ELSEVIER

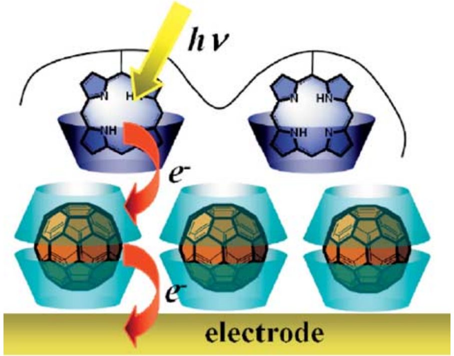
Tetrahedron

Tetrahedron Vol. 61, No. 21, 2005

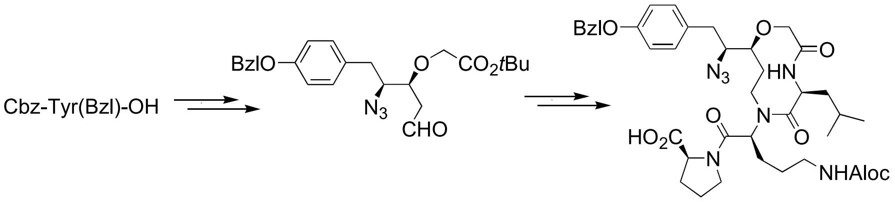
Contents

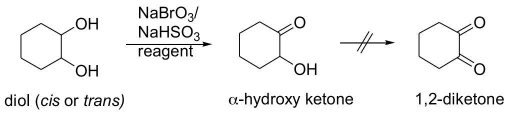
REPORT

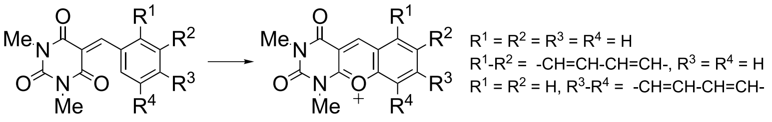
Photocurrent-generators constructed of supramolecular mono/multilayer containing fullerenes are reviewed. The report contains 129 references.

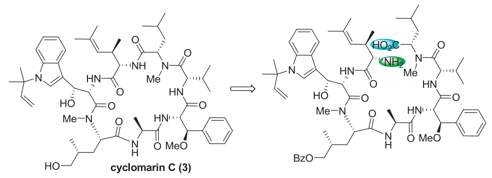


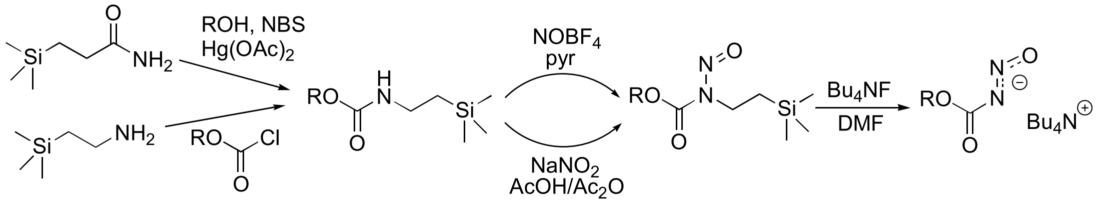
ARTICLES



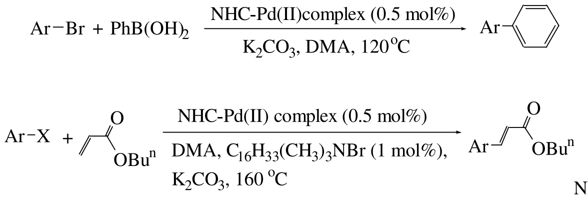


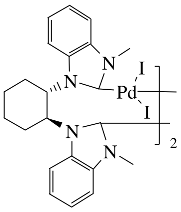






In the presence of a novel dimeric bidentated NHC–Pd(II) complex, Suzuki–Miyaura reaction and Heck-type cross-coupling reaction proceeded smoothly in DMAC to give the coupled products in good yields.



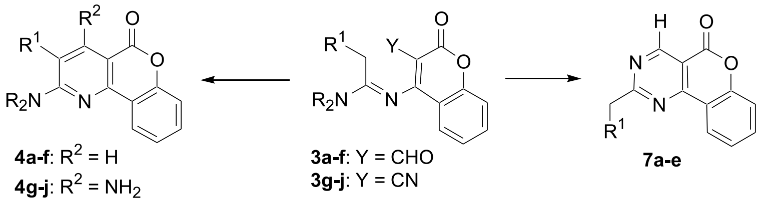


NHC-Pd(II) complex **5**

Substituted coumarin amidines: useful building blocks for the preparation of [1]benzopyrano[4,3-*b*]pyridin-5-one and [1]benzopyrano[4,3-*d*]pyrimidin-5-one derivatives

pp 4957–4964

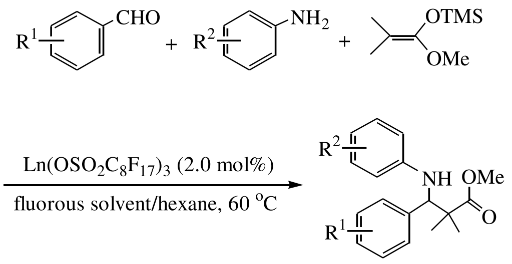
Egle M. Beccalli, Alessandro Contini and Pasqualina Trimarco*



Mannich-type reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane with arylaldehydes and aromatic amines catalyzed by perfluorinated rare earth metal salts in fluorous phase

pp 4965–4970

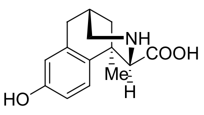
Min Shi,* Shi-Cong Cui and Ying-Hao Liu



Stereoselective synthesis of new modified conformationally constrained L-tyrosine analogue with potential applications to SH2 domain ligands

pp 4971–4981

Fa Liu, Tai-Shan Hu and Zhu-Jun Yao*



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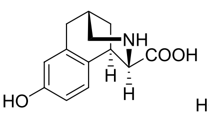

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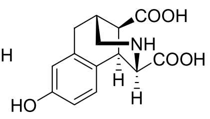
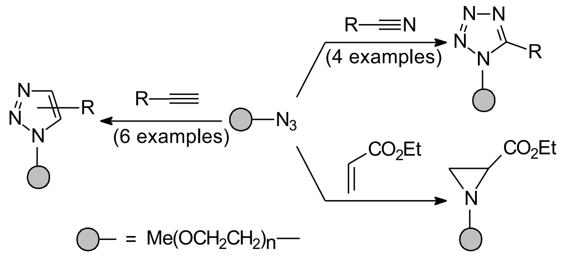


3

1,3-Dipolar cycloadditions of MeOPEG-bounded azides

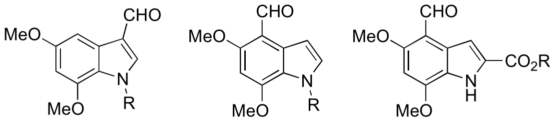
pp 4983–4987

Giorgio Molteni* and Paola Del Buttero



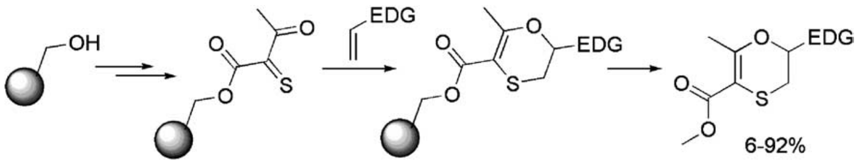
Regioselective reactivity of some 5,7-dimethoxyindoles **pp 4989–5004**

Glenn C. Condie, Michelle F. Channon, Andrew J. Ivory, Naresh Kumar and David StC. Black*



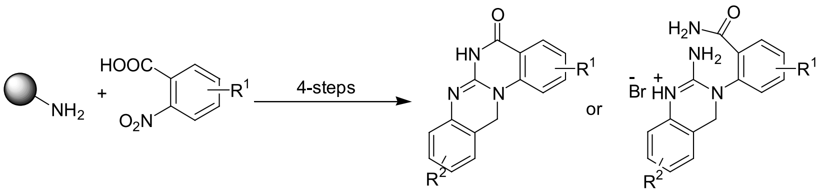
Hetero Diels–Alder reactions (HDAR) of α,α′-dioxothiones on solid support **pp 5005–5010**

Stefano Menichetti,* Mattia Mori and Cristina Nativi



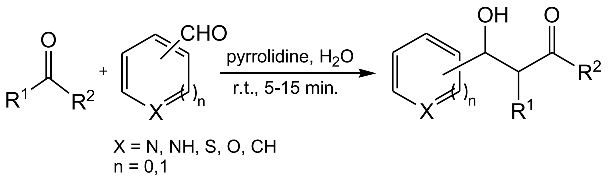
Base catalyzed intramolecular transamidation of 2-aminoquinazoline derivatives on solid phase **pp 5011–5018**

Rajesh K. Grover, Amit P. Kesarwani, Gaurav K. Srivastava, Bijoy Kundu* and Raja Roy*



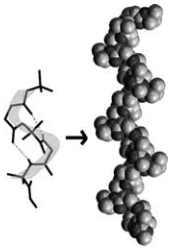
Electron deficiency of aldehydes controls the pyrrolidine catalyzed direct cross-aldol reaction of aromatic/heterocyclic aldehydes and ketones in water **pp 5019–5025**

Swapandeep Singh Chimni* and Dinesh Mahajan



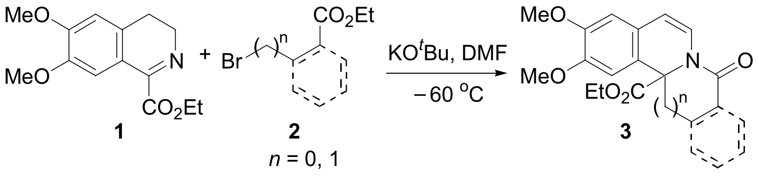
Can a consecutive double turn conformation be considered as a peptide based molecular scaffold for supramolecular helix in the solid state? **pp 5027–5036**

Apurba Kumar Das, Arijit Banerjee, Michael G. B. Drew, Sudipta Ray, Debasish Haldar and Arindam Banerjee*



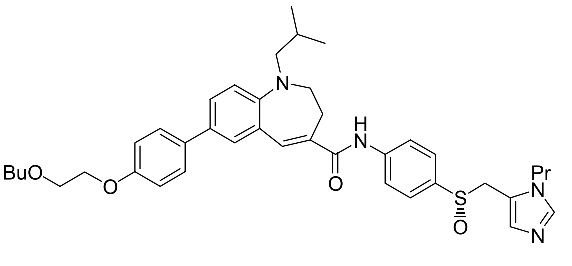
A novel 3,4-dihydroisoquinoline annulation: expedient access to isoquinoline heterocycles **pp 5037–5042**

Wei-Dong Z. Li* and Hua Yang



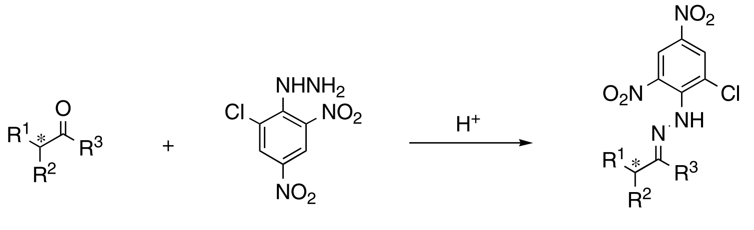
Unusual asymmetric oxidation of sulfide; the diastereoselective oxidation of prochiral sulfide-chiral acid salt with hydrogen peroxide without metal **pp 5043–5048**

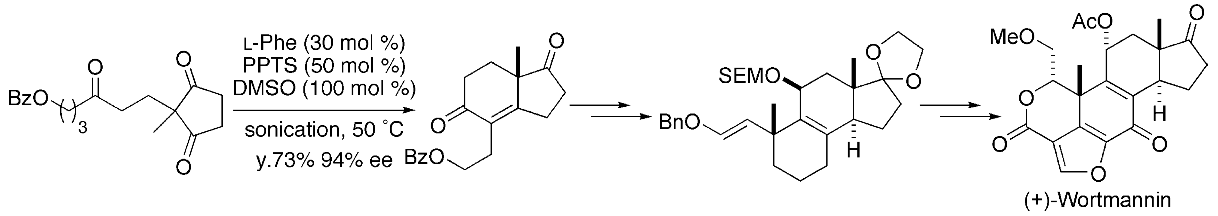
Tomomi Ikemoto,* Atsuko Nishiguchi, Tatsuya Ito and Hiroyuki Tawada

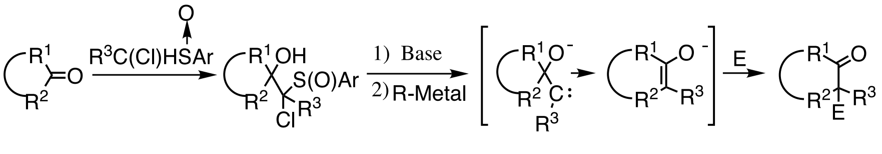


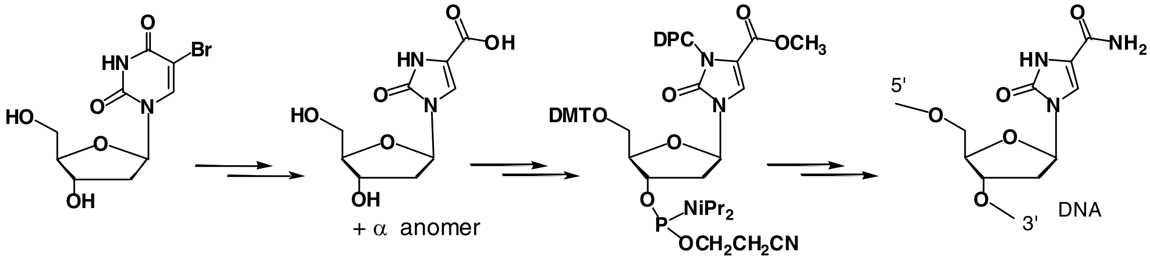
Chlorodinitrophenylhydrazine, a useful crystalline agent for absolute configuration determination of various chiral ketones **pp 5049–5055**

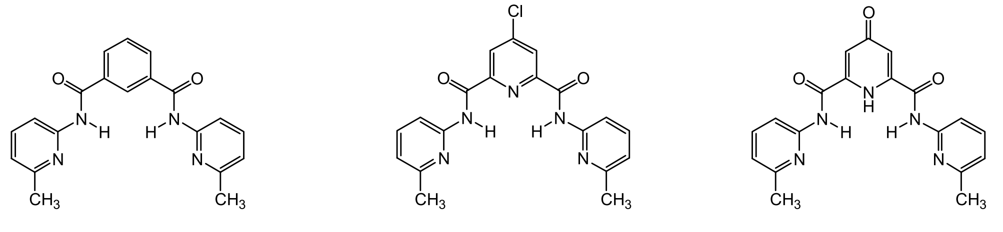
Yasushi Kawai,* Motoko Hayashi and Norihiro Tokitoh









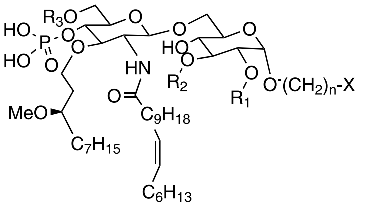


Binding constants for the complexes formed between synthetic hosts and six ureido derivatives have been quantified, theoretically by MM calculations and experimentally by NMR titrations.

Synthesis of lipid A analogues containing glucose instead of glucosamine and their LPS-antagonistic activities

pp 5101–5122

Masao Shiozaki,* Yukiko Watanabe, Yuji Iwano, Toshio Kaneko, Hiromi Doi, Daisuke Tanaka, Takaichi Shimozato and Shin-ichi Kurakata



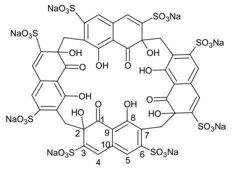
Compounds synthesized: R_1 = n-$C_{12}H_{25}$, n-$C_{14}H_{29}$, (R)-$(CH_2)_2CH(OH)C_{11}H_{23}$, $COCH_2COC_{11}H_{23}$ or -$(CH_2)_2COC_{11}H_{23}$; R_2 = n-$C_{10}H_{21}$ or n-$C_{12}H_{25}$; R_3 = H or CH_3; n = 1, 2 or 3; X = COOH , $OP(O)(OH)_2$ or $P(O)(OH)_2$

The LPS-antagonistic activities of synthetic ten compounds toward human whole blood and galactosamine loaded C3H/HeN mice were measured.

A ^{1}H NMR study on the complexation of tetraalkylammonium cations, mono and diprotonated amines, and amino acids with a derivatized cyclotetrachromotropylene in an aqueous solution

pp 5123–5129

Bo-Long Poh* and Chin Mean Teem

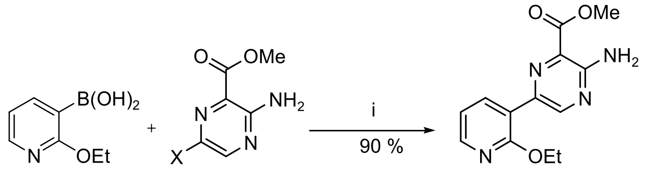


A water-soluble macrocyclic host for organic cations in an aqueous medium.

2-Ethoxy-3-pyridylboronic acid: a versatile reagent for the synthesis of highly-functionalised 3-aryl/heteroaryl-pyridines via Suzuki cross-coupling reactions

pp 5131–5135

Amy E. Thompson, Andrei S. Batsanov, Martin R. Bryce,* Nezire Saygili, Paul R. Parry and Brian Tarbit



i. Reagents and conditions: $Pd(PPh_3)_2Cl_2$, 1,4-dioxane, Na_2CO_3 (1 M), 20 °C, 16 h.

OTHER CONTENTS

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

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Supramolecular design of photocurrent-generating devices using fullerenes aimed at modelling artificial photosynthesis

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[a]RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[b]Graduate School of Materials Science, Nara Institute of Science and Technology, Takayama, Ikoma, Nara 630-0192, Japan
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Contents

1. Introduction

The importance of unconventional design principles for solar cells with functional flexibility and cost efficiency has been recognized from the viewpoint of energy and environmental problems. Conventional solar cells using semiconducting materials such as silicon have contributed greatly to modern society. They are not, however, satisfactory in terms of economic cost and energy conversion efficiency.[1–4] Recently, molecular science has proposed solar cells using organic compounds to serve the growing social demands for energy. Organic solar cells could benefit from diverse functions of modifiable organic compounds, in addition to offering cost advantages relative to inorganic/silicon-based photovoltaic materials. Their component molecules have steadily been made multifunctional because a scheme for organic photocurrent generation has been put forth. For example, not only the efficiency for energy conversion, but also the light absorption wavelength (light color), form, and durability of devices can be controlled using synthetic approaches. The use of photocurrent-generating devices comprising organic molecules is anticipated to widen in scope and functionality.

Regarding organic solar cells, photocurrent conversion is

Keywords: Photocurrent; Cyclodextrins; Calixarenes; Fullerenes; Porphyrins; Electron-transfer; SAM; LB.

