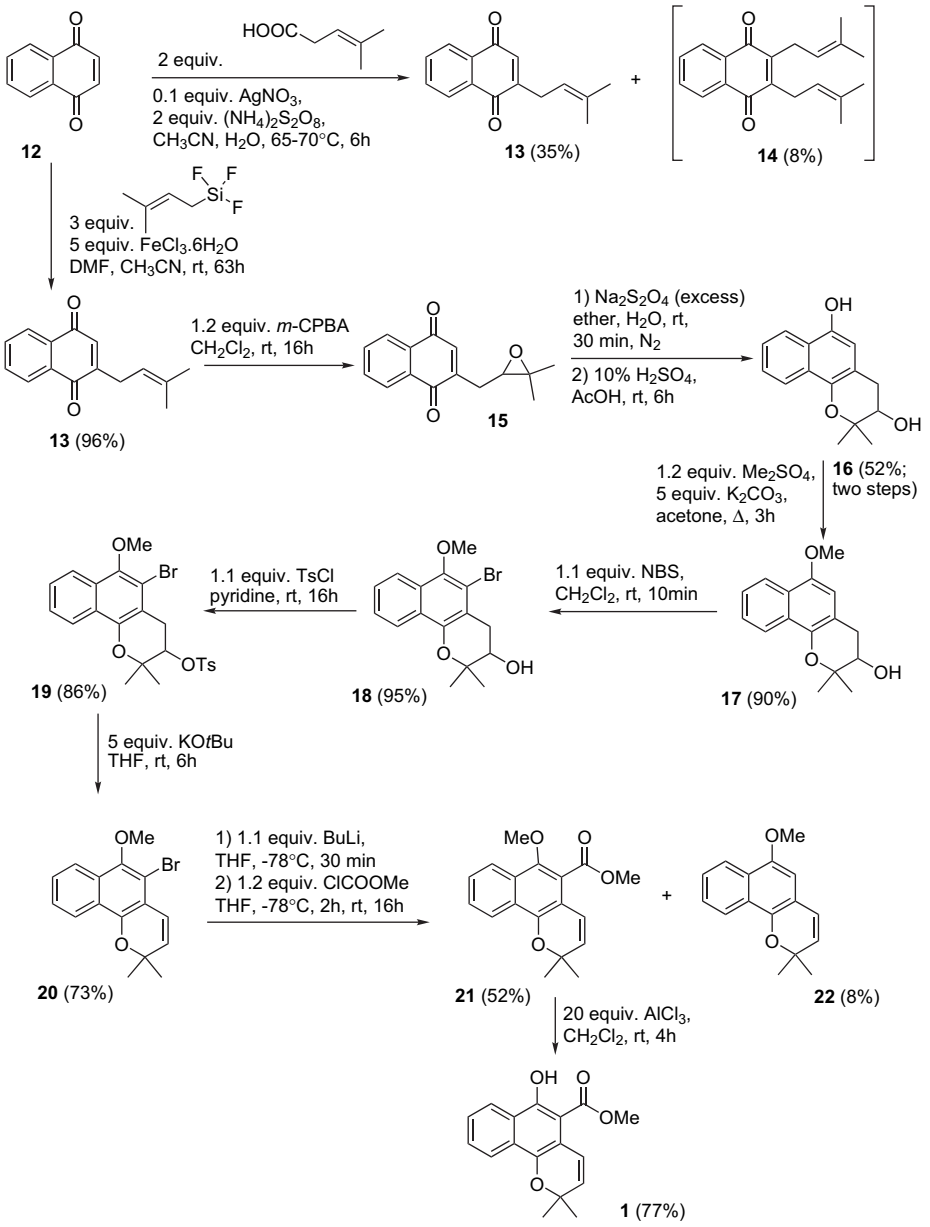
2. Results and discussion

The synthesis of mollugin **1** is presented in Scheme 2. 2-Prenyl-1,4-naphthoquinone **13**, the starting material for the synthesis of mollugin **1**, was reported previously to be available in a yield of 58% by radical prenylation of 1,4-naphthoquinone **12** with 4-methyl-3-pentenoic acid[15] in the presence of silver nitrate and ammonium persulfate.[16] In our hands, using these published reaction conditions, mixtures of starting material **12** together with the monoprenylated and diprenylated 1,4-naphthoquinones **13** and **14** were obtained in variable ratios depending on the applied reaction conditions. Optimisation of the reaction using an excess of allylating carboxylic acid, which was added in portions during the reaction, and purification of the resulting reaction mixtures via chromatography and subsequent recrystallisation afforded 2-prenyl-1,4-naphthoquinone **13** in a maximum yield of 35%. On the other hand, according to a recently published procedure for the monoallylation of quinones, reaction of 1,4-naphthoquinone **12** with prenyltrifluorosilane in the presence of $FeCl_3 \cdot 6H_2O$ as a Lewis acid, afforded the monoprenylated 1,4-naphthoquinone **13** in 98%.[17] Chemoselective epoxidation of the prenylic double bond with *m*-chloroperbenzoic acid in dichloromethane and subsequent reduction of the intermediate epoxide **15** using sodium dithionite in a biphase system with ether and water, followed by acid-catalysed intramolecular cyclisation of the intermediate hydroquinone epoxide with a solution of 10% sulfuric acid in acetic acid afforded 2*H*-naphtho[1,2-*b*]pyran-3,6-diol **16** in an overall yield of 52% (two steps). This diol **16**, which already reveals the basic skeleton of mollugin, was further elaborated via protection of the phenolic hydroxyl group as the methyl ether with dimethyl sulfate and potassium carbonate in refluxing acetone. Bromination of **17** in *ortho*-position of the methoxy substituent with *N*-bromosuccinimide in dichloromethane gave the brominated derivative **18** in 95% yield. However, the dehydration of alcohol **18**, with the objective of introducing a double bond between C(3) and C(4) in the pyran ring was quite troublesome. The alcoholic function of **18**, which is of a neopentylic nature could not be forced to dehydrate under acid-catalysed conditions using either *p*-toluenesulfonic acid or dry oxalic acid in refluxing anhydrous benzene or upon treatment with concentrated hydrochloric acid or sulfuric acid in acetic acid. The dehydration could, however, be accomplished via tosylation of alcohol **18** and subsequent treatment of the tosylate **19** with potassium *tert*-butoxide in dry THF to afford the dehydrated derivative **20** in 73% yield. For the introduction of the methoxycarbonyl group, compound **20** was treated with *n*-butyllithium in dry THF at −78 °C to afford, via bromine–lithium exchange and trapping of the intermediate lithium salt with methyl chloroformate, a mixture of the desired ester **21** in 52% yield, together with 8% of the debrominated naphthopyrane **22**.[18] Treatment of the ester **21** with excess aluminium(III) chloride finally gave mollugin **1** in 77% yield. Recrystallisation from methanol afforded mollugin **1** as yellow-green flakes with physical and spectral data identical to those of the natural product.[1] In this way, mollugin **1** was prepared in 10 steps in a total yield of 11% from 1,4-naphthoquinone **12**.

3. Experimental

3.1. General

^{1}H NMR (270 MHz) and ^{13}C NMR (68 MHz) peak assignments were performed with the aid of the DEPT technique, 2D COSY spectra and HETCOR spectra. Dry tetrahydrofuran (THF) was obtained by distillation from sodium. Diethylether was dried and distilled from sodium. Other solvents were used as received from the supplier.



Scheme 2.