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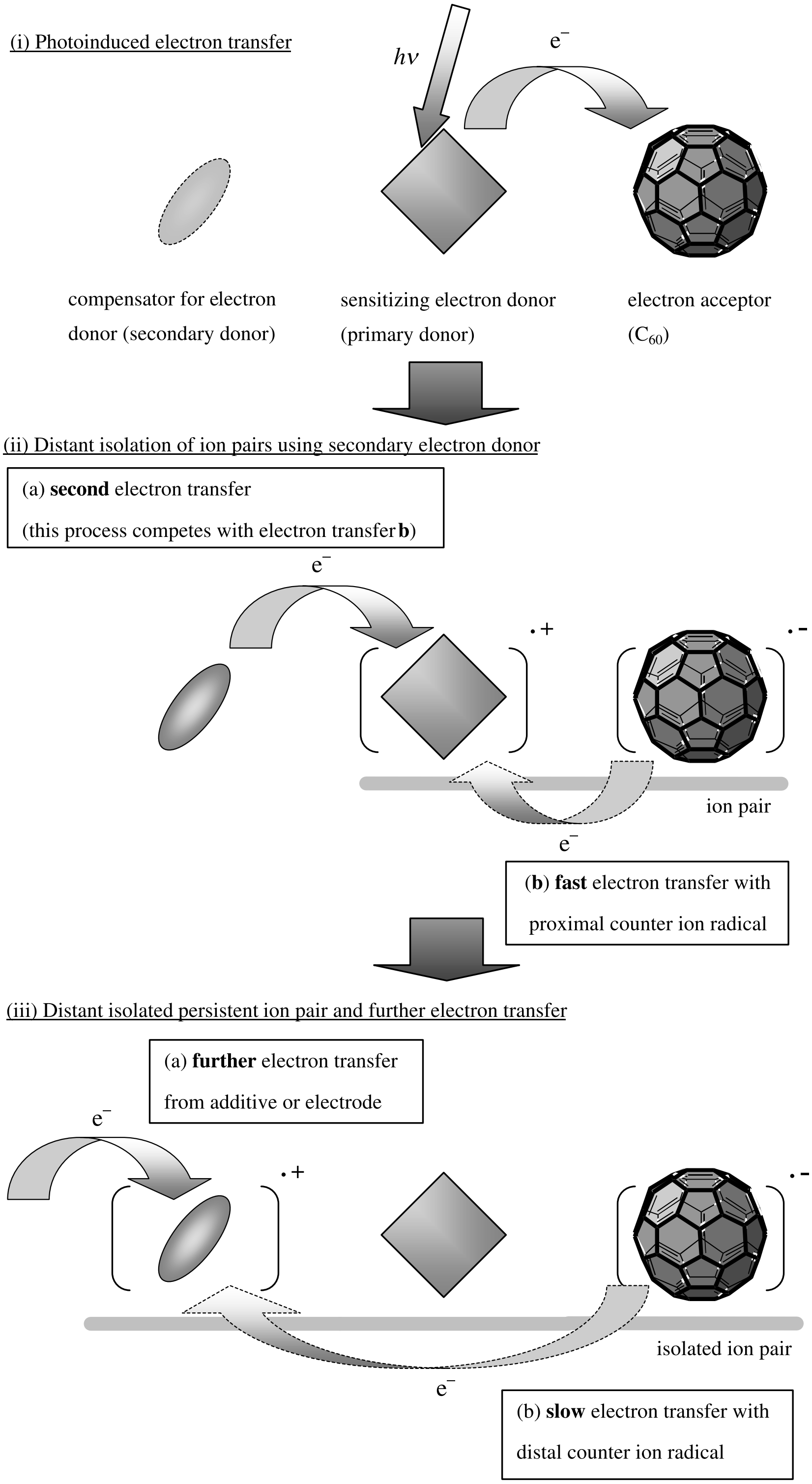
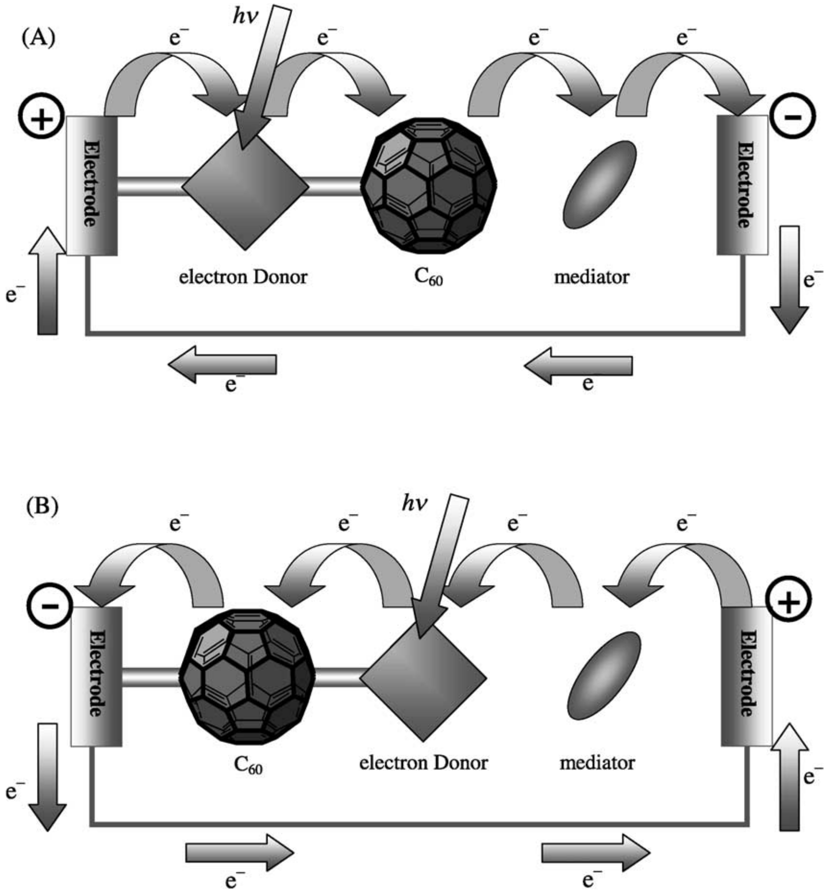
doi:10.1016/j.tet.2005.03.053



Scheme 1. Lifetime extension of charge-separated states by distant isolation of radical ions.

attained by electron flow into the electrode. This important step follows photo-excitation of organic compounds that are adsorbed on the electrode surface. The following two major methods can be shown for the design of organic solar cells: (i) direct interfacial electron injection from a photo-excited sensitizer into the electrode,[5–7] and (ii) electron transfer into the electrode (e.g., indium tin oxide—ITO) after photo-induced generation of long-lived charge separation (i.e., photo-induced formation of intermolecular and intramolecular radical ion pairs) in donor–acceptor organized molecular assemblies.[8–14] The former method includes (i) dye-sensitized semiconductor-based solar cells (i.e., Grätzel cells), which include ultrafast photo-induced electron injection as the core mechanism of light-to-current conversion.[15–16] Regarding Grätzel cells, numerous studies and excellent reviews have been published.[16–20] In this



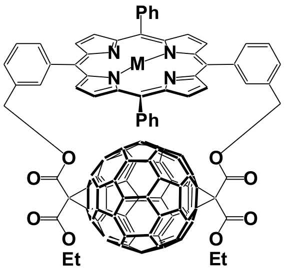
Scheme 2. Artificial photo-energy conversion device mimicking the natural molecular system.

review, the latter technique (ii) will therefore be discussed from the standpoint of organic and physical chemistry.

It is necessary to generate feasible and highly dense charge separation on an electrode surface to realize a highly efficient organic photovoltaic device.[21] Accordingly, a molecular design which satisfies the following prerequisites is being sought through the following avenues:

(1) Control of photo-induced/back electron transfer. Not only acceleration of the inter- and intra-molecular photo-induced electron-transfer process (i.e., improvement of quantum yield), but retardation of its back electron-transfer process (i.e., extension of the charge separation lifetime) should be carried out to collect a plentiful supply of electrons on the electrode surface.[22–24] Put another way, suppression of back electron transfer can increase the total quantum yield for photocurrent generation, because the electron-pouring process competes kinetically with the back electron-transfer process. Moreover, a stable electron-pooling molecule is required as a building block of the photofunctional organized molecular assembly. Electron-pooling molecules must easily receive and hold electrons until the electron is poured into the electrode. Recently, it was reported that fullerenes (especially C_{60}) show high electrochemical performance towards photo-induced electron pooling.[25–26] Other candidates for electron pooling of molecules offer distinct advantages. The topic of photochemical and electrochemical active molecules is too wide, however, to address adequately in this short review which is therefore restricted in scope to efficient photo-energy-convertible molecular arrays composed of fullerenes. (2) Organization of photofunctional molecular assemblies on the surface of the electrode. To absorb photons on the limited surface area of the device effectively (it is naturally regulated by the electrode size), light-harvesting molecules with a high molecular absorption coefficient (dyes) and electrochemically active molecules (donor and acceptor) must be arranged with high density on an electrode. Two general approaches to construct molecular devices exist, one of which is to arrange highly photofunctional molecules that can function independently. These highly photofunctional molecules are synthesized tactically beforehand. The other approach is to control the arrangement of comparatively simple electrochemically and photochemically active molecules, and discover their photochemical functions as organized molecular aggregates.

Regarding prerequisite (1), nature suggests an outstanding strategy. Natural photosynthetic systems convert sunlight into chemical energy, based on the formation of long-lived charge-separated states in highly organized molecular assemblies of biomembranes. The strategy for a lifetime extension of charge-separated states is the distant isolation between the radical cation and anion of charge-separated states, which comprises vectorial, sequential charge-hopping using electron-mediator molecules that are arranged appropriately (Scheme 1).[27] It is possible to build an artificial photo-energy conversion device by applying this strategy of mimicking the natural molecular system for photo-induced electron transfer (Scheme 2).[22–24] On the other hand, regarding the organization of molecular assemblies onto the electrode surface (i.e., prerequisite 2), precise mono and multilayer formation techniques using supramolecular interaction were proposed by trailblazing chemists.[28]



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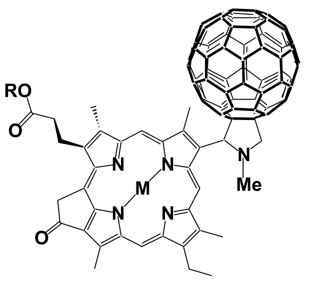
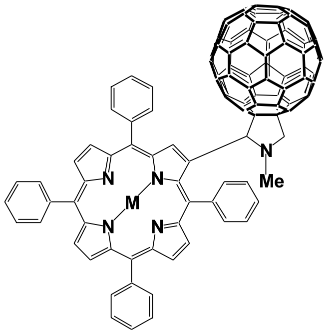
1



M = 2H or Zn, R = H or Me

2



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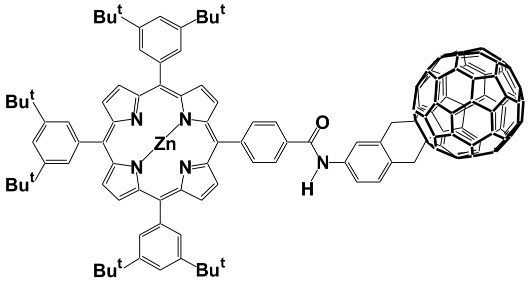

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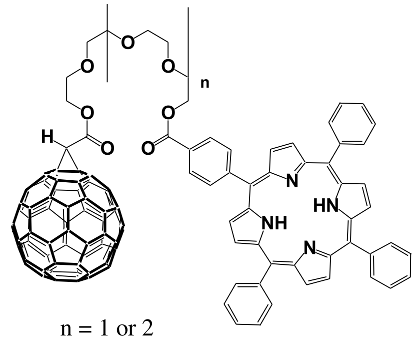

n = 1 or 2

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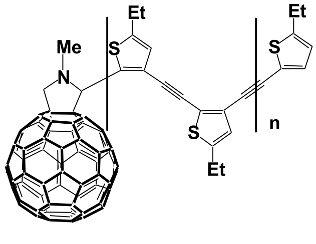
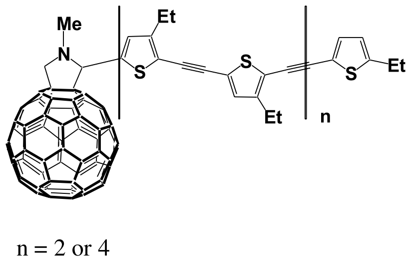


n = 2, 4, or 6

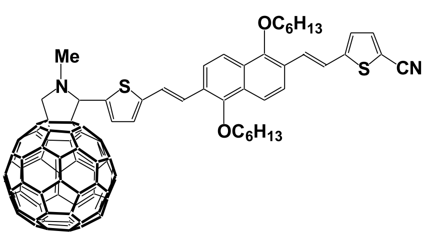
6



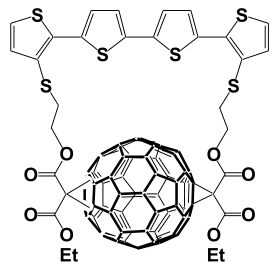
n = 2 or 4

7

The practical development of organic photovoltaic devices necessitates the synthesis of photofunctional molecules that fulfill the above-mentioned prerequisites. It is also necessary to unify these molecules onto the electrode surface. For such purposes, an advantage of the supramolecular design of photovoltaic devices can come into play. The salient feature of supramolecular design is a superiority not only for anchoring the molecule onto the electrode, but also for controlling the molecular functions exhibited as molecular assemblies and aggregates. A recent candidate for examination is the arrangement of previously synthesized functional molecules with the control of a position suitable for showing their ability by supramolecular interaction. This review reports a biomimetic supramolecular design that is intended to solve the above-mentioned problems through the use of fullerene derivatives. Concrete targets are the efficient photo-induced electron accumulation and facile preparation of photofunctional monolayers and multilayers.



8



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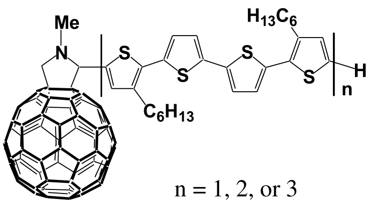
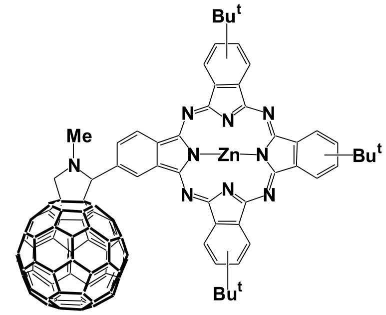
9

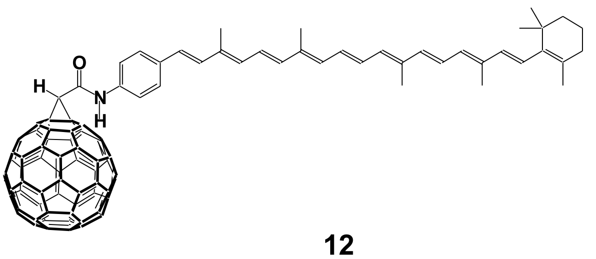


n = 1, 2, or 3

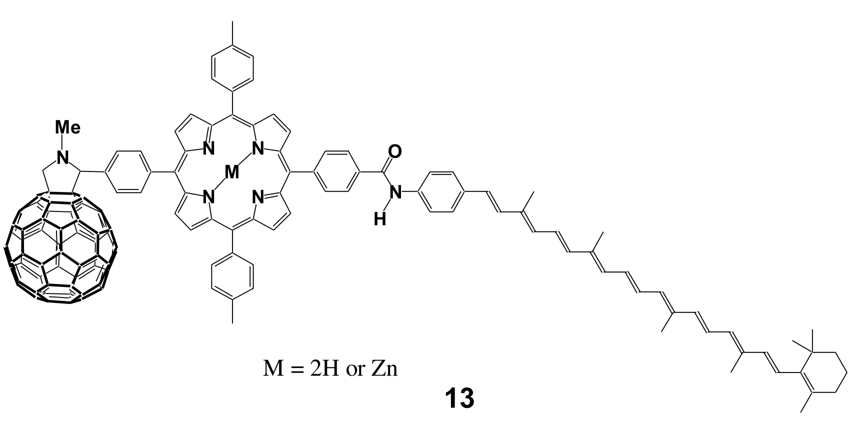
10



11



12



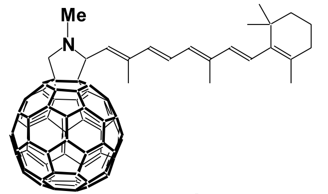
M = 2H or Zn

13

2. Control of photo-induced charge separation and recombination in covalent and non-covalent linked donors and C_{60}

This section describes the molecular design of efficient photo-induced electron pooling in solution. For the construction of an artificial photosynthetic supramolecular system, a design principle of photofunctional molecules showing both highly efficient and long-lived charge separation is needed. Recent progress in photochemistry has simplified the construction of solely photo-induced electron transfer between the electron donor and acceptor (i.e., photo-induced charge separation) with high quantum yield using the Rehm–Weller equation[29] or Marcus theory.[30] In practice, according to these laws, photo-induced electron-transfer systems can be designed with sufficient reproducibility by setting up photochemical parameters such as redox potentials of molecules, photo-excitation energy of sensitizer, and distance between the electron donor and acceptor. In addition, it has been disclosed that highly efficient photo-induced electron transfer can be built using C_{60}, which has a high electron-accepting ability and stability against photochemical and electrochemical redox procedures,[31] that is, photo-induced charge separation can take place with a high quantum yield by connecting C_{60} and suitable photosensitizing electron donors such as porphyrins **1**–**5**,[31b,32] thiophenes **6**–**10**,[33] phthalocyanines **11**,[34] and carotenoids/retinols **12**–**14**,[35] and other derivatives **15**–**19**.[36] The efficiency for photocurrent generation is, however, not necessarily improved by increasing the quantum yield for photo-induced electron transfer. This is a necessary precondition for photocurrent conversion and the control of back electron transfer (i.e., charge recombination) is another factor. Back electron transfer will decrease the total yield for photocurrent generation, because the electron-pouring process into the electrode is in kinetic competition with the back electron-transfer process. All photochemical reactions using photogenerated radical ions proceed in parallel with back electron transfer (i.e., reverse process of photo-induced electron transfer), and the control of back electron transfer is therefore a fundamental subject in photochemical reactions started from photo-induced electron transfer.

The design of a molecular system that can control both forward and back electron transfer (i.e., photo-induced charge separation and charge recombination) is considerably difficult: placing an electron donor near an acceptor with covalent linking will generally promote back electron transfer, just as it does forward photo-induced electron transfer. Natural photosynthesis systems are closely examined to satisfy these requirements. More specifically, retardation of charge recombination can be established by keeping the radical ion pairs distantly isolated.[27] The invention of a molecular photo-energy converter therefore needs to be attempted not only by choosing suitable photofunctional building blocks, but through an examination of the three-dimensional arrangement and structure of the molecules.[8–14] It is therefore necessary to arrange the electron-donor, -acceptor, and -mediating molecules in a suitable position for electron relay. In a connected system of C_{60} and a photosensitizing electron donor, two methods have been proposed recently to realize retardation of charge recombination. Some typical reports are described below.



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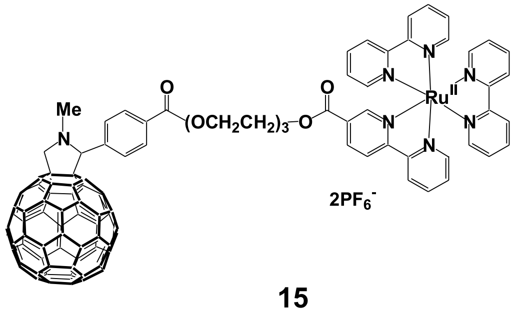

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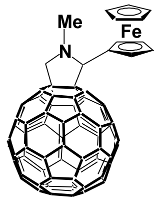

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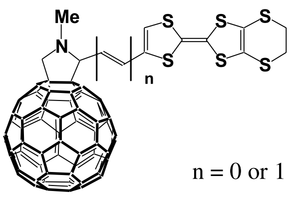

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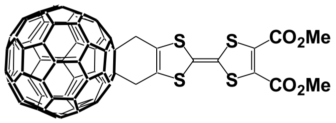

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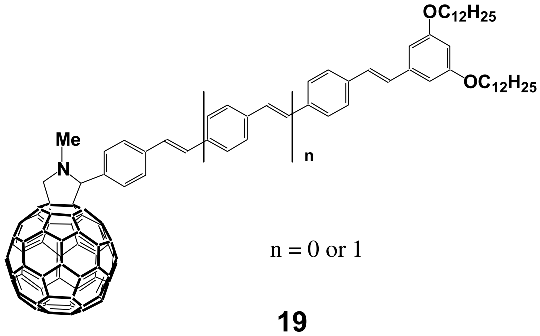

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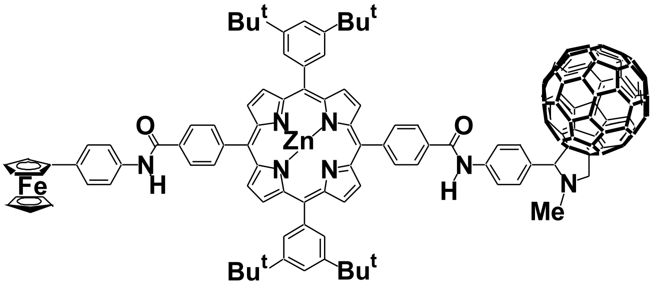
2.1. Donor–acceptor covalently linked systems

Intramolecular electron-transfer processes of donor–acceptor covalently linked systems have been studied intensively in anticipation of the development of molecular photovoltaic devices.[37–39] From the inspiration for biomimetics of photosynthesis, efficient photovoltaic devices are expected to be created by building a monolayer system showing vectorial photo-electron pooling. One method of obtaining a photofunctional membrane is a combination of preparation of a covalently linked donor–acceptor array that performs highly efficient charge separation within a molecule and arrangement of the donor–acceptor array asymmetrically onto the electrode surface.



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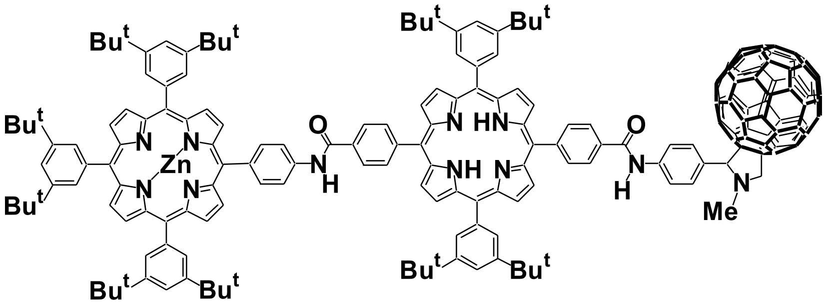

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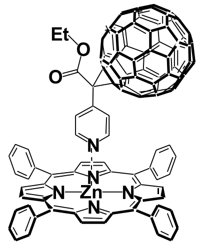
Donor–acceptor dyad systems showing rapid intramolecular photo-induced charge separation have been realized by covalent linking of C_{60} and porphyrins.[32] Based on this past knowledge, interesting trials have been made by applying a C_{60}-porphyrin-linked dyad to the multistep electron-transfer system.[22] Recently, Sakata, Imahori and other researchers have prepared a variety of porphyrin-fullerene-linked triads to mimic the photosynthetic multistep electron transfer found in natural photosynthesis systems. The C_{60}-zinc porphyrin-ferrocene triad (C_{60}-ZnP–Fc) **20** displays lifetime extension of a charge-separated state without lowering its efficiency.[39b] This excellent method of lifetime extension is based on secondary intramolecular electron transfer to a porphyrin cation radical from ferrocene as an additional donor. Photodynamics in a triad can be determined by measuring the transient absorption of radical ions of each photofunctional unit. The most predominant pathway of photo-induced charge separation in the C_{60}-ZnP–Fc triad was reported as follows: primary photo-induced electron transfer occurs mainly from excited states of the porphyrin to produce a transient charge-separated state (C_{60}^{-}-ZnP^{+}–Fc) in a high quantum yield (summation of quantum yields for all possible pathways is almost 1.0). The transient charge-separated state (C_{60}^{-}-ZnP^{+}–Fc) undergoes a secondary electron transfer between ZnP^{+} and the Fc moiety to yield quantitatively the resulting charge-separated state (C_{60}^{-}-ZnP–Fc^{+}) which persists up to 10 μs in polar solvents. This outstanding long-lived charge separation was realized by isolating the radical ion pairs. On the other hand, a ZnP-free base porphyrin–C_{60} triad **21** was also reported to exhibit sequential energy transfer and charge separation that mimic both the antenna function in the light-harvesting complex and the subsequent charge separation function in the reaction center.[40] These examples clearly demonstrate that precise control of electron transfer is possible by connecting each photofunctional molecule with C_{60} using covalent bonding. Prototypes of photovoltaic devices have been built by arranging these donor–acceptor-linked molecules on the electrode surfaces as a self-assembled monolayer (SAM). Mention is made in a later section regarding a photovoltaic device built using an SAM.

2.2. Donor–acceptor supramolecular linked systems

It is difficult to control the fast intramolecular back electron-transfer rates between a photogenerated radical cation and anion in a covalently linked system because advanced synthetic techniques are required for making sequential electron-transfer systems. The formation of long-lived charge separation has been reported, especially in high-tactically covalently-linked donor–acceptor and electron-mediator systems described in the previous section. Recently, an interesting use has been reported of photosensitizing molecules that are connected with functional combination units such as aromatic amines (pyridines



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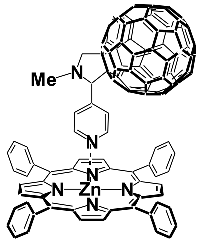

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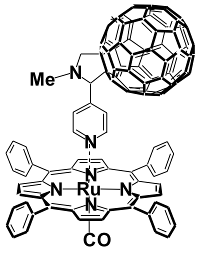

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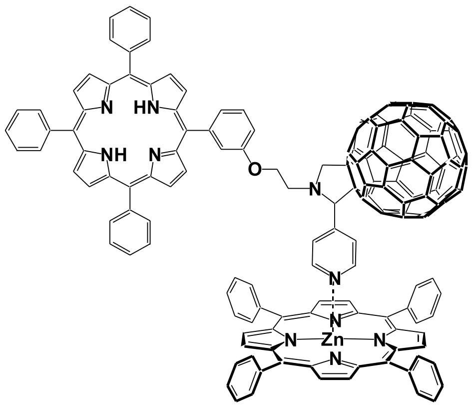

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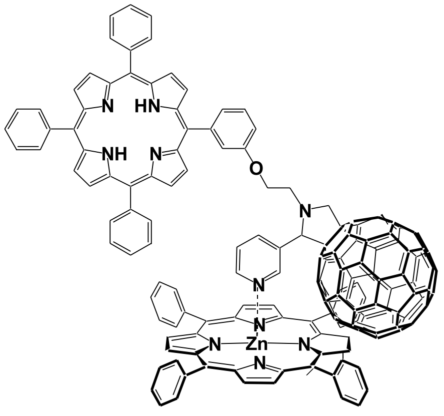

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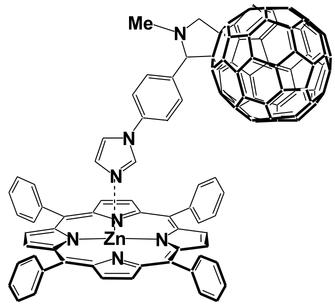

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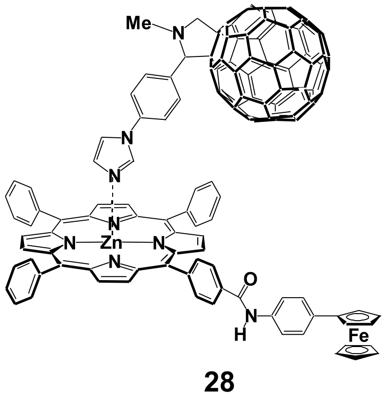

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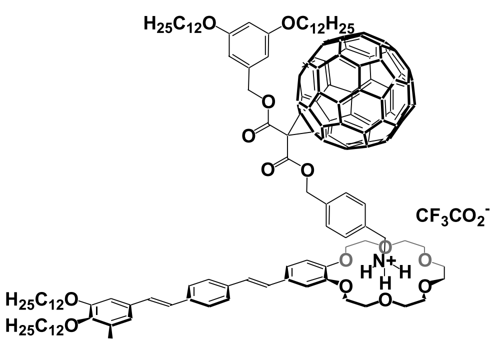
22–26,[41] and imidazoles **27–28**[42]), crown ethers **29–32**,[43] and calixarenes.[44] These sensitizers can be connected further with photochemical and electrochemical functional molecules using their combination units by supramolecular interactions such as ligand–metal coordination, hydrogen bonding, and host–guest inclusion. Photochemical properties and reactivities of sensitizers can be controlled by making supramolecular linkages with photofunctional and electrofunctional molecules. For example, two research groups led by Da Ros and D'Souza have reported an axially coordinated linkage between a metal to pyridine/imidazole unit connected with C_{60}.[42,45] These molecules show an effective forward intramolecular electron transfer supported with axial ligand–metal interaction (quantum yield of ca. 1.0) and a relatively slow back electron transfer. Through excitation of zinc porphyrin as a photosensitizing donor, efficient forward electron transfer occurs to the axially bound C_{60}. Supramolecular triads are also formed by axial coordination of fullerene to a covalently linked zinc porphyrin-ferrocene **28**.[42b] In this system, forward electron transfer is followed by a hole transfer from the ZnP^{+} to the ferrocene entity to generate the C_{60}^{-}-ZnP–Fc^{+} as a long-lived charge-separated state. These photo-induced charge separation and charge recombination processes can be examined in dyads and triads using time-resolved transient absorption and fluorescence lifetime measurements.

Efficient intramolecular photo-induced electron transfer and controlled intermolecular back electron transfer can be established compatibly if the creation of dyads that possess the abilities both of connection and dissociation is realized using supramolecular design. The core mechanism of retardation of back electron transfer in supramolecular dyads is based on the dissociation of dyads after



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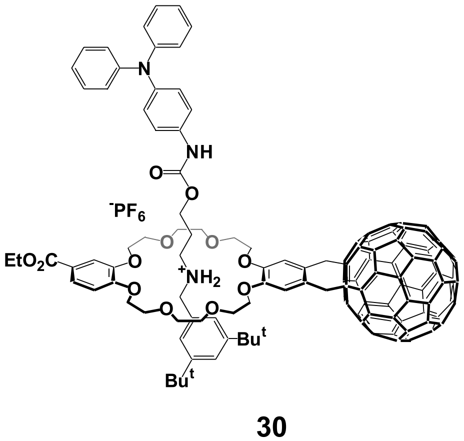

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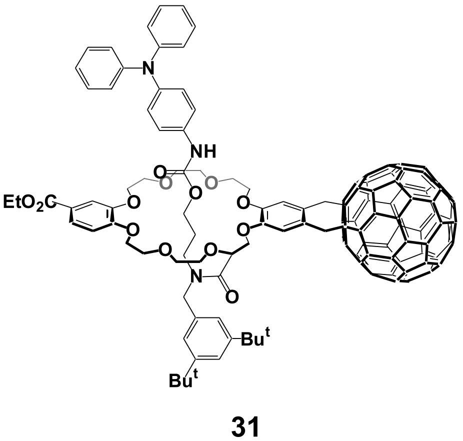

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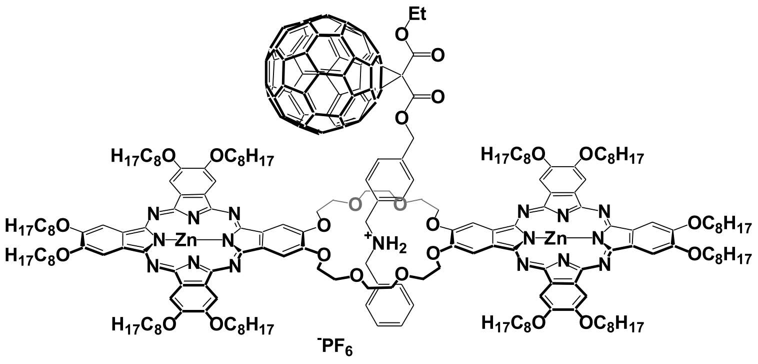

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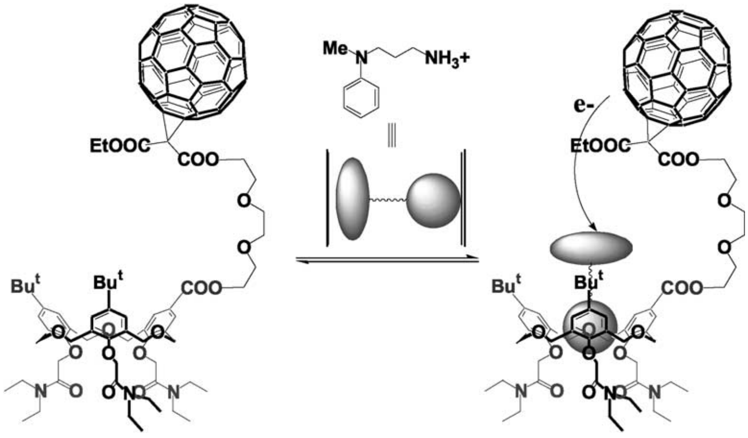
photo-induced forward electron transfer by breaking of supramolecular linking between the donor and acceptor.

Recently, we have constructed a novel non-covalently linked photoreactive dyad by applying supramolecular assembly using host–guest inclusion.[44c] A C_{60}-calixarene covalently linked molecule (calix-C_{60}) and an aniline–ammonium covalently linked molecule were synthesized. By connecting with calixarene, C_{60} is given the ability to interact with the primary alkylammonium unit. The ammonium moiety can be included in the calixarene moiety.[46] In a solution of calix-C_{60} and ammonium–aniline, the calixarene moiety of calix-C_{60} forms a donor–acceptor dyad via the cation-π-type inclusion of the ammonium moiety. In the calix-C_{60}/ammonium–aniline dyad system, photoinduced C_{60}-anion radical formation was observed in high quantum yield (0.95). Transient spectroscopies confirmed that the electron transfer proceeds through dissociation of the exciplex of ^{3}(C_{60}-aniline) in the calix-C_{60}/ammonium–aniline system (Scheme 3).

On the other hand, controlled experiments in the C_{60}/ammonium–aniline system (without a calixarene moiety) have shown a negligible quantum yield for electron transfer (<0.05). By applying an interaction between calixarene and ammonium, the electron transfer is accelerated in the dyad, greatly increasing the quantum yield for electron transfer. The produced radical anion of the C_{60} moiety persists for about 1 ms and the back electron-transfer process completely obeys second-order kinetics. These observations



Scheme 3. Photo-induced electron transfer in calix-C_{60}/ammonium–aniline supramolecular dyad system.

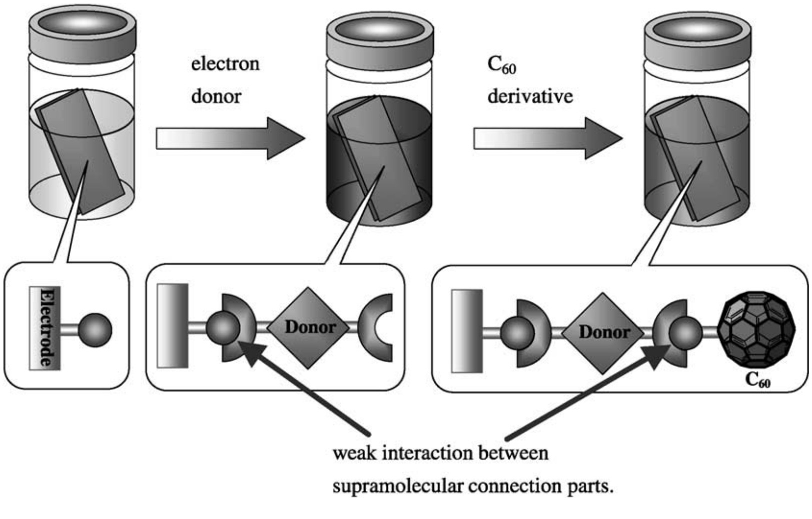
indicate that the radical anion of the C_{60} moiety and the radical cation of the aniline moiety are solvated separately after forward electron transfer. These findings show that fast intramolecular forward electron transfer, followed by slow intermolecular back electron transfer after breaking the linkage between donor and acceptor, is realized also in a C_{60}-donor-connected system using host–guest inclusion. With such a supramolecular design principle, replacement of each building block is comparatively easy. Through the application of these supramolecular dyad and triad systems to the surface of electrodes, the design of photovoltaic devices can be realized through an exhaustive study on various donors, acceptors, sensitizers, and mediators (Scheme 4).

3. Highly dense deposition of an incorporated donor–acceptor array on electrodes

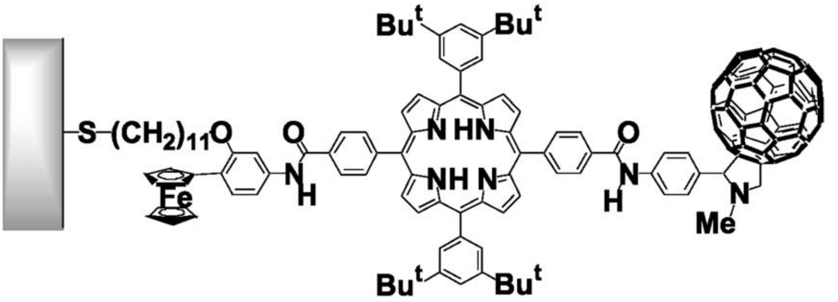
This section describes examples of research that is intended to develop the highly dense deposition of a covalently linked donor–acceptor array. It is necessary to absorb light to the full extent of the limited surface area of photovoltaic devices in addition to control of the above-mentioned electron transfer to realize efficient photocurrent conversion, that is, extremely dense functional molecules anchored onto the electrode are needed. Few research reports regarding these molecules, however, exist at the present time and three such reports are described below. In particular, a prominent trial production of photocurrent conversion devices has actually been performed using SAM techniques.

3.1. Self-assembled monolayers (SAMs)

Uosaki[46] and Sakata[47] have expounded the application of SAMs to photovoltaic systems that can provide highly ordered structures on the electrode surface. The essential utility of SAMs is for binding functional molecules with gold surfaces using a sulfur–gold linkage.[28b,48] For the surface of metal oxides such as indium-tin-oxide (ITO), binding using a silica–oxygen linkage has been mainly applied recently.[49] Generally, a thiol unit (–SH, which can interact with the gold surface) and a siloxy unit (–SiOH, which can interact with the metal oxides) are introduced asymmetrically into the end of the functional molecules as a connecting part. The connected functional molecule is adsorbed on a surface in a certain direction. SAMs therefore offer great advantages to construct vectorial electron-transfer systems.



Scheme 4. Phased preparation of photovoltaic cell using supramolecular array.



Scheme 5. Self-assembled monolayer of the ferrocene–porphyrin–C_{60} triad on a gold electrode.