3.1.1. 2-(3-Methyl-2-butenyl)-1,4-naphthoquinone (13). Method A:[15] a solution of 1,4-naphthoquinone (**12**) (0.09 mol, 15.94 g), 4-methyl-3-pentenoic acid[15] (0.09 mol, 12.83 g of technical grade, i.e., 80% pure) and silver nitrate (9 mmol, 2 g) in acetonitrile (100 ml) and demineralised water (200 ml) were heated at 65–70 °C, and to the stirred solution was added dropwise, over a period of 2 h, a solution of ammonium persulfate (0.09 mol, 20.52 g) in demineralised water (50 ml). Stirring was continued at the same temperature for 1 h. A second portion of 4-methyl-3-pentenoic acid (0.045 mol, 6.40 g of 80% technical grade) was added and to the resulting mixture, a second portion of ammonium persulfate (0.09 mol, 20.52 g) in demineralised water (50 ml) was added dropwise over a period of 2 h and the stirred mixture was kept at the same temperature for 1 h. Afterwards the reaction mixture was cooled to room temperature, poured in water (500 ml) and extracted with ethyl acetate. The combined organic extracts were washed with a saturated solution of sodium hydrogen carbonate, dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography over a short column of silica gel using ethyl acetate/petroleum ether (1:9) as eluent gave first a residual amount of 1,4-naphthoquinone (**10**) followed by a mixture of 2-(3-methyl-2-butenyl)-1,4-naphthoquinone (**13**) and 2,3-bis(3-methyl-2-butenyl)-1,4-naphthoquinone (**14**), which eluted together as a second fraction from the column (R_f=0.35). The latter fraction was further purified by recrystallisation from petroleum ether to afford *2-(3-Methyl-2-butenyl)-1,4-naphthoquinone* **13** (7.12 g, 35%) as yellow needles, mp 58–58.5 °C (lit.[16] mp 60–61 °C). ^{1}H NMR ($CDCl_3$): δ 1.67 (3H, s, CH_3), 1.79 (3H, s, CH_3), 3.28 (2H, m, CH_2), 5.19–5.26 (1H, m, CH=CMe_2), 6.77 (1H, t, J=1.6 Hz, H-3), 7.69–7.76 (2H, m, H-6 and H-7), 8.04–8.12 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 17.81 (CH_3), 25.79 (CH_3), 28.01 (CH_2), 118.29 (CH=Me_2), 126.02 and 126.50 (C-5 and C-8), 132.15 (=C_{quat}), 132.33

(=C_{quat}), 133.55 and 133.60 (C-6 and C-7), 134.61 (C-3), 136.33 (=C_{quat}), 150.76 (=C_{quat}), 185.23 (2×C=O). IR (NaCl): ν_{max} 1659 (C=O), 1619 (C=O), 1595 (C=C) cm^{-1}. MS *m/z* (%): 226 (M+, 24), 211 (46), 183 (9), 146 (62), 41 (100). Anal. Calcd for $C_{15}H_{14}O_2$: C 79.62%, H 6.24%. Found: C 79.48%, H 6.33%

3.1.2. 2,3-Bis(3-Methyl-but-2-enyl)-1,4-naphthoquinone (14). ^{1}H NMR ($CDCl_3$): δ 1.68 (6H, d, J=1.3 Hz, 2×CH_3), 1.79 (6H, s, 2×CH_3), 3.36 (4H, d, J=6.9 Hz, 2×CH_2), 5.01 (2H, t×q, J=6.9, 1.3 Hz, 2×=CH), 7.68 (2H, m, 2×CH_{ar}), 8.07 (2H, m, 2×CH_{ar}). ^{13}C NMR ($CDCl_3$): δ 18.18 (2×CH_3), 25.87 (2×CH_3), 2.14 (2×CH_2), 119.94 (2×CH), 126.30 (2×CH_{ar}), 132.29 (2×C_{quat}), 133.39 (2×CH_{ar}), 133.89 (2×C_{quat}), 146.04 (2×C_{quat}), 185.26 (2×C=O). IR (KBr): ν_{max} 1659 (C=O), 1614 (C=C), 1592 (C=C). MS (ES^+) *m/z* (%): 295 ($M+H^+$, 100). Anal. Calcd for $C_{20}H_{22}O_2$: C 81.60%, H 7.53%. Found: C 81.48%, H 7.61%

Method B:[17] to a mixture of 1,4-naphthoquinone (**12**) (0.63 mmol, 100 mg) and $FeCl_3 \cdot H_2O$ (3 mmol, 810 mg) in DMF (9 ml) and acetonitrile (3 ml), was added prenyltrifluorosilane (1.8 mmol, 300 mg) and the mixture was stirred overnight at room temperature. The reaction mixture was poured in water and extracted with ethyl acetate. The extract was washed with water and dried ($MgSO_4$). Evaporation of the solvent in vacuo and purification of the residue by means of flash chromatography on silica gel using ethyl acetate/petroleum ether (1/9) as eluent gave **13** (140 mg, 96%) as a pale yellow solid.

3.1.3. 2-(2,3-Epoxy-3-methylbutyl)-1,4-naphthoquinone (15). To a cooled (0 °C) solution of 2-prenyl-1,4-naphthoquinone (**13**) (1 mmol, 0.23 g) in dichloromethane (10 ml) was added *m*-chloroperbenzoic acid (1.2 mmol, 0.28 g) and the mixture was stirred for 16 h at room temperature. The solvent was evaporated in vacuo and the residual white-yellow solid was dissolved in ether, washed with 2 M sodium hydroxide and then with water, dried ($MgSO_4$) and evaporated in vacuo to afford the crude epoxide **15** (170 mg, 70%, purity>95%) as a yellow oil, which was used as such in the next step. ^{1}H NMR ($CDCl_3$): δ 1.37 (6H, s, 2×CH_3), 2.63–2.73 (1H, m, C$H_a$$H_b$), 2.91–3.04 (2H, m, $CH_a$$H_b$ and CH–O), 6.93 (1H, s, H-3), 7.70–7.77 (2H, m, H-6 and H-7), 8.02–8.11 (2H, m, H-5 and H-8). ^{13}C NMR ($CDCl_3$): δ 18.83, 24.65, 29.33, 58.65, 61.45, 126.11, 126.56, 132.00, 133.69, 133.82, 135.85, 147.90, 184.62, 184.83. IR (NaCl): ν_{max} 1660 (C=O), 1623 (C=O). MS *m/z* (%): 242 (M+, 2), 184 (100), 156 (50).

3.1.4. 2,2-Dimethyl-3,4-dihydro-2*H*-naphtho[1,2-*b*]-pyran-3,6-diol (16). To a cooled (0 °C) solution of 2-prenyl-1,4-naphthoquinone (**13**) (10 mmol, 2.3 g) in dichloromethane (100 ml) was added *m*-chloroperbenzoic acid (12 mmol, 2,8 g) and the mixture was stirred for 16 h at room temperature. The solvent was evaporated in vacuo and the residual solid was dissolved in ether (100 ml), washed with 2 M sodium hydroxide and the ether solution, under a nitrogen atmosphere, was vigourously stirred for 30 min with a 20% solution of sodium dithionite in water (100 ml), while the yellow colour of the solution slowly became pale. The ether phase was separated by decantation and evaporated in vacuo. The residue was dissolved in acetic acid (100 ml) and the stirred solution was mixed with a 10% solution of sulfuric acid in water (50 ml), while stirring was continued for 6 h under a nitrogen atmosphere. The reaction mixture was poured in water and extracted with dichloromethane. The combined organic extracts were washed with a saturated solution of sodium hydrogen carbonate and then with brine, dried ($MgSO_4$) and concentrated in vacuo to a residual volume of 30 ml. The solution was kept overnight at −20 °C causing product **16** to precipitate as small white transparent cubes (1.26 g, 52%), mp 173.5–174.8 °C. ^{1}H NMR (acetone-d_6): δ 1.34 (3H, s, CH_3), 1.49 (3H, CH_3), 2.79 (1H, dd, J_{AB}=16.7 Hz, J_d=7.6 Hz, C$H_a$$H_b$), 3.06 (1H, dd, J_{AB}=16.7 Hz, J_d=5.6 Hz, $CH_a$$H_b$), 3.88–3.95 (1H, m, CH–OH), 4.26 (1H, d, J=5.6 Hz, CH–OH), 6.64 (1H, s, H-5), 7.43–7.51 (2H, m, H-8 and H-9), 8.12–8.19 (2H, m, H-7 and H-10), 8.43 (1H, s, phenolic-OH). ^{13}C NMR (acetone-d_6): δ 20.02 (CH_3), 26.19 (CH_3), 32.51 (CH_2), 70.22 (CHOH), 77.63 (CMe_2), 10.61 (C-5), 114.77 (=C_{quat}), 122.08 and 122.75 (C-7 and C-10), 125.08 and 125.95 (C-8 and C-9), 125.62 (=C_{quat}), 126.92 (=C_{quat}), 141.23 (=C–O), 146.97 (=C–O). IR (KBr): ν_{max} 3511 (OH), 3236 (OH), 1639, 1600, 1107, 1335, 1272, 1140, 1052, 766 cm^{-1}. MS *m/z* (%): 244 (M+, 63), 211 (49), 173 (81), 43 (100). Anal. Calcd for $C_{15}H_{16}O_3$: C 73.75%, H 6.60%. Found: C 73.42%, H 6.66%.