A representative example was realized by Sakata and Imahori, namely the construction of wet-type photovoltaic cells using SAMs on a gold electrode.[50] This system comprised a well-oriented self-assembled monolayer of covalently-linked triads containing C_{60}, porphyrin, and ferrocene, and an alkanethiol unit (Scheme 5). The surface coverage of SAMs systems can be analyzed using a cyclic voltammetric experiment. The adsorbed amount on the gold electrode was calculated to be 1.9×10^{-10} mol cm^{-2}. In this C_{60}-zinc porphyrin–ferrocene system, well-packed triads with an almost perpendicular orientation were observed on the gold surface. Through irradiation of the triad-modified gold electrode, photocurrent generation was observed in the presence of electron carriers such as oxygen and methylviologen.[50b,c] In this system, an electron obtained from long-lived charge separation in the triad is carried to the counter electrode by the diffusing electron carriers. The quantum yield for photocurrent generation was reported as 0.25, implying that the higher efficiency is attributable to the vectorial electron transfer in SAMs of covalently linked molecules.

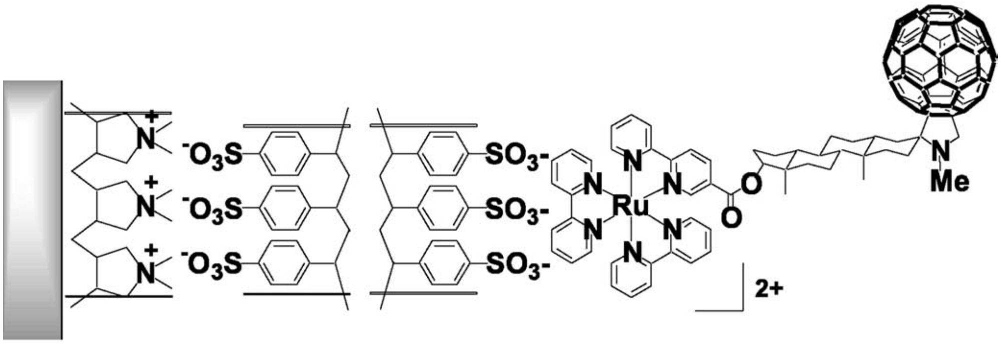
3.2. Layer-by-layer deposition

Supramolecular accumulation of a covalently linked dyad on an electrode has been proposed. Guldi et al. reported the layer-by-layer deposition of a C_{60}-donor-linked dyad on the ITO electrode.[51] A cationic-charged C_{60}-tris(2,2′-bipyridine)ruthenium(II)complex dyad was synthesized through the covalent attachment of each photofunctional building block. In this system, the dyad is adsorbed on the anion-covered ITO surface by a coulombic interaction using the cationic charge of the Ru complex moiety. Anion covering of the ITO was achieved sequentially in two steps: pre-covering of the hydrophilic ITO surface by anchoring a cationic poly(diallyldimethylammonium) (PDDA) and subsequent anion covering by adsorption of poly(styrene-4-sulfonate) (PSS). Coulombic layer-by-layer deposition allows the formation of a durable photoresponsive multilayer (Scheme 6). Successive multilayer deposition of the dyad onto the ITO substrate is also possible using coulombic interactions, short-range van der Waals forces, and hydrophobic interactions. Through irradiation of the dyad-modified electrode, progressive intensification of the photocurrent was measured while increasing the number of layers.

3.3. Langmuir–Blodgett (LB) films

The LB fabrication technique allows the deposition of heteromolecular multilayers onto the electrode surface.[52] Construction of an interlayer vectorial photo-induced electron transfer in a stack of photofunctional and electrofunctional monolayers can be realized through the fabrication of LB membranes. The molecular design is carried out by introducing LB-layer-forming substituents such as long aliphatic chains into photofunctional molecules.[53] An advantage of the LB membrane systems is their convenience for synthesizing component parts for membranes which have a relatively simple structure, compared with covalently linked molecules that have a high-tactically structured donor–acceptor.

As an example of an up-and-coming system, the C_{60}-LB-membrane system reported by Lemmetyinen et al. incorporates an alternating LB system comprising a phytochlorin-C_{60} dyad and poly(3-hexylthiophene) layers.[54] These experiments carried out not only anchoring of the covalently linked dyad onto an electrode, but also construction of an interlayer electron-transfer system



Scheme 6. Spontaneous self-assembly of composite nanostructure films with the ruthenium(II)–polypyridyl–C_{60} dyads.

between the dyad layer and an additionally-layered functional molecule (thiophene derivatives) using the LB method. In this system, an interlayer vectorial hole transfer to the poly(3-hexylthiophene) layer takes place, following intramolecular photo-induced electron transfer between the photo-excited phytochlorin moiety and the C_{60} moiety. A poly(3-hexylthiophene) layer can act as a secondary electron donor following the primary photo-induced electron transfer by donating an electron to the phytochlorin cation. The longer distance between the radical cation and anion, and the longer lifetime of the charge separation (up to seconds) were established by virtue of interlayer vectorial secondary electron transfer. Long-lived charge separation also results from charge migration in the molecular chain of the thiophene polymer. Interlayer functionalization, which is based on reciprocally photo-induced processes between stacked monolayers, points to a simple design method for devices using organized molecular assembly.

Until now, we have explored the lamination of electrodes with highly dense functional molecules and, in particular, the construction of photocurrent conversion devices by systematically arranging donor–acceptor connected molecules in SAMs was clearly demonstrated. Difficulty remains, however, in the synthesis of highly structured functional dyads or triads with a connection part for SAMs. Using these molecular design principles, further functional extensions of covalently linked molecules are possible, but we presume that the synthetic difficulty is further increased. On the other hand, LB film technology has been reported as a useful method for preparing photofunctional layers on electrodes. Highly advanced techniques of production and coping with environmental instability, however, are needed to utilize C_{60}-containing LB films. Recently, design principles for organized molecular aggregates on electrodes have been proposed for convenience and facilitation of procedures for obtaining functional membranes.

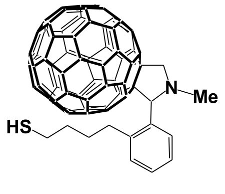
4. Preparation of a photocurrent generator using organized molecular assembly on electrodes: interlayer electron transfer

Considering the cost for research and development of a photocurrent conversion device, it is desirable to develop a simpler method for photofunctional membrane preparation. An advantage of organic solar cell systems is that various photofunctional building blocks can be chosen properly according to their use. As described in the previous section, the construction of interlayer electron-transfer systems presents one solution. This section describes supramolecular assembly of C_{60}, because the monolayer is expected allow development of an interlayer electron-transfer system.

4.1. Formation of C_{60} monolayer onto the electrode surface

Reports of monolayer formation of C_{60} or its derivatives, which are expected to become the foundation of the interlayer electron-transfer system on the electrode, are described below. As one method of C_{60} monolayer generation onto the surface of substrates/electrodes, this subsection describes (a) SAM or chemical modification, (b) coordinate covalent bonding, (c) LB membrane using C_{60} modification, (d) LB membrane using host–guest inclusion, and (e) an alternate adsorption method using coulombic interaction.

Next, we describe C_{60} fixation onto the substrate by SAM/chemical modification. This method is not classified into supramolecular assembling, but it is effective for the easy preparation of C_{60}-modified substrates. The SAM method using a connecting part such as a thiol unit or a siloxy unit is an important technique for anchoring molecules on a gold or ITO electrode.[55] Examples of C_{60}-thiol-linked molecules are **33–35**.



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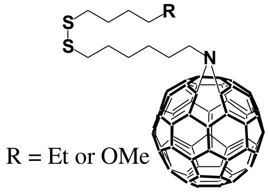

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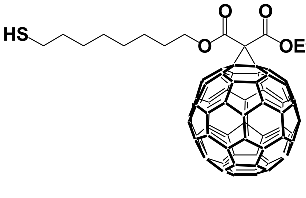

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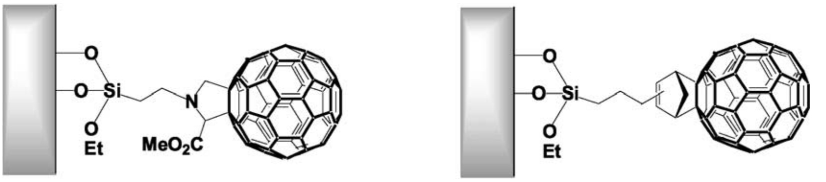
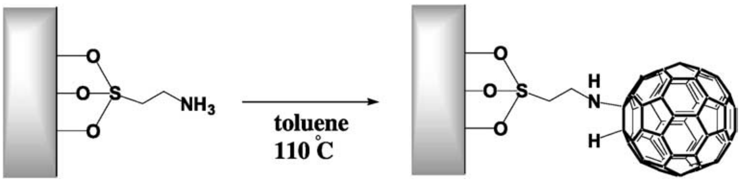
As described previously, SAM methods have also been applied with striking success to the fixation of C_{60}-donor covalently connected molecules such as dyads or triads. Reportedly, the SAM technique can play an important role in anchoring C_{60} derivatives. Aside from that mentioned above, other research efforts have been geared towards the immobilization of C_{60} onto substrates. Two procedures for C_{60} deposition have been reported by making a covalent linkage between C_{60} and the substrate. One method is to prepare a C_{60} derivative, which has a connection part in advance, and the other is to modify the substrate with a substituent group which can react with C_{60}. As an example of the former procedure, Maggini et al. have reported the deposition of a C_{60}-triethoxysilyl covalently-linked molecule on a silica gel surface.[56] Thermally generated covalent bonds between Si–O allow the fixation of the C_{60} moiety (Scheme 7).

In an example of the latter procedure, Mirkin and Yoon covered a glass substrate with 3-aminopropyl groups by treating with (3-aminopropyl)triethoxysilane (APS).[57] This surface lamination preceded thermal treatment in the presence of C_{60} (Scheme 8). Alkylamines readily undergo N–H addition to form C_{60} double bonds.[58]

Examples of experiments using a similar concept are: the fixation of C_{60} on an Si–H functionalized SiO_2 surface using C–Si bond formation by hydrosilylation[59]



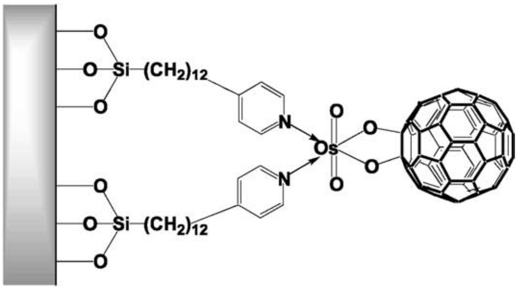
Scheme 7. Deposition of C_{60}-triethoxysilyl covalently-linked molecule on a silica gel.



Scheme 8. Reaction of C_{60} with glass-bound 3-aminopropylsilyl groups.

and on an alkylamine-functionalized gold surface using C–N bond formation by C_{60}-amine addition.[57,60] The amine-inserted fullerene can undergo a second insertion of an amine group. It has therefore been demonstrated that APS-coated zeolite crystals can be deposited on the C_{60}-coated substrate as a closely packed monolayer. A similar procedure can be expanded further into dyads using electron donors with an amino group instead of zeolite crystals.

Smith et al. reported the deposition of C_{60} using coordinate covalent bonding as an example of supramolecular assembly of C_{60}.[61] The quartz surface was modified by the addition of a pyridyl-terminated alkoxysilane monolayer that forms a $(pyridyl)_2$–OsO_4–C_{60} coordination complex. The pyridyl-terminated SAM on the substrate can produce linkages with C_{60} through sequential treatment with (i) OsO_4 and (ii) C_{60} in toluene. By this method, C_{60} is modified by an osmium complex, thereby allowing coordinate bonding with pyridyl groups through the osmium complex moiety (Scheme 9).



Scheme 9. Self-assembled monolayer of $(pyridyl)_2$–Os–C_{60} coordination compound.

On the other hand, the LB-method is a technique cited in relatively early reports. Diederich et al. reported LB monolayer and multilayer formation using a covalently attached molecule between methano-C_{60} and benzo[2.2.2]-cryptand **36–38** with sodium ion as an ionic guest molecule.[62] Their study attained the construction of an LB membrane using a cryptand-bound metal ion as a hydrophilic group and using methano-C_{60} as a hydrophobic group. In this way, an LB monolayer of C_{60} derivatives can be produced at the air–water interface by applying a molecular design that introduces a polar moiety to C_{60}. Reportedly, malonic acid **39**,[63] quaternary ammonium **40**,[64] and metal ion-chelating EDTA **41**[65] were also connected with C_{60} and facilitate LB film formation. It was also reported that a C_{60}-bearing artificial lipid **42** can constitute an LB film.[66] On the other hand, host–guest inclusion of C_{60} by an azacrown ether **43**,[67] or calix[8]arene **44**[68] derivatives can produce LB-forming materials. One important feature of these host–guest techniques is that C_{60} can be arranged on the substrate without chemical modification of the C_{60}.

A layer-by-layer coulombic deposition technique, which was mentioned in Section 3.2, can be applied for the deposition of ionic C_{60} derivatives.[51] It is expected that the alternate adsorption method can build interlayer electron-transfer systems relatively simply. Monolayer formation with a higher performance will be realized by the use of chemically unmodified C_{60}, if this technique is combined with the host–guest technique.[69] The next subsection describes a facile supramolecular assembling of flawless C_{60} and photofunctional molecules onto electrodes, with particular emphasis on our latest results.

4.2. Facile preparation of a durable membrane for interlayer electron-transfer systems using alternate adsorption

We have recently found that cationic charges can be imparted to C_{60} without chemical modification by forming a host–guest 2:1 (host:C_{60}) inclusion complex with hexa-cationic-homo-oxacalix[3]arene. The resultant cationic C_{60} complex is capable of solubilizing C_{60} into water.[69] Through the adsorption of the cationic inclusion complex of C_{60}, a C_{60} monolayer can be prepared easily onto an anion-covered Au surface (Scheme 10).

This C_{60} monolayer shows specific redox signals in cyclic voltammetry. From the peak current of the first reduction, surface coverage by the C_{60} complex can be estimated reasonably[70] to be 1.7×10^{-10} mol cm^{-2}. The amount of coverage by C_{60} was 1.4-fold higher than that using

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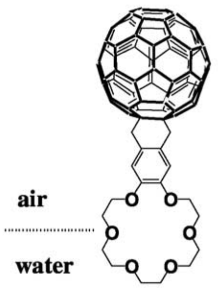

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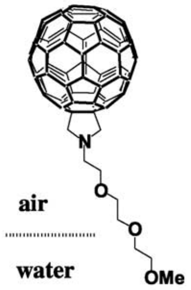

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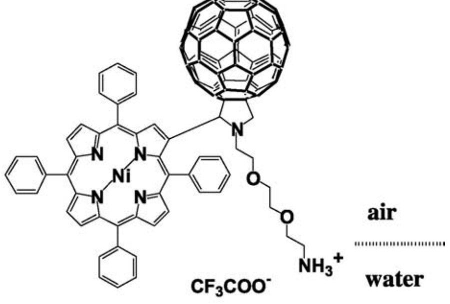

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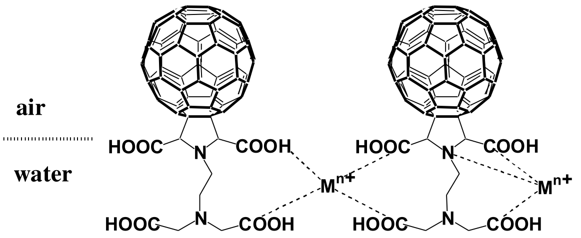

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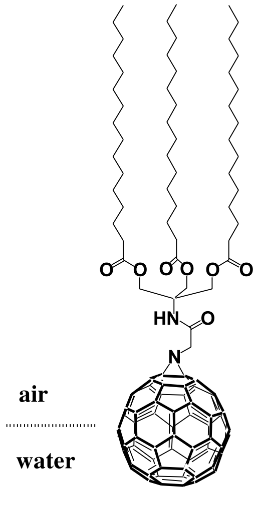

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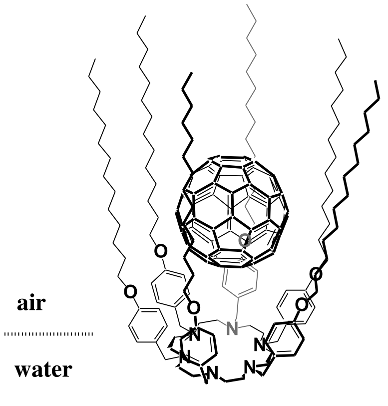

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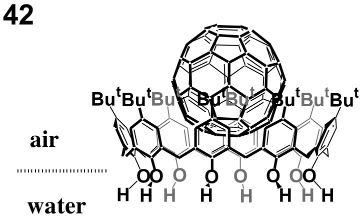

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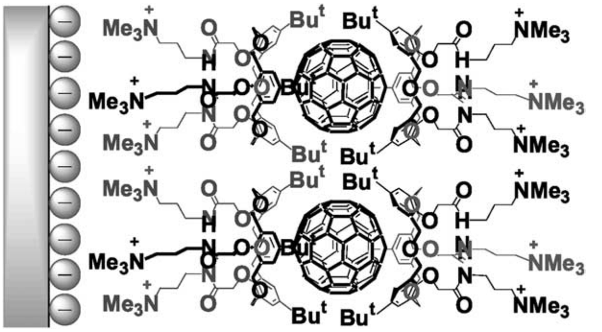


Scheme 10. Self-assembled monolayer of calixarene·C_{60} complex on gold electrode.

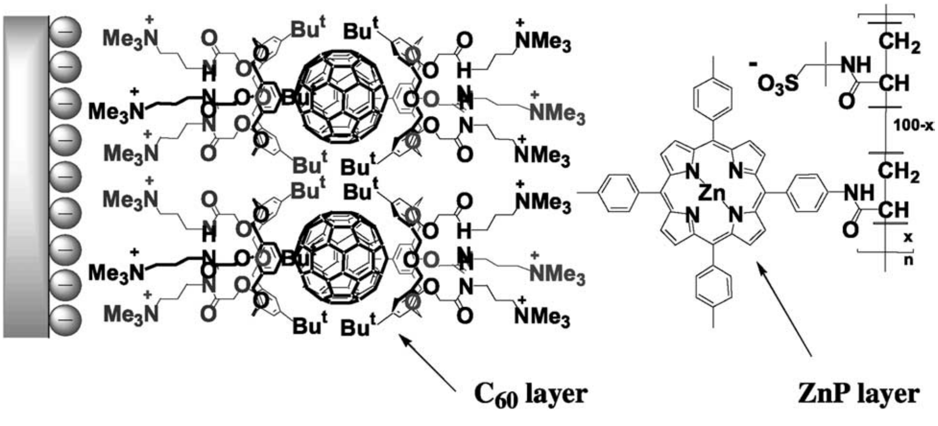
adsorption by conventional SAM methods. Using this technique, the high-density accumulation of C_{60} was simple. Moreover, using this method, C_{60} can be arranged on the substrate without chemical modification and self aggregation of C_{60}. Host–guest inclusion is an extremely effective method to retain photochemical and electrochemical properties of C_{60} that are partially impaired by chemical modification and self aggregation. An actual application—the preparation of a photovoltaic device comprising a bilayer between an anionic photofunctional donor and a cationic C_{60} (acceptor)—is described below.

On completion of the arrangement of the cationic C_{60} complex onto the surface, the anion-covered electrode is enclothed by cationic charges, thereby preventing further adsorption of the cationic C_{60} complex. Additionally, another anionic functional layer can be deposited on the cationic C_{60} monolayer. This adsorption process can be repeated alternately using reversely charged compounds. The process can allow the preparation of a stacked functional multilayer. An oriented multilayer system of C_{60} photofunctional molecules can be constructed by this supramolecular, stacked design. For example, the facile preparation of a novel C_{60}/ZnP bilayer system is achieved through electrostatic force (Scheme 11) and, thereby, an efficient interlayer vectorial photocurrent generation is established.[71] In the C_{60}/ZnP bilayer system, the surface concentration was estimated to be 1.4×10^{-10} mol cm^{-2} and the surface concentration of ZnP units was estimated to be 5.5×10^{-10} mol cm^{-2}.

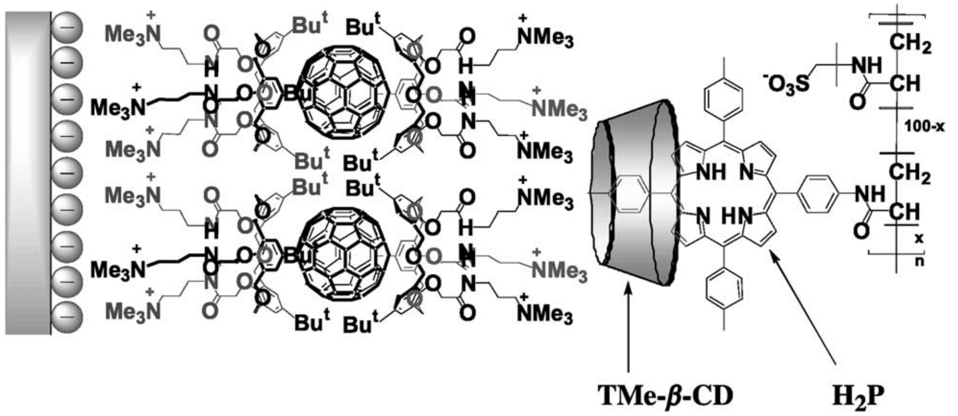
Photocurrent measurements were carried out for the C_{60}/ZnP bilayer system and the bilayer deposited on the modified ITO electrode. For the electrochemical experiments in water, we used 0.1 M Na_2SO_4 as the supporting electrolyte, 50 mM ascorbic acid (AsA) as an electron sacrificer, a Pt counterelectrode, and an Ag/AgCl (3 M NaCl) reference electrode. The action spectrum of photocurrent generation indicates that the efficiency for photocurrent generation in the C_{60}/ZnP bilayer system is increased markedly in comparison with that in the C_{60} monolayer system. These results show that our non-covalently linked donor–acceptor system features sequential photocurrent flow: ZnP units → C_{60} units → ITO electrode, as in the case of the covalently linked dyad or triad systems reported previously. These photoresponsive phenomena are repeatedly reversible and, moreover, the films were sufficiently robust. The quantitative quantum yield can be estimated as 10 and 21% for the C_{60} monolayer and C_{60}/ZnP bilayers, respectively. These findings suggest that our alternate adsorption method is extremely useful for a novel and simple preparation of photocurrent generators. This method can be extended further into multicomponent systems such as non-covalently linked triads or tetrads by a convenient alternate adsorption method.

4.2.1. Supramolecular control of electron-transfer systems.

Facile preparations of the functional multilayer are expected to permit the improvement of photoresponsibility through a comparatively easy procedure. By alternate adsorption, described previously, a photoresponsive bilayer of C_{60}/free base porphyrin (H_2P) can be prepared in a similar manner to the C_{60}/ZnP system. Measurements of UV–vis absorption spectra indicate that the C_{60} units are included individually by calixarenes. The porphyrin units, however, produce self aggregates considerably in the C_{60}/H_2P bilayer. Self aggregation of photosensitizing units invites self quenching of photo-excited states and the quantum yield for electron transfer decreases remarkably. Trimethyl-β-cyclodextrin was then used as a host molecule to isolate a porphyrin unit.[72] Cyclodextrins can include various organic molecules including porphyrin derivatives in their cavity. For the C_{60}/aggregated H_2P system, the isolated-H_2Por layer can be prepared simply through the addition of trimethyl-β-cyclodextrin(TMe-β-CD) to



Scheme 11. SAM of calixarene·C_{60} complex (first layer) and ZnP pendant polymer (second layer) on ITO electrode.



Scheme 12. SAM of calixarene·C_{60} complex (first layer) and H_2P·TMe-β-CD complex (second layer) on ITO electrode.

the solution of H_2P-polymer at the time of alternate adsorption (Scheme 12). Although the quantum yield for photocurrent generation in the absence of trimethyl-β-cyclodextrin was 0.15, in the presence of trimethyl-β-cyclodextrin the quantum yield improves by 1.3-fold to 0.20. The alternative adsorption not only simplifies the construction of donor–acceptor multilayer systems, but also allows the control of electron transfer.

5. Conclusions and perspectives

This review has reported recent research on the molecular control of electron transfer and the highly dense deposition onto an electrode. For constructing photovoltaic devices, these targets cannot be considered independently. For an efficient organic photocurrent generator that is based on photo-induced charge separation, the relative position of each building block is controlled by covalent bonding and supramolecular interaction. Numerous options to design organic photovoltaic devices are presented, considering the strategy of interlayer electron transfer in addition to intramolecular processes. Moreover, in order to reduce research costs, it is desirable that the replacement of building blocks in an organized molecular assembly can be put into practice on an ‘as-needed’ basis. The simplification of device preparation can therefore be seen as an important target in organic photocurrent conversion. A supramolecular reaction design is expected to respond to this requirement. Such a design allows combinatorial approaches using various functional molecules. On the other hand, sufficient light absorption is securable not only by anchoring functional molecules as a monolayer with high density. For sufficient photon collection, intralayer and interlayer energy transfer using antenna molecules or photosensitizers must be considered. In addition, for a higher multicomponent membrane system with increased membrane thickness, it may be necessary to contrive a mechanism of electron flow perpendicularly through a multilayer. Many challenges are still posed for the realization of an organic solar cell, but we believe that solving these problems one by one will ultimately pave the way to its actual use.

References and notes

1. Shah, A.; Torres, P.; Tscharner, R.; Wyrsch, N.; Keppner, H. *Science* **1999**, *285*, 692.
2. Dresselhaus, M. S.; Thomas, I. L. *Nature* **2001**, *414*, 332.
3. Nelson, J. *Science* **2001**, *293*, 1059.
4. Sheats, J. R. *J. Mater. Res.* **2004**, *19*, 1974.
5. He, J.; Benkoe, G.; Korodi, F.; Polivka, T.; Lomoth, R.; Kermark, B.; Sun, L.; Hagfeldt, A.; Sundström, V. *J. Am. Chem. Soc.* **2002**, *124*, 4922.
6. Zhang, L.; Yang, J.; Wang, L.; Yang, G.-Z.; Weng, Y.-X. *J. Phys. Chem. B* **2003**, *107*, 13688.
7. Furube, A.; Katoh, R.; Yoshihara, T.; Hara, K.; Murata, S.; Arakawa, H.; Tachiya, M. *J. Phys. Chem. B* **2004**, *108*, 12583.
8. Matsuo, T. *J. Photochem.* **1985**, *29*, 41.
9. Grätzel, M. *NATO ASI Ser., Ser. C: Math. Phys. Sci.* **1987**, *214*, 435.
10. Kondo, T.; Ito, T.; Nomura, S.; Uosaki, K. *Thin Solid Films* **1996**, *284–285*, 652.
11. Sakata, Y.; Imahori, H.; Tsue, H.; Higashida, S.; Akiyama, T.; Yoshizawa, E.; Aoki, M.; Yamada, K.; Hagiwara, K.; Taniguchi, S.; Okada, T. *Pure Appl. Chem.* **1997**, *69*, 1951.
12. Miyashita, T.; Aoki, A.; Abe, Y. *Mol. Cryst. Liq. Cryst. Sci. Technol., Sec. A: Mol. Cryst. Liq. Cryst.* **1999**, *327*, 77.
13. Imahori, H.; Sakata, Y. *Eur. J. Org. Chem.* **1999**, 2445.
14. Imahori, H. *Org. Biomol. Chem.* **2004**, *2*, 1425.
15. Grätzel, M. *Nature* **2001**, *414*, 338.
16. Grätzel, M. *J. Photochem. Photobiol., C: Photochem. Rev.* **2003**, *4*, 145.
17. Arakawa, H.; Sayama, K. *Res. Chem. Intermed.* **2000**, *26*, 145.
18. Bisquert, J.; Cahen, D.; Hodes, G.; Ruehle, S.; Zaban, A. *J. Phys. Chem. B* **2004**, *108*, 8106.
19. Wang, Z.-S.; Kawauchi, H.; Kashima, T.; Arakawa, H. *Coord. Chem. Rev.* **2004**, *248*, 1381.
20. Schwarzburg, K.; Ernstorfer, R.; Felber, S.; Willig, F. *Coord. Chem. Rev.* **2004**, *248*, 1259.
21. Moser, J. E.; Bonhote, P.; Walder, L.; Grätzel, M. *Chimia* **1997**, *51*, 28.
22. Gust, D.; Moore, T. A.; Moore, A. L.; Lee, S. J.; Bittersmann, E.; Luttrull, D. K.; Rehms, A. A.; DeGraziano, J. M.; Ma, X. C. *Science* **1990**, *248*, 199.
23. Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435.
24. Mataga, N.; Miyasaka, H. *Adv. Chem. Phys.* **1999**, *107*, 431.
25. Bott, A. W. *Curr. Sep.* **1991**, *10*, 137.
26. (a) Allemand, P. M.; Koch, A.; Wudl, F.; Rubin, Y.; Diederich,

F.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L. *J. Am. Chem. Soc.* **1991**, *113*, 1050. (b) Dubois, D. K.; Kadish, M.; Flanagan, S.; Haufler, R. E.; Chibante, L. P. F.; Wilson, L. J. *J. Am. Chem. Soc.* **1991**, *113*, 4364. (c) Konishi, T.; Sasaki, Y.; Fujitsuka, M.; Toba, Y.; Moriyama, H.; Ito, O. *J. Chem. Soc., Perkin Trans. 2* **1999**, 551.

27. (a) McDermott, G.; Prince, S. M.; Freer, A. A.; Hawthornthwaite-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W. *Nature* **1995**, *374*, 517. (b) Olson, J. M. *Photochem. Photobiol.* **1998**, *67*, 61. (c) Zouni, A.; Witt, H.-T.; Kern, J.; Fromme, P.; Kraub, N.; Saenger, W.; Orth, P. *Nature* **2001**, *409*, 739. (d) Jordan, P.; Fromme, P.; Witt, H.-T.; Klukas, O.; Saenger, W.; Krauß, N. *Nature* **2001**, *411*, 909.
28. For example (a) Untereker, D. F.; Lennox, J. C.; Wier, L. M.; Moses, P. R.; Murray, R. W. *J. Electroanal. Chem. Interface Electrochem.* **1977**, *81*, 309. (b) Delamarche, E.; Michel, B.; Biebuyck, H. A.; Gerber, C. *Adv. Mater.* **1996**, *8*, 719.
29. Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.
30. (a) Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155. (b) Marcus, R. A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1111.
31. For example (a) Gabe, Y.; Urano, Y.; Kikuchi, K.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 3357. (b) Schuster, D. I.; Cheng, P.; Jarowski, P. D.; Guldi, D. M.; Luo, C.; Echegoyen, L.; Pyo, S.; Holzwarth, A. R.; Braslavsky, S. E.; Williams, R. M.; Klihm, G. *J. Am. Chem. Soc.* **2004**, *126*, 7257.
32. (a) Bourgeois, J.-P.; Diederich, F.; Echegoyen, L.; Nierengarten, J.-F. *Helv. Chim. Acta* **1998**, *81*, 1835. (b) Armaroli, N.; Marconi, G.; Echegoyen, L.; Bourgeois, J.-P.; Diederich, F. *Chem. Eur. J.* **2000**, *6*, 1629. (c) Tkachenko, N. V.; Rantala, L.; Tauber, A. Y.; Helaja, J.; Hynninen, P. H.; Lemmetyinen, H. *J. Am. Chem. Soc.* **1999**, *121*, 9378. (d) Kuciauskas, D.; Lin, S.; Seely, G. R.; Moore, A. L.; Moore, T. A.; Gust, D.; Drovetskaya, T.; Reed, C. A.; Boyd, P. D. W. *J. Phys. Chem.* **1996**, *100*, 15926. (e) Imahori, H.; Hagiwara, K.; Aoki, M.; Akiyama, T.; Taniguchi, S.; Okada, T.; Shirakawa, M.; Sakata, Y. *J. Am. Chem. Soc.* **1996**, *118*, 11771. (f) Baran, P. S.; Monaco, R. R.; Khan, A. U.; Schuster, D. I.; Wilson, S. R. *J. Am. Chem. Soc.* **1997**, *119*, 8363.
33. (a) Obara, Y.; Takimiya, K.; Aso, Y.; Otsubo, T. *Tetrahedron Lett.* **2001**, *42*, 6877. (b) Guldi, D. M.; Luo, C.; Swartz, A.; Gomez, R.; Segura, J. L.; Martin, N.; Brabec, C.; Sariciftci, N. S. *J. Org. Chem.* **2002**, *67*, 1141. (c) Van Hal, P. A.; Beckers, E. H. A.; Meskers, S. C. J.; Janssen, R. A. J.; Jousselme, B.; Blanchard, P.; Roncali, J. *Chem. Eur. J.* **2002**, *8*, 5415. (d) Fujitsuka, M.; Masuhara, A.; Kasai, H.; Oikawa, H.; Nakanishi, H.; Ito, O.; Yamashiro, T.; Aso, Y.; Otsubo, T. *J. Phys. Chem. B* **2001**, *105*, 9930.
34. Loi, M. A.; Denk, P.; Hoppe, H.; Neugebauer, H.; Winder, C.; Meissner, D.; Brabec, C.; Sariciftci, N. S.; Gouloumis, A.; Vazquez, P.; Torres, T. *J. Mater. Chem.* **2003**, *13*, 700.
35. (a) Imahori, H.; Cardoso, S.; Tatman, D.; Lin, S.; Noss, L.; Seely, G. R.; Sereno, L.; Chessa De Silber, J.; Moore, T. A. *Photochem. Photobiol.* **1995**, *62*, 1009. (b) Moore, A. L.; Moore, T. A.; Gust, D.; Silber, J. J.; Sereno, L.; Fungo, F.; Otero, L.; Steinberg-Yfrach, G.; Liddell, P. A.; Hung, S.-C.; Imahori, H.; Cardoso, S.; Tatman, D.; Macpherson, A. N. *Pure Appl. Chem.* **1997**, *69*, 2111. (c) Bahr, J. L.; Kuciauskas, D.; Liddell, P. A.; Moore, A. L.; Moore, T. A.; Gust, D. *Photochem. Photobiol.* **2000**, *72*, 598. (d) Yamazaki, M.; Araki, Y.; Fujitsuka, M.; Ito, O. *J. Phys. Chem. A* **2001**, *105*, 8615.
36. (a) Maggini, M.; Donò, A.; Scorrano, G.; Prato, M. *J. Chem. Soc., Chem. Commun.* **1995**, 845. (b) Maggini, M.; Karlsson, A.; Scorrano, G.; Sandona, G.; Farnia, G.; Prato, M. *J. Chem. Soc., Chem. Commun.* **1994**, 589. (c) Martín, N.; Sánchez, L.; Seoane, C.; Andreu, R.; Garín, J.; Orduna, J. *Tetrahedron Lett.* **1996**, *37*, 5979. (d) Llacay, J.; Mas, M.; Molins, E.; Veciana, J.; Powell, D.; Rovira, C. *Chem. Commun.* **1997**, *7*, 659.
37. For example (a) Fujihira, M.; Sakomura, M. *Thin Solid Films* **1989**, *179*, 471. (b) Eichen, Y.; Nakhmanovich, G.; Epshtein, O.; Ehrenfreund, E. *J. Phys. Chem. B* **2000**, *104*, 770.
38. (a) Paddon-Row, M. N. *Acc. Chem. Res.* **1994**, *27*, 18. (b) Wegewijs, B.; Verhoeven, J. W. *Adv. Chem. Phys.* **1999**, *106*, 221.
39. (a) Safonov, I. G.; Baran, P. S.; Schuster, D. I. *Tetrahedron Lett.* **1997**, *38*, 8133. (b) Imahori, H.; Tamaki, K.; Guldi, D. M.; Luo, C.; Fujitsuka, M.; Ito, O.; Sakata, Y.; Fukuzumi, S. *J. Am. Chem. Soc.* **2001**, *123*, 2607. (c) Imahori, H.; Mori, Y.; Matano, Y. *J. Photochem. Photobiol., C: Photochem. Rev.* **2003**, *4*, 51. (d) Imahori, H.; Kashiwagi, Y.; Hasobe, T.; Kimura, M.; Hanada, T.; Nishimura, Y.; Yamazaki, I.; Araki, Y.; Ito, O.; Fukuzumi, S. *Thin Solid Films* **2004**, *451*, 580.
40. Luo, C.; Guldi, D. M.; Imahori, H.; Tamaki, K.; Sakata, Y. *J. Am. Chem. Soc.* **2000**, *122*, 6535.
41. (a) D'Souza, F.; Deviprasad, G. R.; Zandler, M. E.; El-Khouly, M. E.; Fujitsuka, M.; Ito, O. *J. Phys. Chem. B* **2002**, *106*, 4952. (b) Guldi, D. M.; Ramey, J.; Martinez-Diaz, M. V.; de la Escosura, A.; Torres, T.; Da Ros, T.; Prato, M. *Chem. Commun.* **2002**, 2774.
42. (a) D'Souza, F.; Deviprasad, G. R.; Zandler, M. E.; Hoang, V. T.; Klykov, A.; Van Stipdonk, M.; Perera, A.; El-Khouly, M. E.; Fujitsuka, M.; Ito, O. *J. Phys. Chem. A* **2002**, *106*, 3243. (b) D'Souza, F.; Smith, P. M.; Gadde, S.; McCarty, A. L.; Kullman, M. J.; Zandler, M. E.; Itou, M.; Araki, Y.; Ito, O. *J. Phys. Chem. B* **2004**, *108*, 11333.
43. (a) Diederich, F.; Gómez-López, M. *Chimia* **1998**, *52*, 551. (b) Gutiérrez-Nava, M.; Nierengarten, H.; Masson, P.; Van Dorsselaer, A.; Nierengarten, J.-F. *Tetrahedron Lett.* **2003**, *44*, 3043. (c) Sandanayaka, A. S. D.; Sasabe, H.; Araki, Y.; Furusho, Y.; Ito, O.; Takata, T. *J. Phys. Chem. A* **2004**, *108*, 5145.
44. (a) Kawaguchi, M.; Ikeda, A.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 1* **1998**, 179. (b) Shinkai, S.; Ikeda, A. *Gazz. Chim. Ital.* **1997**, *127*, 657. (c) Konishi, T.; Ikeda, A.; Kishida, T.; Rasmussen, B. S.; Fujitsuka, M.; Ito, O.; Shinkai, S. *J. Phys. Chem. A* **2002**, *106*, 10254.
45. (a) Da Ros, T.; Prato, M.; Carano, M.; Ceroni, P.; Paolucci, F.; Roffia, S.; Valli, L.; Guldi, D. M. *J. Organomet. Chem.* **2000**, *599*, 62. (b) Da Ros, T.; Prato, M.; Guldi, D. M.; Ruzzi, M.; Pasimeni, L. *Chem. Eur. J.* **2001**, *7*, 816.
46. (a) Araki, K.; Inada, K.; Otsuka, H.; Shinkai, S. *Tetrahedron* **1993**, *49*, 9465. (b) Araki, K.; Hashimoto, N.; Otsuka, H.; Shinkai, S. *J. Org. Chem.* **1993**, *58*, 5958. (c) Masci, B. *Tetrahedron* **1995**, *51*, 5459.
47. (a) Uosaki, K.; Kondo, T.; Zhang, X.-Q.; Yanagida, M. *J. Am. Chem. Soc.* **1997**, *119*, 8367. (b) Kondo, T.; Yanagida, M.; Zhang, X.-Q.; Uosaki, K. *Chem. Lett.* **2000**, 964.
48. (a) Akiyama, T.; Imahori, H.; Ajawakom, A.; Sakata, Y. *Chem. Lett.* **1996**, 907. (b) Imahori, H.; Norieda, H.; Ozawa, S.; Ushida, K.; Yamada, H.; Azuma, T.; Tamaki, K.; Sakata, Y. *Langmuir* **1998**, *14*, 5335. (c) Imahori, H.; Ozawa, S.; Ushida, K.; Takahashi, M.; Azuma, T.; Ajavakom, A.; Akiyama, T.; Hasegawa, M.; Taniguchi, S.; Okada, T.; Sakata, Y. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 485.