3.1.5. Synthesis of 6-methoxy-2,2-dimethyl-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-3-ol (17). A mixture of 2,2-dimethyl-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-3,6-diol (**16**) (4.5 mmol, 1.1 g), dimethyl sulfate (5.4 mmol, 0.68 g) and potassium carbonate (22.5 mmol, 3.1 g) in acetone (50 ml) was heated under reflux for 3 h, cooled to room temperature, filtered and evaporated in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (1:4) gave pure **17** (1.05 g, 90%). Recrystallisation from ethyl acetate/petroleum ether (1:9) afforded **17** as white cubes, mp 122.3–122.8 °C. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, s, CH_3), 1.46 (3H, s, CH_3), 1.92 (1H, br s, OH), 2.85 (1H, dd, J_{AB}=17.2 Hz, J=4.6 Hz, C$H_a$$H_b$), 3.16 (1H, dd, J_{AB}=17.2 Hz, J=4.8 Hz, $CH_a$$H_b$), 3.85–3.89 (1H, m, CH–OH), 3.94 (3H, s, MeO), 6.45 (1H, s, H-5), 7.44–7.49 (2H, m, H-8 and H-9), 8.14–8.19 (2H, m, H-7 and H-10). ^{13}C NMR ($CDCl_3$): δ 22.32 (CH_3), 24.47 (CH_3), 32.15 (CH_2), 55.67 (MeO), 69.83 (CH–OH), 76.71 (CMe_2), 105.21 (C-5), 111.52 (=C_{quat}), 121.52 and 121.69 (C-7 and C-10), 125.21 and 125.87 (C-8 and C-9), 125.42 (=C_{quat}), 126.18 (=C_{quat}), 141.26 (=C–O), 149.27 (=C–O). IR (KBr): ν_{max} 3313 (OH), 1631, 1598, 1458, 1387, 1273, 769 cm^{-1}. MS *m/z* (%): 258 (M+, 6), 200 (19), 105 (100). Anal. Calcd for $C_{16}H_{18}O_3$: C 74.39%, H 7.02%. Found: C 74.28%, H 7.13%.

3.1.6. 5-Bromo-6-methoxy-2,2-dimethyl-2,3-dihydro-2*H*-naphtho[1,2-*b*]-pyran-3-ol (18). 6-Methoxy-2,2-dimethyl-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-3-ol (**17**) (2 mmol, 0.51 g) was dissolved in dichloromethane (50 ml, dichloromethane was washed twice with concentrated sulfuric acid and filtered over sodium carbonate before use, to eliminate all traces of ethanol) and to this stirred solution, *N*-bromosuccinimide (2.2 mmol, 0.39 g) was added portionwise over a period of 10 min. The organic solution was washed successively with water, 5% solution of sodium hydrogen sulfite, saturated solution of sodium hydrogen carbonate

and finally with brine, dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (1:4) afforded **18** (0.64 g, 95%) as a brown oil, which slowly solidified, mp 87–88 °C. ^{1}H NMR ($CDCl_3$): δ 1.36 (3H, s, CH_3), 1.43 (3H, s, CH_3), 2.09 (1H, br s, OH), 2.89 (1H, dd, J_{AB}=17.5 Hz, J=5.0 Hz, CH_aH$_b$), 3.10 (1H, dd, J_{AB}=17.5 Hz, J=5.3 Hz, CH$_a$$H_b$), 3.88 (1H, m, CH–OH), 3.93 (3H, s, MeO), 7.44–7.52 (2H, m, H-8 and H-9), 8.00–8.03 and 8.18–8.21 (each 1H, each m, H-7 and H-10). ^{13}C NMR ($CDCl_3$): δ 21.92 (CH_3), 24.40 (CH_3), 33.35 (CH_2), 61.28 (MeO), 77.10 (CMe_2), 112.88 (=C_{quat}), 116.12 (=C_{quat}), 121.74 and 122.30 (C-7 and C-10), 125.32 (=C_{quat}), 125.82 and 126.72 (C-8 and C-9), 127.62 (=C_{quat}), 145.08 (=C–O), 146.83 (=C–O). IR (KBr): ν_{max} 3313 (OH), 1574, 1450, 1366, 1350, 1142, 1082, 991, 767 cm^{-1}. MS *m/z* (%): 336/8 (M+, 40), 303/5 (17), 265/7 (28), 264/6 (33), 49 (100). Anal. Calcd for $C_{16}H_{17}BrO_3$: C 56.99%, H 5.08%. Found: C 57.28%, H 5.01%.

3.1.7. 5-Bromo-6-methoxy-2,2-dimethyl-3-tosyloxy-3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran (19). A solution of 5-bromo-6-methoxy-2,2-dimethyl-2,3-dihydro-2*H*-naphtho-[1,2-*b*]-pyran-3-ol (**18**) (1.9 mmol, 0.64 g), and *p*-toluenesulfonyl chloride (2.1 mmol, 0.40 g) in pyridine (5 ml) was stirred for 16 h in a flask fitted with a calcium chloride tube. The solution was diluted with ether (100 ml), washed twice with 2 M HCl and then with a saturated solution of sodium hydrogen carbonate and then with brine, dried ($MgSO_4$) and evaporated in vacuo to afford **17** (0.80 g, 86%, purity>95%) as a brown oil, which was used without purification in the next step. An analytical sample of compound **19** was obtained using chromatography on silica gel with ethyl acetate/petroleum ether (1:4) as eluent to afford **19** as a light brown oil, which slowly solidified, mp 124 °C. ^{1}H NMR ($CDCl_3$): δ 1.32 (3H, s, CH_3), 1.36 (3H, s, CH_3), 2.45 (3H, s, CH_3-Ar), 2.87 (1H, dd, J_{AB}=17.8 Hz, J_d=6,1 Hz, CH_aH$_b$), 3.15 (1H, dd, J_{AB}=17.8 Hz, J_d=5.3 Hz, CH$_a$$H_b$), 3.92 (3H, s, MeO), 4.75 (1H, t, $J\approx$6 Hz, CH-OTs), 7.34 (2H, d, J=7.9 Hz, 2×=CH), 7.44–7.54 (2H, m, H-8 and H-9), 7.80 (2H, d, J=8.3 Hz, 2×=CH), 7.99–8.15 (2H, m, H-7 and H-10). ^{13}C NMR ($CDCl_3$): δ 21.67 (CH_3), 21.79 (CH_3), 24.67 (CH_3), 30.73 (CH_2), 61.28 (MeO), 75.27 (CH-OTs), 78.87 (CMe_2), 111.62 (=C_{quat}), 115.15 (C_{quat}), 121.76 and 122.23 (C-7 and C-10), 125.12 (=C_{quat}), 125.96 and 126.92 (C-8 and C-9), 127.71 (=C_{quat}), 127.85 (2×=CH), 129.94 (2×CH), 133.78 (=C_{quat}), 144.74 (=C_{quat}), 145.10 (=C–O), 147.01 (=C–O). IR (KBr): ν_{max} 1571, 1450, 1350, 1189, 906, 863, 765 cm^{-1}. MS *m/z* (%): 490/2 (M+, 40), 336/8 (12), 318/20 (23), 303/5 (62), 264/6 (30), 224 (30), 91 (53), 43 (100). Anal. Calcd for $C_{23}H_{23}BrO_5S$: C 56.22%, H 4.72%. Found: C 56.11%, H 4.91%.