49. Fadeev, A. Y.; McCarthy, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 12184.
50. (a) Imahori, H.; Yamada, H.; Ozawa, S.; Sakata, Y.; Ushida, K. *Chem. Commun.* **1999**, 1165. (b) Imahori, H.; Yamada, H.; Nishimura, Y.; Yamazaki, I.; Sakata, Y. *J. Phys. Chem. B* **2000**, *104*, 2099. (c) Imahori, H.; Norieda, H.; Yamada, H.; Nishimura, Y.; Yamazaki, I.; Sakata, Y.; Fukuzumi, S. *J. Am. Chem. Soc.* **2001**, *123*, 100.
51. Luo, C.; Guldi, D. M.; Maggini, M.; Menna, E.; Mondini, S.; Kotov, N. A.; Prato, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 3905.
52. (a) Zasadzinski, J. A.; Viswanathan, R.; Madsen, L.; Garnaes, J.; Schwartz, D. K. *Science* **1994**, *263*, 1726. (b) Aoki, A.; Miyashita, T. *J. Electroanal. Chem.* **1999**, *473*, 125.
53. Caminati, G.; Gabrielli, G.; Ricceri, R.; Turro, C.; Turro, N. J. *Thin Solid Films* **1996**, *284*, 718.
54. Alekseev, A. S.; Tkachenko, N. V.; Tauber, A. Y.; Hynninen, P. H.; Osterbacka, R.; Stubb, H.; Lemmetyinen, H. *Chem. Phys.* **2002**, *275*, 243.
55. (a) Shi, X.; Caldwell, W. B.; Chen, K.; Mirkin, C. A. *J. Am. Chem. Soc.* **1994**, *116*, 11598. (b) Imahori, H.; Azuma, T.; Ozawa, S.; Yamada, H.; Ajavakom, A.; Norieda, H.; Sakata, Y.; Ushida, K. *Chem. Commun.* **1999**, 557. (c) Imahori, H.; Azuma, T.; Ajavakom, A.; Norieda, H.; Yamada, H.; Sakata, Y. *J. Phys. Chem. B* **1999**, *103*, 7233. (d) Shon, Y.-S.; Kelly, K. F.; Halas, N. J.; Lee, T. R. *Langmuir* **1999**, *15*, 5329. (e) Enger, O.; Nuesch, F.; Fibbioli, M.; Echegoyen, L.; Pretsch, E.; Diederich, F. *J. Mater. Chem.* **2000**, *10*, 2231. (f) Gu, T.; Whitesell, J. K.; Fox, M. A. *J. Org. Chem.* **2004**, *69*, 4075.
56. (a) Bianco, A.; Gasparrini, F.; Maggini, M.; Misiti, D.; Polese, A.; Prato, M.; Scorrano, G.; Toniolo, C.; Villani, C. *J. Am. Chem. Soc.* **1997**, *119*, 7550. (b) Nie, B.; Rotello, V. M. *J. Org. Chem.* **1996**, *61*, 1870.
57. Chen, K.; Caldwell, W. B.; Mirkin, C. A. *J. Am. Chem. Soc.* **1993**, *115*, 1193.
58. (a) Wudl, F. *Acc. Chem. Res.* **1992**, *25*, 157. (b) Wudl, F.; Hirsch, A.; Khemani, K. C.; Suzuki, T.; Allemand, P.-M.; Koch, A.; Eckert, H.; Srdanov, G.; Webb, H. M. In *Fullerenes: Synthesis. Properties and Chemistry of Large Carbon Clusters*; Hammond, G. S., Kuck, V. J., Eds.; American Chemical Society: Washington, DC, 1992; p 161.
59. Planeix, J.-M.; Coq, B.; de Menorva, L.-C.; Medina, P. *Chem. Commun.* **1996**, 2087.
60. (a) Choi, S. Y.; Lee, Y.-J.; Park, Y. S.; Ha, K.; Yoon, K. B. *J. Am. Chem. Soc.* **2000**, *122*, 5201. (b) Higashi, N.; Inoue, T.; Niwa, M. *Chem. Commun.* **1997**, 1507.
61. Chupa, J. A.; Xu, S.; Fischetti, R. F.; Strongin, R. M.; McCauley, J. P., Jr.; Smith, A. B., III; Blasie, J. K.; Peticolas, L. J.; Bean, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 4383.
62. Jonas, U.; Cardullo, F.; Belik, P.; Diederich, F.; Gügel, A.; Harth, E.; Herrmann, A.; Isaacs, L.; Müllen, K.; Ringsdorf, H.; Thielgen, C.; Uhlmann, P.; Vasella, A.; Waldraff, C. A. A.; Walter, M. *Chem. Eur. J.* **1995**, *1*, 243.
63. (a) Wei, T.-X.; Zhai, J.; Ge, J.-H.; Gan, L.-B.; Huang, C.-H.; Luo, G.-B.; Ying, L.-M.; Liu, T.-T.; Zhao, X.-S. *Appl. Surf. Sci.* **1999**, *151*, 153. (b) Patel, H. M.; Didymus, J. M.; Wong, K. K. W.; Hirsch, A.; Skiebe, A.; Lamparth, I.; Mann, S. *Chem. Commun.* **1996**, 611.
64. (a) Felder, D.; Nava, M. G.; Carreon, M. D. P.; Eckert, J.-F.; Luccisano, M.; Schall, C.; Masson, P.; Gallani, J.-L.; Heinrich, B.; Guillon, D.; Nierengarten, J.-F. *Helv. Chim. Acta* **2002**, *85*, 288. (b) Felder, D.; Carreon, M. D. P.; Gallani, J.-L.; Guillon, D.; Nierengarten, J.-F.; Chuard, T.; Deschenaux, R. *Helv. Chim. Acta* **2001**, *84*, 1119. (c) Zhang, S.; Rio, Y.; Cardinali, F.; Bourgogne, C.; Gallani, J.-L.; Nierengarten, J.-F. *J. Org. Chem.* **2003**, *68*, 9787.
65. (a) Nakashima, N.; Ishii, T.; Shirakusa, M.; Nakanishi, T.; Murakami, H.; Sagara, T. *Chem. Eur. J.* **2001**, *7*, 1766. (b) Guldi, D. M.; Zilbermann, I.; Anderson, G. A.; Kordatos, K.; Prato, M.; Tafuro, R.; Valli, L. *J. Mater. Chem.* **2004**, *14*, 303.
66. Zhang, W.; Shi, Y.; Gan, L.; Wu, N.; Huang, C.; Wu, D. *Langmuir* **1999**, *15*, 6921.
67. (a) Diederich, F.; Effing, J.; Jonas, U.; Jullien, L.; Plesnivy, T.; Ringsdorf, H.; Thilgen, C.; Weinstein, D. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1599. (b) Zhang, X.; Zhang, R.; Shen, J.; Zou, G. *Macromol. Rapid Commun.* **1994**, *15*, 373.
68. (a) Castillo, R.; Ramos, S.; Cruz, R.; Martinez, M.; Lara, F.; Ruiz-Garcia, J. *J. Phys. Chem.* **1996**, *100*, 709. (b) Bortchagovsky, E.; Yurchenko, I.; Kazantseva, Z.; Humlicek, J.; Hora, J. *Thin Solid Films* **1998**, *313–314*, 795. (c) Nostro, P. L.; Casnati, A.; Bossoletti, L.; Dei, L.; Baglioni, P. *Colloids Surf. A* **1996**, *116*, 203.
69. Ikeda, A.; Hatano, T.; Kawaguchi, M.; Shinkai, S.; Suenaga, H. *Chem. Commun.* **1999**, 1403.
70. Hatano, T.; Ikeda, A.; Sano, M.; Kanekiyo, Y.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 2* **2000**, 909.
71. Ikeda, A.; Hatano, T.; Shinkai, S.; Akiyama, T.; Yamada, S. *J. Am. Chem. Soc.* **2001**, *123*, 4855.
72. (a) Ikeda, A.; Hatano, T.; Konishi, T.; Shinkai, S. *Tetrahedron* **2003**, *59*, 3537. (b) Konishi, T.; Ikeda, A.; Asai, M.; Hatano, T.; Shinkai, S.; Fujitsuka, M.; Ito, O.; Tsuchiya, Y.; Kikuchi, J. *J. Phys. Chem. B* **2003**, *107*, 11261.

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Synthesis of a β-turn mimetic suitable for incorporation in the peptide hormone LHRH

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Abstract—LHRH is a decapeptide hormone which plays a central role in neuroendocrinology. Conformational studies have suggested that LHRH may adopt a β-turn involving residues 5–8 when bound to its receptor. A β-turn mimetic with side chains corresponding to those of a Tyr-Gly-Leu-Orn tetrapeptide has therefore been synthesized for incorporation at positions 5–8 in LHRH. In the turn mimetic, residues *i* and $i+1$ are connected by a $\psi[CH_2O]$ isostere instead of an amide bond, while a covalent ethylene bridge replaces the hydrogen bond which is often found between residues *i* and $i+3$ in β-turns. The turn mimetic was assembled from three types of building blocks: an azido aldehyde, an Fmoc protected amino acid and a protected dipeptide amine.

1. Introduction

Luteinizing hormone-releasing hormone (LHRH), pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, plays a key role in the mammalian reproductive system.[1] The decapeptide hormone is secreted in pulses from the hypothalamus and stimulates the anterior pituitary gland to release the gonadotropic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). After the sequence of LHRH was determined in 1971,[1–4] intensive studies directed towards obtaining knowledge of structure–activity relationships as well as receptor binding have been performed.[5,6] Such studies are of interest in development of therapeutic agents for treatment of a variety of hormone dependent disorders, such as breast and prostate cancer.[7,8] The use of peptides, such as LHRH, as drugs is often limited due to rapid degradation by proteolytic enzymes, low bioavailability and rapid secretion. Design of peptidomimetics which resemble the secondary structure of biologically active peptides is one way to circumvent these problems.[9–11] Such, peptidomimetics are valuable not only for design of new pharmaceuticals, but also serve to help in elucidating the biologically active conformations of peptides, and provide additional structure–activity relationship data.[12–14]

β-Turns are formed by four consecutive amino acid residues and constitute one of the most common secondary structure elements found in peptides and proteins.[15,16] In addition, β-turns have often been indicated as recognition elements involved in intermolecular interactions that regulate many important functions in organisms.[17,18] Therefore, β-turns have received a great deal of attention and a large number of β-turn mimetics have been reported. In order to achieve high-affinity and selective binding to a targeted receptor, the conformation of the mimetic should accurately resemble that of the peptide backbone in the turn and the compound must also mimic side chains which are essential for receptor-binding.[19,10] The existence of a β-turn involving residues Tyr^5-Gly-Leu-Arg^8 in LHRH has been suggested by conformational energy calculations[20] and various physiochemical methods.[21,22] Furthermore, replacement of the Tyr^5-Arg^8 segment by a sterically hindered β-lactam,[23] which is a model of a type II′ β-turn, resulted in an analogue that was more potent than LHRH both in vitro and in vivo. In addition, a highly potent and orally active nonpeptide antagonist at the human LHRH receptor based on a type II β-turn model has been developed.[24] Therefore, introduction of a β-turn mimetic having correct side chain functionalities will provide invaluable insight into the structural features essential for binding of LHRH to its receptor. As a first step towards this goal we now report an approach which provides access to a conformationally restricted β-turn mimetic for incorporation in place of residues 5–8 in LHRH.

2. Results and discussion

Our group recently reported results from a project aimed at design and synthesis of a novel type of β-turn mimetics, and

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Keywords: Peptidomimetic; β-Turn; LHRH; Peptide hormone.

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exemplified the approach by incorporation of a mimetic into Leu-enkephalin.[25,26] In these mimetics the amide bond between residue *i* and *i*+1 of the β-turn is replaced by a methylene ether isostere, while the *i*→*i*+3 hydrogen bond which stabilizes the turn has been exchanged for a covalent ethylene bridge, thereby forming a 10-membered β-turn mimetic (cf. **1**, Fig. 1). We now describe the synthesis of β-turn mimetic **2** which has side-chains corresponding to those of a Tyr-Gly-Leu-Orn tetrapeptide (Fig. 2). Mimetic **2** is intended for incorporation at positions 5–8 in LHRH and in order to simplify the synthesis ornithine was chosen in place of arginine, which is found at position 8 in LHRH. This choice was made since it is possible to convert the side chain of ornithine to that of arginine on solid phase after incorporation of the mimetic in LHRH.[27] Mimetic **2** was designed to allow assembly from three building blocks; azido aldehyde **3**, dipeptide amine **4** and Fmoc-Leu-OH **5**. Building block **3** corresponds to Tyr and Gly at positions *i* and *i*+1 of the turn and the azido group serves as a precursor of the amino group of residue *i*. *N*-Alkylated amino acid derivatives, such as residue *i*+3 in mimetic **1** (Fig. 1), are more susceptible to epimerization during activation and coupling than the ordinary amino acids.[28,29] Therefore, it was decided to avoid this potential problem by including Pro[9] of LHRH when preparing the turn mimetic and dipeptide amine **4** was chosen instead of an ornithine building block. The *tert*-butyldiphenyl silyl protected hydroxyl group in **4** corresponds to the carboxyl group of the proline residue.

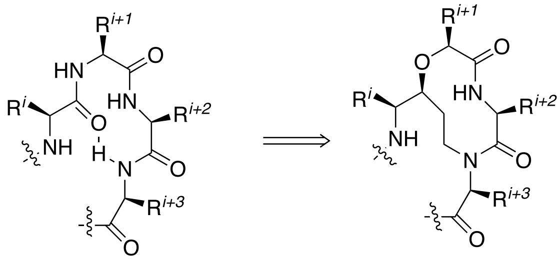

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Figure 1. Design of a β-turn mimetic. In this mimetic, a methylene ether isostere replaces the amide bond between resides *i* and *i*+1 in the turn and a covalent ethylene bridge connects residues *i* and *i*+3, which are often hydrogen bonded in β-turns.

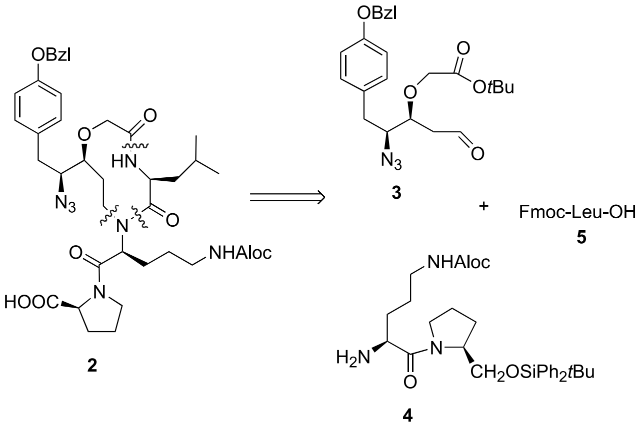
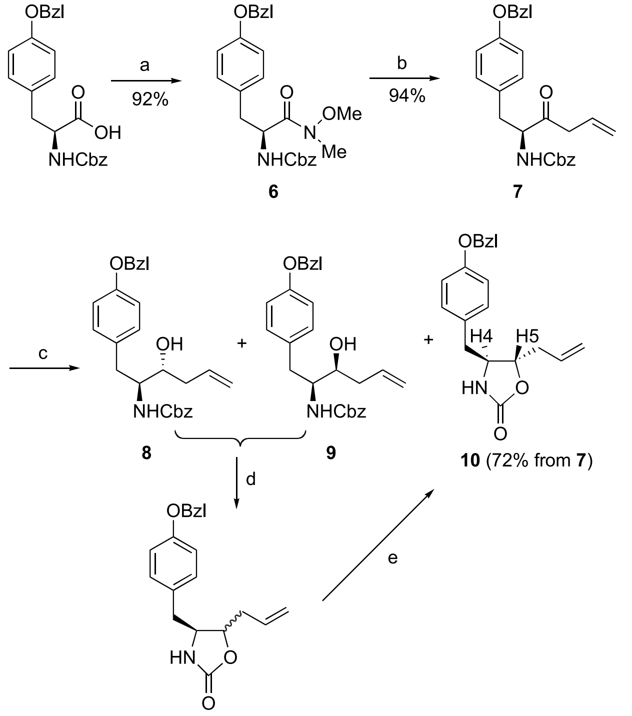
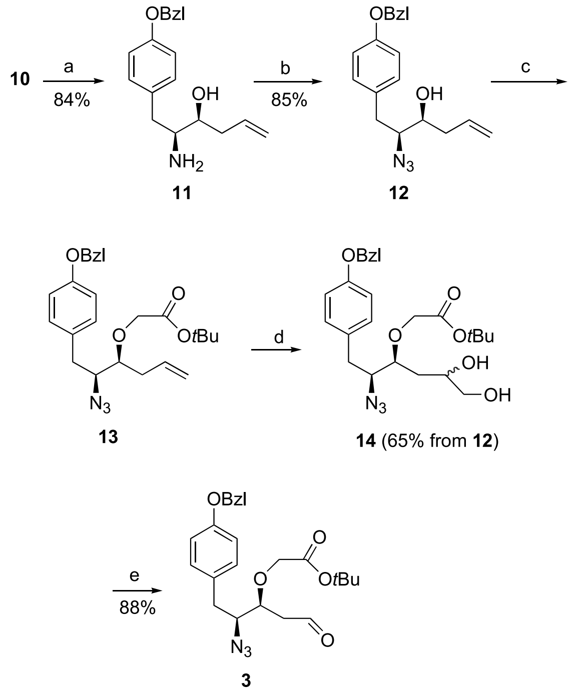


Figure 2. A retrosynthetic analysis shows that the target mimetic **2** may be assembled from three building blocks; azido aldehyde **3**, dipeptide amine **4** and Fmoc-Leu-OH (**5**).

The key building block, azido aldehyde **3**, was prepared from the commercially available Cbz-Tyr(Bzl)-OH (Schemes 1 and 2). This was transformed into a mixed anhydride by addition of isobutyl chloroformate and *N*-methyl



Scheme 1. (a) *i*BuOCOCl, NMM, MeONHMe·HCl, CH_2Cl_2, −15 °C→rt, 2 h, 92%. (b) Allylmagnesium bromide, THF, −78 °C, 1.5 h, 94%. (c) K-Selectride, THF, −78 °C 19 h. (d) and (e) THF:MeOH:aq KOH (7.5 N) (4:2:1), rt, 5 h, flash column chromatography, 72% of **10** over steps (c)–(e).