3.1.8. 5-Bromo-6-methoxy-2,2-dimethyl-2*H*-naphtho-[1,2-*b*]pyran (20). To a cooled (0 °C) solution of 5-bromo-6-methoxy-2,2-dimethyl-3-tosyloxy-3,4-dihydro-2*H*-naphtho-[1,2-*b*]pyran (**19**) (1.3 mmol, 0.66 g) in dry tetrahydrofuran (20 ml) was added potassium *tert*-butoxide (6.5 mmol, 0.73 g), and the reaction mixture was kept at room temperature for 6 h in a flask fitted with a calcium chloride tube. The reaction mixture was quenched by the addition of 1 M HCl (100 ml) and the aqueous solution was extracted with ether. The combined organic extracts were washed with a saturated solution of sodium hydrogen carbonate, dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (5:95) afforded **20** (0.32 g, 73%) as a white solid. Recrystallisation from methanol gave **20** as light yellow needles, mp 59.5–60 °C. ^{1}H NMR ($CDCl_3$): δ 1.51 (6H, s, 2×CH_3), 3.95 (3H, s, MeO), 5.72 (1H, d, J=9.9 Hz, H-3), 6.81 (1H, d, J=9.9 Hz, H-4), 7.44–7.52 (2H, m, H-8 and H-9), 7.99–8.03 and 8.16–8.19 (each 1H, each m, H-7 and H-10). ^{13}C NMR ($CDCl_3$): δ 27.48 (2×CH_3), 61.28 (MeO), 76.48, (CMe_2), 112.65 (=C_{quat}), 115.18 (=C_{quat}), 121.81 and 122.51 (C-7 and C-10), 121.90 (C-4), 125.08 (=C_{quat}), 125.84 and 127.02 (C-8 and C-9), 128.30 (=C_{quat}), 130.31 (C-3), 145.60 (=C–O), 146.72 (=C–O). IR (KBr): ν_{max} 1632, 1556, 1355, 1270, 1163, 1129, 1081, 766 cm^{-1}. MS *m/z* (%): 318/20 (M+, 27), 303/5 (100), 288/90 (19), 225 (28). Anal. Calcd for $C_{16}H_{15}BrO_2$: C 60.21%, H 4.74%. Found: C 60.09%, H 4.91%.

3.1.9. Synthesis of 6-methoxy-2,2-dimethyl-2*H*-naphtho-[1,2-*b*]pyran (22) and methyl 6-methoxy-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5-carboxylate (21). A solution of 5-bromo-6-methoxy-2,2-dimethyl-2*H*-naphtho[1,2-*b*]-pyran (**20**) (0.53 mmol, 170 mg) in dry tetrahydrofuran (5 ml) was cooled to −78 °C and to the stirred solution, in a nitrogen atmosphere, was added dropwise a solution of *n*-butyllithium (2.5 M) in hexane (0.58 mmol, 0.23 ml). After 30 min at this temperature, a solution of methyl chloroformate (0.64 mmol, 60 mg) in dry THF (1 ml) was added and the reaction mixture was kept for an additional 2 h at −78 °C. Afterwards, the reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was poured in 1 M HCl and extracted with ether. The combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (5:95) as eluent afforded first 6-methoxy-2,2-dimethyl-2*H*-naphtho-[1,2-*b*]pyran (**22**)[18] (R_f=0.29, 10 mg, 8%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.49 (6H, s, 2×CH_3), 3.94 (3H, s, MeO), 5.64 (1H, d, J=9.5 Hz, H-3), 6.39 (1H, d, J=9.5 Hz, H-4), 6.50 (1H, s, H-5), 7.42–7.49 (2H, m, H-8 and H-9), 8.13–8.16 (2H, m, H-7 and H-10). MS *m/z* (%): 240 (M+, 25), 225 (100), 210 (10). Using the same solvent combination, methyl 6-methoxy-2,2-dimethyl-2*H*-naphtho-[1,2-*b*]pyran-5-carboxylate (**21**) (R_f=0.13, 90 mg, 57%) was collected as a second fraction and appeared as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.51 (6H, s, 2×CH_3), 3.96 (3H, s, MeO), 3.99 (3H, s, MeO), 5.68 (1H, d, J=9.9 Hz, H-3), 6.42 (1H, d, J=9.9 Hz, H-4), 7.47–7.53 (2H, m, H-8 and H-9), 8.01–8.06 and 8.18–8.22 (each 1H, each m, H-7 and H-10). ^{13}C NMR ($CDCl_3$): δ 27.65 (2×CH_3), 52.40 (MeO), 63.49 (MeO), 76.44 (CMe_2), 112.36 (=C_{quat}), 119.80 (=CH), 120.52 (=C_{quat}), 122.46 (=CH), 122.59 (=CH), 126.75 (=CH), 126.83 (=CH), 127.78 (=C_{quat}), 130.24 (=CH), 144.89 (=C–O), 147.53 (=C–O), 167.78 (C=O). IR (NaCl): ν_{max} 1731 (C=O) cm^{-1}. MS *m/z* (%): 298 (M+, 7), 283 (13), 143 (88), 84 (92), 49 (100). Anal. Calcd for $C_{18}H_{18}O_4$: C 72.47%, H 6.08%. Found: C 72.22%, H 5.84%.

3.1.10. Mollugin (1). To a cooled (0 °C) solution of methyl 6-methoxy-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5-carboxylate

(**21**) (0.69 mmol, 220 mg) in dry dichloromethane (20 ml) was added aluminium(III) chloride (13.8 mmol, 1.84 g) and the reaction mixture was stirred for 4 h in a flask fitted with a calcium chloride tube. The reaction mixture was quenched by the addition of water (50 ml) (cooling!) and the aqueous solution was further diluted with 1 M HCl (50 ml), extracted with dichloromethane, dried ($MgSO_4$) and evaporated in vacuo. Flash chromatography on silica gel using ethyl acetate/petroleum ether (5:95) afforded mollugin (**1**) (150 mg, 77%) as a yellow powder. Recrystallisation from methanol gave mollugin (**1**) as yellow-green flakes, mp 129.5–131 °C (lit.[1] mp 128.8 °C). The spectral data of mollugin (**1**) were in complete accordance with those reported for the natural mollugin. ^{1}H NMR ($CDCl_3$): δ 1.48 (6H, s, 2×CH_3), 4.00 (3H, s, MeO), 5.66 (1H, d, J=9.9 Hz, H-3), 7.09 (1H, d, J=9.9 Hz, H-4), 7.46–7.53 and 7.57–7.63 (each 1H, each m, H-8 and H-9), 8.15–8.18 and 8.34–8.38 (each 1H, each m, H-7 and H-10), 12.17 (1H, s, OH), ^{13}C NMR ($CDCl_3$): δ 26.83 (2×CH_3), 52.29 (MeO), 74.61 (CMe_2), 102.19 (=C_{quat}), 112.54 (=C_{quat}), 121.90 (=CH), 122.30 (=CH), 124.06 (=CH), 125.05 (=C_{quat}), 126.27 (=CH), 128.98 (=C_{quat}), 129.32 (=CH), 141.54 (=C–O), 156.48 (=C–O), 172.49 (C=O), IR (KBr): ν_{max} 1651 (C=O), 1449, 1360, 1342, 1238, 769 cm^{-1}. MS m/z (%): 284 (M+, 23), 252 (32), 237 (85), 84 (69), 49 (100). Anal. Calcd for $C_{17}H_{16}O_4$: C 71.82%, H 5.67%. Found: C 71.63%, H 5.77%.

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Unexpected dimerization during hydrogenation of 2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-ones

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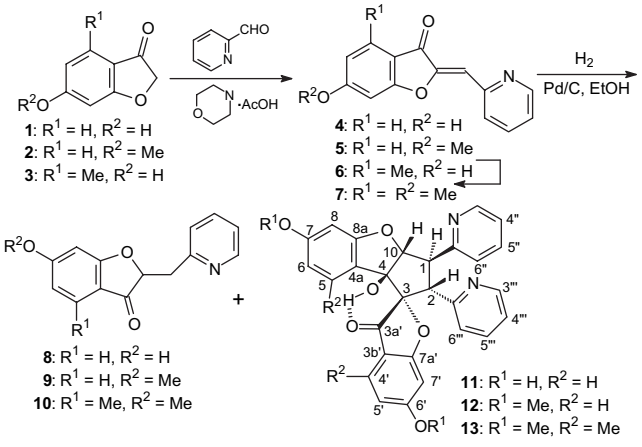
Abstract—Palladium catalyzed hydrogenation of 2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-ones (**1**–**3**) gave besides the expected 2,α-dihydro products **8**–**10** pentacyclic dimers formed by an attack of a semihydrogenated species on the substrate.

1. Introduction

For testing purposes a series of 2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-ones (**4**–**6**) were prepared by condensation of 3(2*H*)-benzofuran-3-ones (**1**–**3**) with pyridine-2-aldehyde in the presence of morpholine acetate (Scheme 1).[1]



Scheme 1.

Alkene **5** was subjected to catalytic hydrogenation over palladium-on-charcoal in ethanol. Owing to its poor solubility the substrate was added as a solid to the ethanolic suspension of the prehydrogenated catalyst. Unexpectedly hydrogen uptake completely stopped after the absorption of approximately 60% of the theoretically calculated volume. Chromatography of the product gave, apart from the expected 6-methoxy-2-(2-pyridylmethyl)-3(2*H*)-benzofuran-3-one (**9**), a substance with a mp 180 °C that even after several recrystallizations showed two complete sets of ^{1}H NMR signals in a 1:1 intensity ratio for the aromatic protons of the benzofuran and pyridine rings, as well as signals for a contiguous set of three aliphatic protons.

2. Results and discussion

2.1. Structural studies

Detailed spectroscopic studies summarized in Table 1 showed that the by-product of the hydrogenation of compound **5** was a dimer of structure **12** (see Scheme 1) in which one of the carbonyl groups was transformed into an alcohol. ^{1}H–^{1}H connectivities and the presence of five different spin systems were observed in the ^{1}H–^{1}H COSY spectrum, which also permitted complete ^{1}H signal assignment. The position of the H-5 signal (δ 6.57 ppm) indicated that, when compared to that of H-4′ (δ 7.50 ppm), H-5 was not *peri* to a carbonyl group. Furthermore, there was only one signal in the ^{13}C NMR spectrum, which could be assigned to a ketone. Long-range ^{1}H–^{13}C connectivities (HMBC spectrum) of the quaternary ^{13}C signals at 97.3 and 92.5 ppm (assigned to C-3 and C-4) gave further evidence for the postulated structure (**12**). The NOESY spectrum suggested that there was no steric proximity neither between H-1 and H-2 nor between H-1 and H-10. This indicated a trans–trans disposition, which was in agreement with the measured ^3J coupling constants.

Keywords: 2-Pyridylmethylene-3(2*H*)-benzofuran-3-ones; Hydrogenation; Dimerization.
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Table 1. NMR data for compound **12**

Position	δ_H, Multiplicities, intensities, and coupling constants	^1H–^1H COSY cross-peaks	δ_C	Significant HMBC correlations of the ^{13}C signal
1	4.63, dd, (1H), 12.8, 6.5 Hz	2-H, 10-H	54.2	2-H, 6″-H
2	4.88, d, (1H), 12.8 Hz	1-H	58.0	1-H, 6‴-H
3			97.3	2-H, 10-H
4			92.5	5-H, 10-H
4a			117.9	6-H, 8-H
5	6.56, d, (1H), 8.3 Hz	6-H	124.1	
6	6.26, dd, (1H), 8.3, 2.0 Hz	5-H, 8-H	107.6	8-H
7			162.2	5-H, 6-H, 8-H
8	6.42, d, (1H), 2 Hz	6-H	96.4	5-H, 6-H
8a			162.5	5-H, 6-H, 8-H, 10-H
10	5.35, d, (1H), 6.5 Hz	1-H	99.2	1-H, 2-H
3a′			198.0	2-H, 5′-H
3b′			114.8	4′-H, 5′-H, 7′-H
4′	7.49, d, (1H), 8.8 Hz	5′-H	125.1	
5′	6.52, dd, (1H), 8.8, 2.0 Hz	4′-H, 7′-H	112.4	4′-H, 7′-H
6′			168.8	4′-H, 7′-H, OCH_3
7′	6.10, d, (1H), 2.0 Hz	5′-H	95.5	4′-H
7a′			174.1	4′-H, 7′-H
1″			158.7	1-H, 2-H, 10-H, 4″-H
3″	8.59, dd, (1H), 4.9, 1.9 Hz	7.10	149.8	4″-H, 5″-H
4″	7.10, dd, (1H), 7.5, 4.9 Hz	8.59, 7.55	122.1	
5″	7.55, ddd, (1H), 7.5, 7.5, 1.9 Hz	7.10, 7.39	136.4	3″-H
6″	7.39, d, (1H), 7.5 Hz	7.55	124.3	
1‴			153.4	1-H, 2-H, 4‴-H
3‴	8.33, dd, (1H), 5.2, 1.5 Hz	6.91	148.8	4‴-H, 5‴-H
4‴	6.91, dd, (1H), 7.5, 5.2 Hz	8.33, 7.32	122.3	
5‴	7.32, ddd, (1H), 7.5, 7.5, 1.5 Hz	6.91, 7.06	136.0	3‴-H
6‴	7.06, d, (1H), 7.5 Hz	7.32	123.8	
OH	5.15, br s, (1H)			
OCH_3	3.74, s, (3H)		55.7	
OCH_3'	3.71, s, (3H)		56.1	

A total of 32 ^{13}C signals was observed, that confirmed the dimeric structure of **12**. Total signal assignment was possible on the basis of HMBC connectivities.

Chelation between the carbonyl and hydroxyl groups was indicated by a slowly developing color reaction with iron(III) chloride[2] and a bathochromic shift (ν_{CO} 1704→1672 cm^{-1}) of the carbonyl absorption[3] relative to that in the parent compound (**2**) enabling the assignment of the disposition of the spiro system.