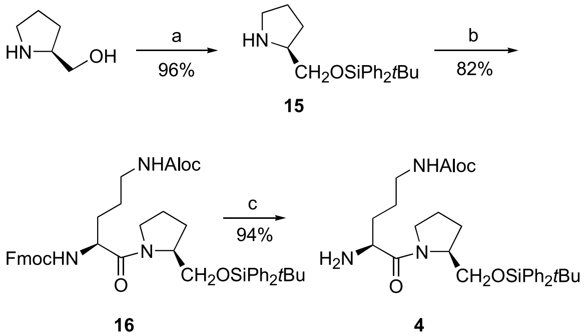
Scheme 2. (a) EtOH/KOH (aq, 1 M) (1:1), reflux, 6 h, 84%. (b) TfN_3, DMAP, $CuSO_4$, CH_2Cl_2, rt, 2 h, 85%. (c) $BrCH_2CO_2tBu$, Bu_4NHSO_4, benzene:aq NaOH (50%) (1:1), rt, 1 h. (d) OsO_4, NMO, THF/acetone:H_2O (1:1:1), rt, 2.5 h, 65% over two steps. (e) $Pb(OAc)_4$, Na_2CO_3, benzene, 0 °C→rt, 88%.

morpholine, followed by reaction with *N*,*O*-dimethylhydroxylamine to give Weinreb amide **6** (92%). Grignard reaction[30] of **6** with allylmagnesium bromide furnished ketone **7** (94%) which was reduced using K-selectride to give a mixture of **8**, **9**, and **10**. *Anti* and *syn* amino alcohols **8** and **9** were difficult to separate from each other by flash column chromatography, whereas *anti*-oxazolidinone **10** was readily separated from **8** and **9**. After removal of **10**, the mixture of **8** and **9** was converted to a mixture of syn and *anti*-oxazolidinones by treatment with aqueous KOH in a mixture of THF and methanol. Purification by chromatography then gave **10** in 72% overall yield from **7**. The stereochemistry of *anti*-oxazolidinone **10** was established by comparing the NOESY spectrum of **10** with that of the corresponding *syn*-isomer. The spectrum of **10** did not show any cross-peak for H-4 and H-5 in the oxazolidinone ring, whereas the *syn*-isomer showed a strong cross-peak between these two hydrogen atoms.

Oxazolidinone **10** was hydrolyzed under alkaline conditions to furnish amino alcohol **11** (Scheme 2). Azido transfer was then performed with freshly prepared triflyl azide in the presence of catalytic amount of $CuSO_4$ to give azido alcohol **12**.[31] This transformation served the dual purposes of introducing an inert amino protective group as well as reducing the steric bulk in the vicinity of the hydroxyl group of **12**. Compound **12** decomposed on standing but could be purified by flash column chromatography if performed rapidly. Due to its instability, **12** was immediately alkylated with *tert*-butyl bromoacetate using phase-transfer catalysis,[32] thus introducing the $i+1$ residue of the turn mimetic. Compound **13** was even more labile than **12** and crude **13** was therefore oxidized immediately to diol **14** by osmium tetraoxide catalysed dihydroxylation (65% from **12**). Cleavage of diol **14** with lead tetraacetate furnished the stable azido aldehyde **3** in 88% yield, thereby providing one of the building blocks required for assembly of the target β-turn mimetic.

Synthesis of a building block corresponding to **4** was first attempted for an Arg-Pro sequence. However, due to purification problems related to the guanidino group of arginine this approach had to be abandoned. Since ornithine can be converted to arginine late in synthetic routes,[27] an Orn-Pro dipeptide was chosen instead of Arg-Pro. A diketopiperazine was formed instead of the desired amine on cleavage of the Fmoc protecting group of Fmoc-Orn(Aloc)-Pro-OMe with morpholine. Therefore, prolinol was chosen as starting material and protected with *tert*-butyldiphenylsilyl chloride to give **15** in high yield (Scheme 3). Coupling of **15** with Fmoc-Orn(Aloc)-OH was then achieved by using HATU[33,34] as coupling reagent in the presence of diisopropylethylamine in DMF. Finally cleavage of the Fmoc group of **16** furnished **4**, that is, the other key building block for synthesis of the β-turn mimetic.

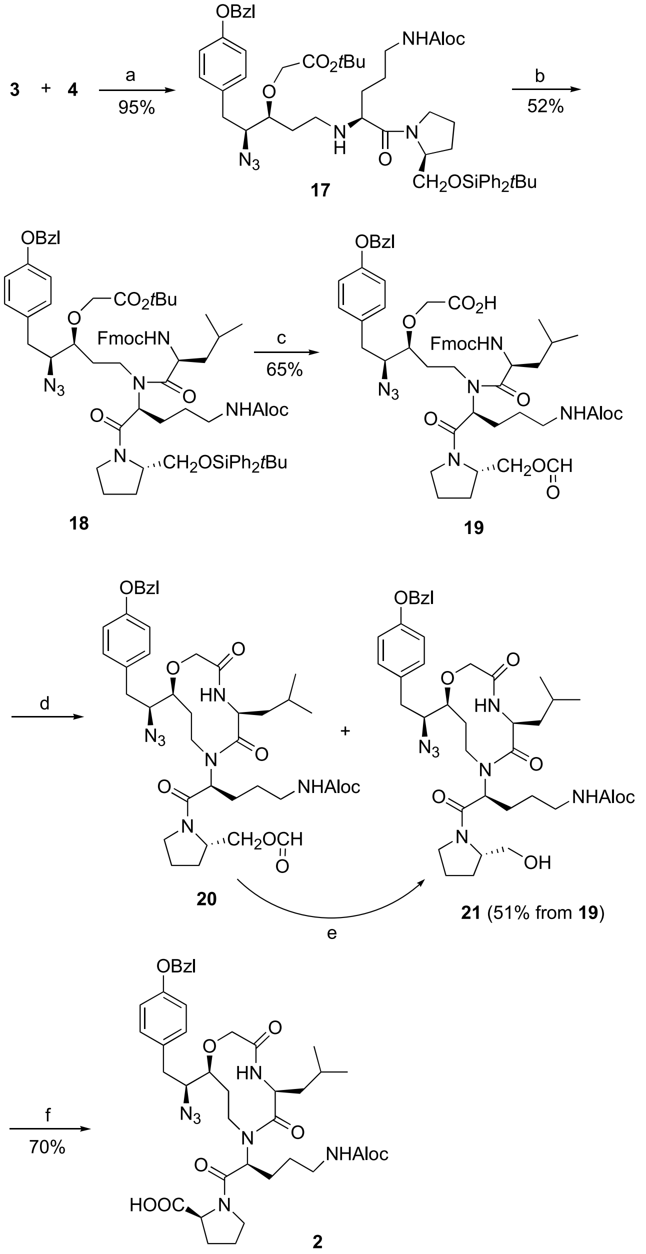
Assembly of the β-turn mimetic began with reductive amination[35] of **3** with **4** using sodium triacetoxyborohydride as reducing agent to give **17** in almost quantitative yield. Introduction of Fmoc-Leu-OH **5** at position $i+2$ in the mimetic turned out to be very difficult, most likely due to steric hindrance. Therefore, large efforts were made to find suitable conditions for acylation of **17** with **5**. When DIC was used as coupling reagent under conditions found to be optimal for acylation of a secondary amine in a synthetic route leading to a γ-turn mimetic,[36] a very low yield of **18** was obtained as a mixture of diastereomers. Conversion of **5** into the corresponding acid chloride and coupling with **17** also led to a diastereomeric mixture of **18**, most likely due to epimerization of the stereogenic center of **5**. Instead **18** was obtained in enantiomerically pure form by use of HATU as coupling reagent in the presence of diisopropylethylamine. In contrast to what can be expected,[37] attempts to cleave the *tert*-butyl ester of **18** without removing the *tert*-butyldiphenylsilyl protecting group failed. The *tert*-butyldiphenylsilyl protecting group was found to be cleaved much faster than the *tert*-butyl ester in formic acid, but fortunately the resulting hydroxyl group was simultaneously protected as a formate so that **19** was formed. The carboxyl group of **19** was then activated as a pentafluorophenyl ester which was added to DBU in hot dioxane under high dilution conditions. This gave a mixture of **20** and **21** in a one-pot procedure[38] involving Fmoc deprotection followed by closure of the ten-membered ring and partial removal of the formate. Compound **20** was easily hydrolyzed to give **21**, which thereby was then obtained in 51% yield from **19** over the three steps. Finally Jones oxidation of **21** furnished the β-turn mimetic **2** which is ready for incorporation in LHRH using solid-phase synthesis (Scheme 4).



Scheme 3. (a) $tBuPh_2SiCl$, imidazole, CH_2Cl_2, rt, 3 h, 96%. (b) Fmoc-Orn(Aloc)-OH, HATU, DIEA, DMF, 0 °C → rt, 2 h, 82%. (c) Morpholine, rt, 3 h, 94%.

3. Conclusions

A β-turn mimetic with side-chains corresponding to a Tyr-Gly-Leu-Orn tetrapeptide, suitable for incorporation at positions 5–8 of the hormone LHRH, has been synthesized. The design of the mimetic is similar to a Tyr-Gly-Gly-Phe mimetic previously incorporated in Leu-enkephalin by us.[25,26] Thus, residues i and $i+1$ are connected by a $\psi(CH_2O($ isostere instead of an amide bond, while a covalent ethylene bridge replaces the hydrogen bond that is often found between the carbonyl group of residue i and the amino group of residue $i+3$ in β-turns. In addition, we have now shown that it is possible to incorporate chiral amino acids at position $i+2$ of this type of β-turn mimetics, that is, the strategy is not limited to glycine which was found at the $i+2$ position in the Leu-enkephalin mimetic. After evaluation of several different procedures it was found that the $i+2$ leucine could be coupled without racemization to a sterically hindered secondary amine using HATU as



Scheme 4. (a) $NaBH(OAc)_3$, DCE, rt, 45 min, 95%. (b) Fmoc-Leu-OH, HATU, DIEA, DMF, 0 °C→rt, 24 h, 52% (70% based on recovered **17**). (c) HCOOH, rt, 19 h, 65%. (d) PfpOH, DIC, EtOAc, 0 °C, 1.5 h, then DBU, dioxane, 100 °C, 4 h. (e) aq LiOH (1 M), THF/MeOH:H_2O (3:1:1), rt, 3 h, 51% for steps (d) and (e). (f) Jones oxidation, 3.5 h, 70%.

coupling reagent in the presence of diisopropylethylamine. In addition, the synthetic strategy was revealed to be compatible with amino acids having protected functionalities in their side-chains both at positions i and $i+4$, such as those of tyrosine and ornithine in the present mimetic.

4. Experimental

4.1. General data

All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH_2Cl_2 and THF were distilled from calcium hydride and sodium-benzophenone, respectively. DMF was distilled and then dried over 3 Å molecular sieves. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light and charring with phosphomolybdic acid in EtOH. Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon).

^1H NMR spectra for compounds **2–21** were recorded at 400 MHz whereas ^{13}C NMR spectra were obtained at 100 MHz for solution in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm) or $CDCl_3$ (δ_C 77.0 ppm) as internal standard] or in MeOH-d_4 [residual CD_2HOD (δ_H 3.31 ppm) or CD_3OD (δ_C 49.2 ppm) as internal standard] at 298 K. Elemental analysis was performed by Mikro Kemi AB, Uppsala, Sweden. Compounds lacking elemental analysis were characterized by high resolution MS and were >95% pure according to ^1H NMR spectroscopy. Ions for positive fast atom bombardment mass spectra were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thioglycerol.

4.1.1. (*S*)-*N*-Methoxyl-*N*-methyl-2-benzyloxycarbonylamino-3-(4-benzyloxyphenyl)-propanoic amide (6). Isobutyl chloroformate (0.845 mL, 6.49 mmol) was added dropwise to a solution of Cbz-Tyr(Bzl)-OH (2.63 g, 6.49 mmol) and *N*-methyl morpholine (NMM, 1.57 mL, 14.3 mmol) in CH_2Cl_2 at −15 °C. After being stirred for 15 min, *N*,*O*-dimethylhydroxylamine·HCl (0.633 g, 6.49 mmol) was added. After a further 30 min the cooling bath was removed and the reaction mixture was stirred at room temperature for additional 1.5 h. The reaction mixture was poured into H_2O (20 mL) and extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were washed sequentially with 0.2 M aq HCl, satd aq $NaHCO_3$ and brine, then dried with $MgSO_4$, filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 3:1→1:1) of the residue gave **6** as a colourless oil (2.69 g, 92%): $[\alpha]_D^{20}=+9.3$ (c 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.45 (10H, m, Ph), 7.09 (2H, d, $J=8.5$ Hz, Ph_{tyr}), 6.90 (2H, d, $J=8.5$ Hz, Ph_{tyr}), 5.52 (1H, d, $J=8.6$ Hz, NH), 5.04–5.13 (5H, m, CH_2Ph, CH_2Ph and CHNH), 3.68 (3H, s, OCH_3), 3.18 (3H, s, NCH_3), 3.05 (1H, ABX type dd, $J=5.8$, 13.6 Hz, Tyr β-H), 2.88 (1H, ABX type dd, $J=7.2$, 13.6 Hz, Tyr β-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 157.7, 155.7, 136.9, 136.3, 130.4, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.4, 114.7, 69.8, 66.6, 61.4, 52.1, 37.7, 32.0; IR (neat) 3298, 1714, 1657 cm^{-1}; HRMS (FAB) calcd for $C_{26}H_{29}N_2O_5$ 449.2079 ($M+H^+$), found 449.2057; Anal. Calcd C, 69.6; H, 6.3; N, 6.2. Found C, 69.7; H, 6.3; N, 6.1.

4.1.2. (*S*)-2-Benzyloxycarbonylamino-1-(4-benzyloxyphenyl)-hex-5-en-3-one (7). Allylmagnesium bromide (1 M solutiom in diethyl ether, 8.35 mL, 8.35 mmol) was added dropwise to a solution of Weinreb amide **6** (1.50 g, 3.34 mmol) in THF (32 mL) at −78 °C. After being stirred for 1.5 h, the reaction was quenched with satd aq NH_4Cl (20 mL) and the mixture was extracted with CH_2Cl_2 (3× 20 mL). The combined organic layers were then washed with brine, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (toluene/ethanol, 20:1→10:1) of the residue gave **7** as a white amorphous solid (1.35 g, 94%): $[\alpha]_D^{20}=+9.6$ (c 0.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ

7.32–7.43 (10H, m, Ph), 7.02 (2H, d, *J*=8.2 Hz, Ph_{tyr}), 6.89 (2H, d, *J*=8.2 Hz, Ph_{tyr}), 5.85 (1H, m, $CH=CH_2$), 5.34 (1H, d, *J*=7.7 Hz, NH), 5.02–5.20 (6H, m, CH_2Ph, CH_2Ph and $CH=CH_2$), 4.63 (1H, m, CHN), 3.19 (1H, ABX type dd, *J*=7.2, 17.3 Hz, $CH_2CH=CH_2$), 3.13 (1H, ABX type dd, *J*=7.2, 17.3 Hz, $CH_2CH=CH_2$), 3.03 (1H, ABX type dd, *J*=6.8, 14.2 Hz, Tyr β-H), 2.96 (1H, ABX type dd, *J*=6.2, 14.2 Hz, Tyr β-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 157.9, 155.6, 136.9, 136.2, 130.2, 129.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.4, 119.4, 115.1, 70.0, 66.9, 60.1, 45.5, 36.8; IR (neat) 3309, 1716, 1689 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{28}NO_4$ 430.1979 $(M+H^+)$, found 430.2024; Anal. Calcd C, 75.5; H, 6.3; N, 3.3. Found C, 75.5; H, 6.3; N, 3.3.

4.1.3. (4*S*,5*S*)-5-Allyl-4-(4-benzyloxybenzyl)-oxazolidin-2-one (10). K-Selectride (1 M solution in THF, 18.0 mL, 18.0 mmol) was added dropwise to a solution of allyl ketone **7** (5.15 g, 12.0 mmol) in THF (82 mL) at −78 °C. After being stirred for 19 h, the reaction was quenched with H_2O (20 mL) and acidified with 10% citric acid to adjust the pH to ∼4. The reaction mixture was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were then washed with brine, dried over $MgSO_4$, filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 10:1→1:1.5) of the residue gave a mixture of **8** and **9** (4.93 g) and pure *anti*-oxazolidinone **10** (0.47 g). Then the mixture of **8** and **9** was stirred in a mixture of THF, MeOH and aq 7.5 M KOH (4:2:1, 140 mL) at room temperature for 5 h and poured into H_2O (130 mL). The phases were separated and the aqueous phase was extracted with EtOAc (4×50 mL). The combined organic phases were washed with brine and dried over $MgSO_4$. Flash column chromatography (heptane/ethyl acetate 10:1→1:1.5) of the residue gave *anti*-oxazolidinone **10** as a white amorphous solid (2.38 g in total, 72% for **7**): $[\alpha]_D^{20}=-63.2$ (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.44 (5H, m, Ph), 7.08 (2H, d, *J*=8.3 Hz, Ph_{tyr}), 6.94 (2H, d, *J*=8.3 Hz, Ph_{tyr}), 5.92 (1H, s, NH), 5.68 (1H, m, $CH=CH_2$), 5.11 (2H, m, $CH=CH_2$), 5.05 (2H, s, CH_2Ph), 4.32 (1H, q, *J*=5.7, 5.7, 5.7 Hz, CHO), 3.68 (1H, q, *J*=6.3, 6.3, 6.3 Hz, CHN), 2.78 (2H, m, Tyr β-H), 2.34 (2H, m, $CH_2CH=CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 158.0, 137.0, 131.5, 130.3, 128.7, 128.3, 128.1, 127.6, 119.4, 115.5, 80.7, 70.1, 58.2, 40.7, 38.7; IR (neat) 3269, 1749, 1687, 1512 cm^{-1}; HRMS (FAB) calcd for $C_{20}H_{22}NO_3$ 324.1679 $(M+H^+)$, found 324.1609; Anal. Calcd C, 74.3; H, 6.6; N, 4.3. Found C, 74.5; H, 6.6; N, 4.3.

4.1.4. (2*S*,3*S*)-2-Amino-1-(4-benzyloxyphenyl)-hex-5-en-3-ol (11). *Anti*-oxazolidinone **10** (1.54 g, 4.76 mmol) was refluxed in a mixture of EtOH (35 mL) and aq KOH (1 M, 35 mL) for 6 h, after which the solvent was evaporated and the residue was partitioned between H_2O (15 mL) and CH_2Cl_2 (15 mL). The aqueous phase was extracted with CH_2Cl_2 (4×30 mL). The combined organic layers were then washed with brine, dried over $MgSO_4$, filtered and concentrated. Flash column chromatography (toluene/ethanol, 10:1→1:2) of the residue furnished amino alcohol **11** as a white amorphous solid (1.18 g, 84%): $[\alpha]_D^{20}=-9.9$ (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.33–7.45 (5H, m, Ph), 7.12 (2H, d, *J*=8.4 Hz, Ph_{tyr}), 6.93 (2H, d, *J*=8.4 Hz, Ph_{tyr}), 5.90 (1H, m, $CH=CH_2$), 5.14 (2H, m, $CH=CH_2$), 5.05 (2H, s, CH_2Ph), 3.48 (1H, m, CHOH), 2.88 (2H, m, $CHNH_2$ and Tyr β-H), 2.47 (1H, ABX type dd, *J*=8.9,12.9 Hz, Tyr β-H), 2.33 (2H, m, $CH_2CH=CH_2$), 2.06 (3H, br s, OH and NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 137.0, 134.9, 131.1, 130.2, 128.6, 127.9, 127.5, 117.5, 115.0, 72.3, 70.0, 56.0, 39.9, 39.2; IR (neat) 3359, 3298, 1610, 1581, 1510 cm^{-1}; HRMS (FAB) calcd for $C_{19}H_{24}NO_2$ 298.1779 $(M+H^+)$, found 298.1798.