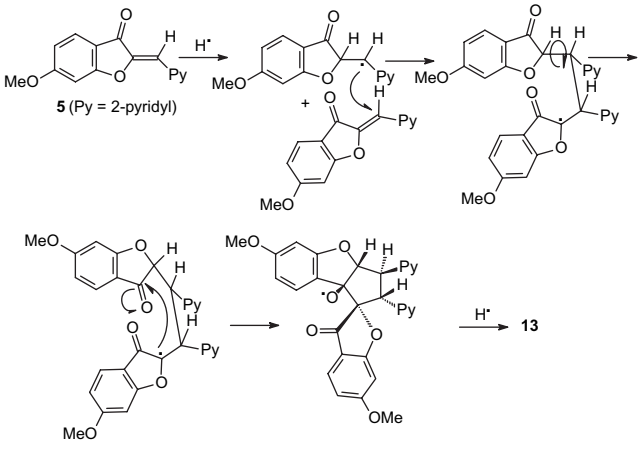
Finally mass spectrometry indicated for **12** a molecular mass of 508 corresponding to 2×**5**+2×H.

2.2. Investigations on other systems

Hydrogenation of compounds **4** and **7** proceeded similarly and provided along with the normal products (i.e., **8** and **10**) the dimers **11** and **13** both showing in their ^1H NMR spectra the duplication of the aromatic and pyridine signals, as well as signals for H-1, H-2, and H-10.

Although reactions other than π-bond saturation, such as hydrogen exchange, double bond migration, and cis–trans isomerization have been reported,[4] to our knowledge no intermolecular reactions accompanying catalytic hydrogenation have been observed.

A tentative mechanism shown in Scheme 2 is proposed for the formation of the dimers as exemplified by the transformation of **5**→**11**.



Scheme 2. Tentative mechanism for the formation of dimer **8**.

This mechanism is in conflict with the still widely accepted classical concept proposed more than 70 years ago by Horiuti and Polányi.[4,5] This postulates that the olefin adsorbed with its π-bond to the catalyst surface assumes, after the addition of one hydrogen atom a half-hydrogenated state, followed by the uptake of the second hydrogen atom. Even if the nature of the interaction of the olefin and the catalyst surface is disputed (dissociative or associative adsorption) it is supposed that the half-hydrogenated species remains on the catalyst until complete saturation.

Our results suggest that there exists a usually latent pathway along with the classical mechanism through which

a half-hydrogenated radical species can attack the olefin precursor. The question whether this occurs in solution or on the surface of the catalyst and how is the second hydrogen atom transferred to the dimeric radical remains open.

The scope of the above dimerization seems to be very narrow. Hydrogenation of 6-hydroxy-2-(3-pyridylmethylene)-3(2*H*)-benzofuran-3-one (**14**), 6-methoxy-2-(4-pyridylmethylene)-3(2*H*)-benzofuran-3-one (**15**), 6-hydroxy-2-(4-hydroxybenzylidene)-3(2*H*)-benzofuran-3-one[6] (**16**), and even of 2-(2-pyridylmethylene)-1-tetralone (**17**) (Fig. 1) only gave the regular dihydro products.

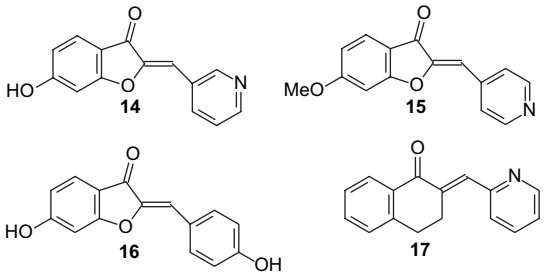


Figure 1.

3. Experimental

3.1. General

Infrared spectra were recorded on a Zeiss Specord IR 74 spectrometer as KBr pellets, ^{1}H (500 MHz) and ^{13}C (125 MHz) spectra were recorded on a Bruker DRX/Avance spectrometer. Fast atom bombardment (FAB/LSIMS) and daughter ion spectra were performed on a Finnigan MAT 95SQ hybrid tandem mass spectrometer. The Cs^+ gun was used at 20 kV and the matrix applied was 3-nitrobenzyl alcohol (NBA). For chromatography silica gel 60 (Merck) was used.

3.1.1. 6-Hydroxy-4-methyl-3(2*H*)-benzofuran-3-one (3). To a solution of orcinol (7.44 g, 60 mmol) and chloroacetonitrile (3.7 mL, 58 mmol) in dry diethyl ether (40 mL) was added powdered anhydrous zinc chloride (5 g). The mixture was cooled to 0 °C and saturated with hydrogen chloride gas. The next day the ether was decanted from the precipitated gummy imine hydrochloride. The rest of the solvent was removed in vacuo and the salt treated boiling water (300 mL). After cooling the precipitate was collected by filtration and dried to give the chloroketone (8.5 g). Without purification the latter was boiled for 15 min with potassium acetate (10 g) in methanol (75 mL). On cooling the product crystallized as needles (5.4 g, 77%), mp 245 °C. ν_{max} 3064, 1664, 1616, 1468, 1388, 1328, 1280, 1264, 1252, 1152, 1116, 1060, 1020, 856, 820, 768, 616, 528, 488 cm^{-1}; δ_H (DMSO-d_6) 2.41 (s, 3H, Me), (4.63, s, 2H, 2-H), 6.30 and 6.32 (2×s, 2H, 4,6-H), 10.77 (br s, 1H, OH). Anal. Calcd for $C_9H_8O_3$: C, 65.85; H, 4.91. Found: C, 65.72; H, 4.96.

3.1.2. 6-Hydroxy-2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-one (4). 6-Hydroxy-3(2*H*)-benzofuran-3-one[7] (**1**) (3.3 g, 22 mmol), pyridine-2-aldehyde (2.4 g 22 mmol), and morpholine acetate (0.6 g) was boiled with stirring in methanol (20 mL) for 2 h. The product was filtered off hot and washed with methanol to give pure **4** as yellow needles (3.5 g, 74%), mp 278–280 °C. ν_{max} 3440, 2352, 1704, 1600, 1564, 1456, 1424, 1400, 1344, 1304, 1252, 1224, 1216, 1152, 1104, 1008, 960, 944, 872, 832, 776, 680, 628, 496, 456 cm^{-1}; δ_H (DMSO-d_6) 6.68 (s, 1H, α-H), 6.74 (dd, *J* 8.2, 1.9 Hz, 1H, 5-H), 6.80 (d, *J* 1.9 Hz, 1H, 7-H), 7.39 (dd, *J* 7.5, 4.5 Hz, 1H, 4′-H), 7.65 (d, *J* 7.5 Hz, 1H, 6′-H), 7.92 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5′-H), 8.15 (d, *J* 8.2 Hz, 1H, 4-H), 8.68 (dd, *J* 4.5, 1.5 Hz, 1H, 3′-H). Anal. Calcd for $C_{14}H_9NO_3$: C, 70.29; H, 3.79; N, 5.86. Found: C, 70.08; H, 3.86; N, 5.70.

3.1.3. 6-Methoxy-2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-one (5). Condensation of 6-methoxy-3(2*H*)-benzofuran-3-one[8] (**2**) with pyridine-2-aldehyde as described under **4** gave the title compound as yellow needles in 70% yield, mp 155–157 °C (from EtOH); ν_{max} 3432, 1704, 1616, 1504, 1440, 1328, 1280, 1196, 1152, 1128, 1104, 1016, 960, 888, 816, 776, 760, 544 cm^{-1}; δ_H ($CDCl_3$) 3.93 (s, 3H, OMe), 6.75 (dd, *J* 8.2, 1.5 Hz, 1H, 5-H), 6.78 (s, 1H, α-H), 6.95 (d, *J* 1.5 Hz, 1H, 7-H), 7.25 (m, 1H, 4′-H), 7.72 (d, *J* 7.5 Hz, 1H, 6′-H), 7.78 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5′-H), 8.09 (d, *J* 8.2 Hz, 1H, 4-H), 8.73 (dd, *J* 4.5, 1.5 Hz, 1H, 3′-H); δ_{13} 56.3, 76.8, 77.2, 77.4, 77.6, 97.2, 111.5, 112.6, 114.7, 123.3, 126.2, 126.7, 136.7, 149.4, 150.4, 152.4, 167.9, 169.5, 183.1; FABMS (NBA): MH^+ 254, daughter ions: *m/z* 239, 226. Anal. Calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.28; H, 4.42; N, 5.61.

3.1.4. 6-Hydroxy-4-methyl-2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-one (6). Condensation of 6-hydroxy-4-methyl-3(2*H*)-benzofuran-3-one (**3**) with pyridine-2-aldehyde as described for **4** gave the title compound as small yellow needles in 43% yield, mp 294–296 °C. ν_{max} 3440, 1696, 1664, 1616, 1580, 1472, 1352, 1288, 1184, 1152, 1052, 1008, 840, 784, 688, 628, 544 cm^{-1}; δ_H (DMSO-d_6) 2.48 (s, 3H, Me), 6.47 and 6.53 (2×d, *J* 2.0 Hz, 2H, 5- and 7-H), 6.60 (s, 1H, α-H), 7.31 (dd, *J* 7.5, 4.5 Hz, 1H, 4′-H), 7.80 (ddd, *J* 7.5, 7.5, 1.0 Hz, 1H, 5′-H), 8.15 (d, *J* 7.5 Hz, 1H, 6′-H), 8.86 (dd, *J* 4.5, 1.0 Hz, 1H, 3′-H). Anal. Calcd for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.06; H, 4.42; N, 5.62.

3.1.5. 6-Methoxy-4-methyl-2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-one (7). 6-Hydroxy-4-methyl-2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-one (**6**) (0.97 g, 3.8 mmol) was stirred in dry acetone (50 mL) in the presence of dry sodium hydrogen carbonate (2.4 g, 28 mmol) and dimethyl sulfate (0.4 mL, 4.3 mmol) for 24 h. Filtration and evaporation of the filtrate, and recrystallization of the residue from EtOH (14 mL) gave **7** as pale yellow needles (0.51 g, 50%), mp 168–169 °C. ν_{max} 2328, 1704, 1616, 1484, 1460, 1352, 1304, 1264, 1216, 1208, 1196, 1148, 1076, 1060, 1008, 832, 788, 768, 608, 544, 408 cm^{-1}; δ_H ($CDCl_3$) 2.62 (s, 3H, Me), 3.86 (s, 3H, OMe), 6.48 and 6.60 (2×d, *J* 2.0 Hz, 1H, 5- and 7-H), 6.90 (s, 1H, α-H), 7.19 (dd, *J* 7.5, 4.5 Hz, 1H, 4′-H), 7.73 (ddd, *J* 7.5, 7.5, 1.0 Hz, 1H, 5′-H), 8.08 (d, *J* 7.5 Hz, 1H, 6′-H), 8.70 (dd, *J* 4.5, 1.0 Hz, 1H, 3′-H). Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.93; H, 4.82; N, 5.30.

3.1.6. 6-Hydroxy-2-(2-pyridylmethyl)-3(2*H*)-benzofuran-3-one (8) and *rel*-1*S*,2*S*,3*S*,4*S*,10*R*-spiro[4,7-dihydroxy-1,2-bis-2-pyridyl-1,2,3,4-tetrahydro-1*H*-9-oxacyclopenta[*a*]indene-2′-(6-hydroxy-3(2*H*)-benzofuran-3-one)] (11). Hydrogenation of **4** (1.08 g, 5 mmol) was performed as described below (compound **9**). Extraction of the crude product with hot acetone (20 mL) gave **8** as colorless plates (0.35 g, 33%), mp 205–208 °C. Evaporation of the mother liquor yielded the dimer **11** as an amorphous powder (0.23 g, 21%), mp 203–204 °C.

Compound **8**: ν_{max} 3424, 3248, 1700, 1612, 1484, 1416, 1328, 1320, 1312, 1264, 1152, 1104, 1072, 1012, 840, 808, 768, 648, 544 cm^{-1}; δ_H (DMSO-d_6) 3.03 (dd, *J* 14.5, 9.5 Hz, 1H, α-H_1), 3.22 (dd, *J* 14.5, 3.8 Hz, 1H, α-H_2), 5.16 (dd, *J* 9.5, 3.8 Hz, 1H, 2-H), 6.42 (d, *J* 1.9 Hz, 1H, 7-H), 6.56 (dd, *J* 8.8, 1.9 Hz, 1H, 4-H), 7.24 (dd, *J* 7.5, 5.5 Hz, 1H, 4′-H), 7.33 (d, *J* 7.5 Hz, 1H, 6′-H), 7.46 (d, *J* 8.8 Hz, 1H, 4-H), 7.72 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5′-H), 8.48 (dd, *J* 5.5, 1.5 Hz, 1H, 3′-H). Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.60; N, 5.81. Found: C, 69.67; H, 4.63; N, 5.86.

Compound **11**: δ_H (DMSO-d_6) 4.30 (dd, *J* 13.2, 6.9 Hz, 1H, 1-H), 4.65 (d, *J* 13.2 Hz, 1H, 2-H), 5.06 (d, *J* 6.9 Hz, 1H, 10-H), 5.71 (br s, 1H, 7′-H), 6.14 (dd, *J* 8.5, 1.9 Hz, 1H, 6-H), 6.24 (d, *J* 1.9 Hz, 1H, 8-H), 6.26 (dd, *J* 8.5, 1.9 Hz, 1H, 5′-H), 6.42 (d, *J* 8.5 Hz, 1H, 5-H), 6.97 (dd, *J* 7.5, 4.5 Hz, 1H, 4‴-H), 7.09 (dd, *J* 7.5 Hz, 1H, 6‴-H), 7.19 (dd, *J* 7.5, 4.5 Hz, 1H, 4″-H), 7.26 (d, *J* 7.5 Hz, 1H, 6″-H), 7.37 (d, *J* 8.5 Hz, 1H, 4′-H), 7.43 (dd, *J* 7.5, 7.5 Hz, 1H, 5‴-H), 7.65 (dd, *J* 7.5, 7.5 Hz, 1H, 5″-H), 8.33 (d, *J* 4.5 Hz, 1H, 3‴-H), 8.53 (d, *J* 4.5 Hz, 1H, 3″-H). Anal. Calcd for $C_{28}H_{20}N_2O_6$: C, 69.99; H, 4.20; N, 5.83. Found: C, 69.80; H, 4.18; N, 5.80.

3.1.7. Hydrogenation of 6-methoxy-2-(2-pyridylmethylene)-3(2*H*)-benzofuran-3-one (5): 6-methoxy-2-(2-pyridylmethyl)-3(2*H*)-benzofuran-3-one (9) and *rel*-1*S*,2*S*,3*S*,4*S*,10*R*-spiro[4-hydroxy-7-methoxy-1,2-bis-2-pyridyl-1,2,3,4-tetrahydro-1*H*-9-oxacyclopenta[*a*]indene-2′-(6-methoxy-3(2*H*)-benzofuran-3-one)] (12). Palladium-on-carbon, (10%, 0.5 g) was prehydrogenated in ethanol (100 mL). The apparatus was opened, **5** (3.0 g, 11.8 mmol) was added as a solid and flushed with hydrogen. After rapid initial absorption hydrogen uptake stopped at about 60% of the theoretical amount (283 mL). After filtration and evaporation of the filtrate the residue was chromatographed (eluant: benzene–ethyl acetate, 2:1) to give 6-methoxy-2-(2-pyridylmethyl)-3(2*H*)-benzofuran-3-one (**9**) as colorless plates (0.7 g, 23%), mp 103–105 °C (from Et_2O) and the dimer **12** as colorless plates (0.31 g, 10%), mp 180–182 °C.

Compound **9**: ν_{max} 2928, 2384, 1620, 1508, 1444, 1376, 1252, 1156, 1028, 784, 616 cm^{-1}; δ_H ($CDCl_3$) 3.11 (dd, *J* 15.5, 9.5 Hz, 1H, α-H_1), 3.14 (dd, *J* 15.5, 3.5 Hz, 1H, α-H_2), 3.84 (s, 3H, OMe), 5.16 (dd, *J* 9.5, 3.5 Hz, 1H, 2-H), 6.59 (d, *J* 1.5 Hz, 1H, 7-H), 6.63 (dd, *J* 8.5, 1.5 Hz, 1H, 5-H), 7.17 (dd, *J* 7.5, 5 Hz, 1H, 4′-H), 7.24 (d, *J* 7.5 Hz, 1H, 6′-H), 7.57 (d, *J* 8.5 Hz, 1H, 4-H), 7.62 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5′-H), 8.57 (dd, *J* 5, 1.5 Hz, 1H, 3′-H). Anal. Calcd for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.62; H, 5.02; N, 5.45.

Compound **12**: ν_{max} 3368, 3064, 3032, 3008, 1672, 1608, 1592, 1500, 1472, 1444, 1288, 1276, 1260, 1200, 1152, 1104, 1064, 1008, 760, 584 cm^{-1}; δ_H ($CDCl_3$) 3.71 and 3.74 (2×s, 2×3H, 2×OMe), 4.63 (dd, *J* 12.8, 6.5 Hz, 1H, 1-H), 4.88 (d, *J* 12.8 Hz, 1H, 2-H), 5.15 (br s, 1H, OH), 5.35 (d, *J* 6.5 Hz, 1H, 10-H), 6.10 (d, *J* 2.0 Hz, 1H, 7′-H), 6.26 (d, *J* 8.3, 2.0 Hz, 1H, 6-H), 6.42 (d, *J* 2.0 Hz, 1H, 8-H), 6.52 (dd, *J* 8.8, 2.0 Hz, 1H, 5′-H), 6.56 (d, *J* 8.3 Hz, 1H, 5-H), 6.91 (dd, *J* 7.5, 5.2 Hz, 1-H, 4‴-H), 7.06 (d, *J* 7.5, 1H, 6‴-H) 7.10 (dd, *J* 7.5, 4.9 Hz, 1H, 4″-H), 7.32 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5‴-H), 7.39 (d, *J* 7.5, 1H, 6″-H), 7.49 (d, *J* 8.8 Hz, 1H, 4′-H), 7.55 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5″-H), 8.33 (dd, *J* 5.2, 1.5 Hz, 1H, 3‴-H), 8.59 (dd, *J* 4.9, 1.9 Hz, 1H, 3″-H); δ_{13} ($CDCl_3$) 54.2 (C-1), 55.7 (OMe), 56.1 (OMe), 58.0 (C-2), 92.5 (C-4), 95.5 (C-7′), 96.4 (C-7), 97.3 (C-3), 99.2 (C-9), 107.6 (C-5), 112.4 (C-5′), 114.8 (C-3b′), 117.9 (C-3b), 122.1 (C-4″), 122.3 (C-4‴), 123.8 (C-6‴), 124.1 (C-4), 124.3 (C-6″), 125.1 (C-4′), 136.0 (C-5‴), 136.4 (C-5″), 148.77 (C-3‴), 148.83 (C-3″), 153.4 (C-1‴), 158.7 (C-1″), 162.2 and 162.5 (C-6 and C-7a), 168.8 (C-6′), 174.1 (C-7a′), 198.0 (C-3a′); FABMS (NBA): MH^+ 509, daughter ions: *m/z* 519, 268. Anal. Calcd for $C_{30}H_{24}N_2O_6$: C, 70.86; H, 4.76; N, 5.51. Found: C, 70.90; H, 4.80; N, 5.55.

3.1.8. 6-Methoxy-4-methyl-2-(2-pyridylmethyl)-3(2*H*)-benzofuran-3-one (10) and *rel*-1*S*,2*S*,3*S*,4*S*,10*R*-spiro[4-hydroxy-7-methoxy-5-methyl-1,2-bis-2-pyridyl-1,2,3,3a-tetrahydro-1*H*-8-oxacyclopenta[*a*]indene-2′-(6-methoxy-4-methyl-3(2*H*)-benzofuran-3-one)] (13). Hydrogenation of **7** (396 mg, 1.48 mmol) was performed as described for **5**. Chromatography of the crude product (eluant: PhMe–EtOAc, 2:1) gave **10** as colorless needles (119 mg, 30%), mp 76–78 °C and (**13**) as an amorphous powder (83 mg, 21%), mp 247–248 °C.

Compound **10**: ν_{max} 3384, 3024, 1656, 1616, 1456, 1440, 1352, 1344, 1332, 1316, 1272, 1200, 1148, 1052, 1040, 944, 808, 752, 696, 544, 496 cm^{-1}; δ_H ($CDCl_3$) 2.52 (s, 3H, CH_3), 3.08 (dd, *J* 15.5, 9.5 Hz, 1H, α-H_1), 3.53 (dd, *J* 15.5, 3.5 Hz, 1H, α-H_2), 3.81 (s, 3H, OCH_3), 5.09 (dd, *J* 9.5, 3.5 Hz, 1H, 2-H), 6.34 (d, *J* 2.0 Hz, 1H, 6-H), 6.37 (m, 1H, 4-H), 7.16 (dd, *J* 7.5, 5.5 Hz, 1H, 4′-H), 7.25 (d, *J* 7.5 Hz, 1H, 6′-H), 7.63 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5′-H), 8.57 (dd, *J* 5.5, 1.5 Hz, 1H, 3′-H). Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.24; H, 5.42; N, 5.41.

Compound **13**: ν_{max} 3424, 3240, 2592, 1960, 1700, 1648, 1616, 1516, 1484, 1428, 1416, 1356, 1312, 1288, 1168, 1152, 1124, 1104, 1084, 1072, 1028, 1012, 976, 904, 860, 840, 820, 808, 792, 768, 760, 752, 660, 648, 636, 624, 612, 596, 584, 576, 520, 504, 464, 440, 428 cm^{-1}; δ_H ($CDCl_3$) 2.15, 2.50 (2×s, 2×Me, 6H), 3.71, 3.73 (2×s, 2×OMe, 6H), 4.63 (dd, *J* 13.0, 6.5 Hz, 1H, 1-H), 4.81 (d, *J* 13.0 Hz, 1H, 2-H), 5.31 (d, *J* 6.5 Hz, 1H, 10-H), 5.64 (br s, 1H, OH), 5.98 (d, *J* 2.0 Hz, 1H, 7′-H), 6.09 (m, 1H, 6-H), 6.21 (m, 1H, 5′-H), 6.24 (d, *J* 2.0 Hz, 1H, 8-H), 6.90 (ddd, *J* 7.5, 5.0, 1.5 Hz, 1H, 4‴-H), 7.05–7.10 (m, 2H, 4″- and 6‴-H), 7.31 (ddd, *J* 7.5, 7.5, 1.5 Hz, 1H, 5‴-H), 7.36 (d, *J* 7.5 Hz, 1H, 6″-H), 7.63 (ddd, *J* 7.5, 7.5, 2.0 Hz, 1H, 5″-H), 8.32 (dd, *J* 5.0, 1.5 Hz, 1H, 3″-H), 8.58 (dd, *J* 5.0, 1.5 Hz, 1H, 3″-H); FABMS (NBA): MH^+ 537, daughter ions: *m/z* 519, 268. Anal. Calcd for

$C_{32}H_{28}N_2O_6$: C, 71.63; H, 5.26; N, 5.22. Found: C, 71.70; H, 5.32; N, 5.18.

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