4.1.5. (2*S*,3*S*)-2-Azido-1-(4-benzyloxy-phenyl)-hex-5-en-3-ol (12). A solution of freshly prepared triflyl azide[31] (14.8 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a solution of **11** (1.05 g, 3.53 mmol), DMAP (216 mg, 1.77 mmol) and $CuSO_4$ (28.2 mg, 0.177 mmol) in CH_2Cl_2 (10 mL) at room temperature. After being stirred for 2 h, the reaction mixture was washed with 10% citric acid (2×), satd aq $NaHCO_3$ (2×) and brine. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 10:1→3:1) of the residue gave azido alcohol **12** as white amorphous solid (0.966 g, 85%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.45 (5H, m, Ph), 7.18 (2H, d, *J*=8.6 Hz, Ph_{tyr}), 6.95 (2H, d, *J*=8.6 Hz, Ph_{tyr}), 5.79 (1H, m, $CH=CH_2$), 5.17 (2H, m, $CH=CH_2$), 5.06 (2H, s, CH_2Ph), 3.62 (1H, m, CHOH), 3.44 (1H, ddd, *J*=3.3, 6.4, 8.2 Hz, CHN_3), 2.99 (1H, ABX type dd, *J*=6.4, 13.8 Hz, Tyr β-H), 2.91 (1H, ABX type dd, *J*=6.4, 13.8 Hz, Tyr β-H), 2.36 (2H, m, $CH_2CH=CH_2$), 1.82 (1H, d, *J*=5.8 Hz, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.8, 137.0, 133.7, 130.3, 129.7, 128.6, 128.5, 127.9, 127.5, 127.5, 127.4, 118.7, 71.3, 70.1, 67.1, 39.1, 36.3; IR (neat) 3365, 3031, 2106, 1610, 1510 cm^{-1}; HRMS (FAB) calcd for $C_{19}H_{21}N_3O_2$ 323.1679 (M^+), found 323.1631.

4.1.6. α-[(4*S*,5*S*)-5-Azido-6-(4-benzyloxyphenyl)-1,2-dihydroxy-hex-4-oxy]-acetic acid *tert*-butyl ester (14). *tert*-Butyl bromoacetate (0.72 mL, 4.89 mmol) was added dropwise to a vigorously stirred mixture of azido alcohol **12** (0.878 g, 2.71 mmol) and tetrabutyl ammonium hydrogen sulphate (257 mg) in benzene–aq 50% NaOH (1:1, 30 mL) at room temperature. After being stirred for 1.5 h, the mixture was separated and the aqueous phase was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give crude O-alkylated azido alcohol **13**. Compound **13** was dissolved in a mixture of THF, acetone and water (1:1:1, 30 mL). OsO_4 (69 mg, 0.21 mmol) and NMO (0.586 g, 4.34 mmol) were added sequentially to the solution. After being stirred for 2.5 h the solution was acidified with 0.2 M aq HCl to adjust the pH to ∼2 and then extracted with EtOAc (4×15 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 and concentrated. Flash column chromatography (heptane/ethyl acetate, 5:1→1:3) of the residue gave azido diol **14** as a colourless oil (0.832 g, 65%): ^1H NMR (400 MHz, $CDCl_3$) δ for a 1:1 diastereomeric mixture 7.30–7.45 (5H, m, Ph), 7.15 (2H, d, *J*=8.5 Hz, Ph_{tyr}), 6.94 (2H, d, *J*=8.5 Hz, Ph_{tyr}), 5.06 (2H, s, CH_2Ph), 4.17–4.28 (1.5H, m, $CH_2COOtBu$), 4.15 (0.5H, m, CHO), 4.05 (0.5H, d, *J*=16.3 Hz, $CH_2COOtBu$), 3.95 (0.5H, m, CHO), 3.76 (0.5H, m, CHO), 3.64 (2.5H, m, CHN_3, CHO and CH_2OH), 3.54 (1H m, CH_2OH), 2.92–3.02 (1H, m, Tyr β-H), 2.58–2.67 (1H, m, Tyr β-H), 1.59–1.83 (2H, m, CH_2CHO), 1.52 (4.5H, s, *t*Bu), 1.49 (4.5H, s, *t*Bu);

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.2, 157.8, 157.8, 137.0, 137.0, 130.2, 130.0, 129.9, 128.6, 127.9, 127.5, 115.1, 115.1, 82.9, 82.8, 78.4, 71.0, 70.1, 68.2, 67.7, 66.8, 66.7, 66.7, 66.0, 35.5, 35.0, 34.3, 33.8, 28.1; IR (neat) 3408, 2106, 1732, 1612, 1512 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{33}N_3O_6Na$ 494.2198 ($M+Na^+$), found 494.2191.

4.1.7. (1*S*,2*S*)-2-Azido-3-(4-benzyloxyphenyl)-1-(2-oxo-ethyl)-propoxy]-acetic acid *tert*-butyl ester (3). Lead tetraacetate (0.237 g, 0.534 mmol) was added to a mixture of azido diol **14** (0.168 g, 0.356 mmol) and Na_2CO_3 (94.3 mg, 0.890 mmol) in benzene (10 mL) at 0 °C. After being stirred for 10 min, the cooling bath was removed and stirring was continued for 1 h. Then the reaction was quenched with 10 drops of ethylene glycol and stirring was continued for 5 min. Et_2O and satd aq $NaHCO_3$ were added and the phases were separated. The aqueous phase was extracted with Et_2O (4×10 mL), the combined organic phases were washed with brine, dried with Na_2SO_4, filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 5:1→3:1) of the residue gave azido aldehyde **3** as a colourless oil (0.138 g, 88%): $[\alpha]_D^{20} = -22.5$ (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (1H, s, CHO), 7.31–7.45 (5H, m, Ph), 7.19 (2H, d, $J=8.3$ Hz, Ph_{tyr}), 6.95 (2H, d, $J=8.3$ Hz, Ph_{tyr}), 5.06 (2H, s, C*H*$_2$Ph), 4.11 (2H, s, C*H*$_2$COO*t*Bu), 4.02 (1H, m, CHN$_3$C*H*O), 3.60 (1H, dt, $J=$ 4.6, 9.2 Hz, CHN$_3$), 3.06 (1H, ABX type dd, $J=4.6$, 14.0 Hz, Tyr β-H), 2.80–2.95 (3H, m, Tyr β-H and C*H*$_2$CHO), 1.49 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.1, 169.2, 157.7, 136.9, 130.2, 129.6, 128.6, 128.5, 127.9, 127.5, 115.0, 81.9, 67.0, 68.9, 65.8, 45.7, 35.3, 28.1; IR (neat) 2107, 1743, 1722 cm^{-1}; HRMS (FAB) calcd for $C_{24}H_{28}N_3O_5$ ($M+H^+$) 438.2021, found 438.2030.

4.1.8. *O*-(*tert*-Butyldiphenylsilyl)-L-prolinol (15). *tert*-Butyldiphenylsilyl chloride (4.92 mL, 21.0 mmol) was added to a solution of L-prolinol (0.987 mL, 10.0 mmol) and imidazole (1.50 g, 22.0 mmol) in CH_2Cl_2 (100 mL) at 0 °C. After being stirred for 3 h the resulting precipitate was filtered off and the reaction was quenched by addition of satd aq NH_4Cl. The aqueous phase was extracted with CH_2Cl_2 (2×20 mL), the combined organic phases were dried with Na_2SO_4, filtered and concentrated. Flash colum chromatography (toluene/ethanol, 10:1→3:1) of the residue gave **15** as a colourless oil (3.26 g, 96%): $[\alpha]_D^{20} = -5.9$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (4H, m, Ph), 7.35–7.44 (6H, m, Ph), 3.65 (1H, ABX type dd, $J=4.9$, 10.0 Hz, CH$_2$OSi), 3.59 (1H, ABX type dd, $J=6.1$, 10.0 Hz, CH$_2$OSi), 3.23 (1H, m, Pro α-H), 2.97 (1H, m, Pro δ-H), 2.85 (1H, m, Pro δ-H), 2.13 (1H, br s, NH), 1.69–1.82 (3H, m, Pro β-H and Pro γ-H), 1.48 (1H, m, Pro β-H), 1.06 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 135.6, 133.7, 129.6, 127.7, 127.7, 66.5, 59.9, 46.5, 27.5, 26.9, 25.4, 19.3; IR (neat) 2856, 1111 cm^{-1}; HRMS (FAB) calcd for $C_{21}H_{30}NOSi$ 340.2079 ($M+H^+$), found 340.2086; Anal. Calcd C, 74.3; H, 8.6; N, 4.1. Found C, 73.9; H, 8.6; N, 4.1.

4.1.9. N^δ-(Allyloxycarbonyl)-N^α-(fluoren-9-ylmethoxy-carbonyl)-L-ornithyl-*O*-(*tert*-butyldiphenylsilyl)-L-prolinol (16). Fmoc-Orn(Aloc)-OH (0.386 g, 0.881 mmol) was activated for 15 min with HATU (0.305 g, 0.801 mmol) in the presence of DIEA (0.308 mL, 1.76 mmol) in DMF (2 mL) at 0 °C. Then **15** (0.275 g, 0.801 mmol) in DMF (2 mL) was added to the solution which was stirred for 3 h before being diluted with water (100 mL) and extracted with CH_2Cl_2 (5×10 mL). The combined organic layers were washed with aq 0.2 M HCl, H_2O, satd aq $NaHCO_3$ and brine, dried with Na_2SO_4, filtered and concentrated. Flash column chromatography (toluene/ethanol, 20:1→10:1) of the residue gave **16** as a white amorphous solid (0.502 g, 82%): $[\alpha]_D^{20} = -35.7$ (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamer ratio 4:1) δ (major rotamer) 7.76 (2H, d, $J=$ 7.4 Hz, Ph), 7.62 (6H, m, Ph), 7.29–7.43 (10H, m, Ph), 5.90 (1H, m, C*H*=CH$_2$), 5.75 (1H, d, $J=8.5$ Hz, Orn α-NH), 5.28 (1H, d, $J=17.1$ Hz, CH=C*H*$_2$), 5.19 (1H, d, $J=$ 17.1 Hz, CH=C*H*$_2$), 4.75 (1H, br s, Orn δ-NH), 4.53 (3H, m, C*H*$_2$CH=CH$_2$ and Orn α-H), 4.37 (2H, m, Fmoc-CH$_2$), 4.26 (1H, m, Pro α-H$_)$, 4.21 (1H, t, $J=6.9$ Hz, Fmoc-CH), 3.85 (1H, m, CH$_2$OSi), 3.72 (1H, m, CH$_2$OSi), 3.64 (1H, m, Pro δ-H), 3.48 (1H, m, Pro δ-H), 3.15 (2H, m, Orn δ-H), 2.08 (2H, m, Pro β-H and Pro γ-H), 1.93 (2H, m, Pro β-H and Pro γ-H), 1.73 (1H, m, Orn β-H), 1.56 (3H, m, Orn β-H and Orn γ-H), 1.06 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 156.2, 143.9, 143.8, 141.3, 135.5, 135.5, 133.4, 133.3, 132.9, 129.7, 127.7, 127.7, 127.7, 127.0, 125.2, 125.1, 119.9, 119.9, 117.5, 66.9, 65.4, 63.3, 58.6, 52.1, 47.6, 47.2, 40.7, 30.7, 26.9, 26.8, 25.5, 24.5, 19.2; IR (neat) 3307, 3278, 3970, 1716, 1633, 1525, 1448 cm^{-1}; HRMS (FAB) calcd for $C_{45}H_{54}N_3O_6Si$ 760.3779 ($M+H^+$), found 760.3768; Anal. Calcd C, 71.1; H, 7.0; N, 5.5. Found C, 70.8; H, 7.0; N, 5.5.

4.1.10. N^δ-(Allyloxycarbonyl)-L-ornithyl-*O*-(*tert*-butyl-diphenylsilyl)-L-prolinol (4). Compound **16** (3.35 g, 4.41 mmol) was stirred in morpholine (24 mL) at room temperature for 2 h. Then the precipitate was filtered off and the filtrate was co-evaporated with toluene. Flash column chromatography (toluene/ethanol, 10:1→1:1) of the residue gave **4** as a colourless oil (2.22 g, 94%): $[\alpha]_D^{20} = -20.8$ (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamer ratio 4:1) δ (major rotamer) 7.62 (4H, m, Ph), 7.34–7.44 (6H, m, Ph), 5.89 (2H, m, Orn δ-NH and C*H*=CH$_2$), 5.28 (2H, m, CH=C*H*$_2$), 4.53 (2H, m, C*H*$_2$CH=CH$_2$), 4.24 (1H, m, Pro α-H), 3.82 (1H, ABX type dd, $J=5.3$, 9.8 Hz, CH$_2$OSi), 3.72 (1H, ABX type dd, $J=2.8$, 9.8 Hz, CH$_2$OSi), 3.53 (2H, m, Orn α-H and Pro δ-H), 3.41 (1H, m, Pro δ-H), 3.14 (2H, m, Orn δ-H), 2.07 (4H, m, Orn α-NH$_2$, Pro β-H and Pro γ-H), 1.81–1.95 (2H, m, Pro β-H and Pro γ-H), 1.58–1.71 (3H, m, Orn γ-H and Orn β-H), 1.49 (1H, m, Orn β-H), 1.04 (9H, s, *t*Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 156.2, 135.5, 135.4, 133.4, 132.9, 132.7, 129.9, 129.6, 128.2, 127.8, 127.6, 127.6, 117.4, 65.3, 63.4, 58.4, 52.6, 47.1, 40.6, 32.4, 26.8, 26.7, 26.3, 24.4, 19.2; IR (neat) 3298, 3070, 1712, 1631, 1531 cm^{-1}; HRMS (FAB) calcd for $C_{30}H_{44}N_3O_4Si$ 538.3079 ($M+H^+$), found 538.3096.

4.1.11. Compound 17. NaBH(OAc)$_3$ (101 mg, 0.478 mmol) was added to a solution of azido aldehyde **3** (132 mg, 0.30 mmol) and primary amine **4** (258 mg, 0.48 mmol) in dichloroethane (7.5 mL) at room temperature. After being stirred for 1 h the reaction was quenched with satd aq $NaHCO_3$ and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered and concentrated. Flash column chromatography (toluene/ethanol, 20:1→5:1) of the residue gave secondary amine **17** as a colourless oil (274 mg, 95%):

$[\alpha]_D^{20} = -31.1$ (*c* 2.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, rotamer ratio 4:1) δ (major rotamer) 7.62 (4H, m, Ph), 7.30–7.45 (11H, m, Ph), 7.18 (2H, d, $J = 8.6$ Hz, Ph_{Tyr}), 6.93 (2H, d, $J = 8.6$ Hz, Ph_{Tyr}), 5.90 (1H, m, $C\mathit{H}{=}CH_2$), 5.25–5.32 (2H, m, $CH{=}C\mathit{H_2}$ and Orn δ-NH), 5.18 (1H, m, $CH{=}C\mathit{H_2}$), 5.05 (2H, s, CH_2Ph), 4.54 (2H, dd, $J = 5.5$, 13.0 Hz, $C\mathit{H_2}CH{=}CH_2$), 4.29 (1H, m, Pro α-H), 4.12 (1H, d, $J = 16.1$ Hz, $C\mathit{H_2}COOtBu$), 4.04 (1H, d, $J = 16.1$ Hz, $C\mathit{H_2}$-$COOtBu$), 3.82 (1H, ABX type dd, $J = 5.6$, 9.8 Hz, CH_2OSi), 3.73 (1H, dd, $J = 2.8$, 9.8 Hz, CH_2OSi), 3.55 (2H, m, Pro δ-H and $CHN_3C\mathit{H}O$), 3.45 (2H, m, Pro δ-H and CHN_3), 3.25 (1H, m, Orn α-H), 3.12 (2H, m, Orn δ-H), 3.02 (1H, ABX type dd, $J = 4.2$, 13.7 Hz, Tyr β-H), 2.77 (1H, ABX type dd, $J = 9.3$, 14.0 Hz, Tyr β-H), 2.69 (1H, m, $C\mathit{H_2}NH$), 2.51 (1H, m, $C\mathit{H_2}NH$), 2.08 (2H, m, Pro β-H and Pro γ-H), 1.91 (2H, m, Pro β-H and Pro γ-H), 1.81 (2H, m, $C\mathit{H_2}CHO$), 1.59 (3H, m, Orn β-H and Orn γ-H), 1.49 (9H, s, $COOtBu$), 1.46 (1H, m, Orn β-H), 1.05 (9H, s, $OSiPh_2tBu$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 169.3, 157.6, 156.2, 133.4, 133.0, 133.4, 133.3, 133.0, 130.2, 129.7, 128.5, 127.9, 127.8, 127.7, 127.6, 127.4, 117.4, 114.9, 81.6, 80.2, 70.0, 68.4, 65.3, 65.2, 63.3, 59.6, 58.3, 47.2, 44.6, 40.7, 35.4, 31.0, 28.1, 27.9, 26.8, 26.6, 24.4, 21.1 19.2; IR (neat) 3325, 3070, 2933, 2860, 2110, 1747, 1722, 1635, 1511 cm^{-1}; HRMS (FAB) calcd for $C_{54}H_{73}N_6O_8Si$ 961.5279 $(M+H^+)$, found 961.5258; Anal. Calcd C, 67.5; H, 7.6; N, 8.7. Found C, 67.4; H, 7.6; N, 8.7.

4.1.12. Compound 18. Fmoc-Leu-OH (**5**, 0.192 g, 0.543 mmol) was activated with diisopropylethylamine (DIEA, 0.19 mL, 1.09 mmol) and HATU (0.188 g, 0.494 mmol) at 0 °C for 30 min in DMF (1.0 mL). Then a solution of **17** (0.475 g, 0.494 mmol) in DMF (2.0 mL) was added. After stirring for 24 h the solution was diluted with CH_2Cl_2 (30 mL) and then poured into H_2O (150 mL) and the aqueous phase was extracted with CH_2Cl_2 (5×10 mL). The combined organic layers were washed with 0.2 M aq HCl, H_2O, satd aq $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4, filtered and concentrated. Flash column chromatography (toluene/ethanol, 30:1 → 15:1 and heptane/ethyl acetate 10:1 → 2:1) of the residue gave **18** as a white amorphous solid (333 mg, 52, 70% yield based on the recovered **17**): $[\alpha]_D^{20} = -54.2$ (*c* 0.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, rotamer ratio 4:1) δ (major rotamer) 7.75 (2H, d, $J = 7.3$ Hz, Ph), 7.60 (6H, m, Ph), 7.29–7.44 (15H, m, Ph), 7.18 (2H, d, $J = 8.1$ Hz, Ph_{Tyr}), 6.90 (2H, d, $J = 8.1$ Hz, Ph_{Tyr}), 5.85 (1H, m, $C\mathit{H}{=}CH_2$), 5.33 (1H, d= 9.0 Hz, Leu α-NH), 5.19–5.26 (2H, m, $CH{=}C\mathit{H_2}$ and Orn α-H), 5.13 (1H, d, $J = 10.0$ Hz, $CH{=}C\mathit{H_2}$), 5.02 (2H, s, CH_2Ph), 4.90 (1H, m, Orn δ-NH), 4.67 (1H, m, Leu α-H), 4.48 (2H, m, $C\mathit{H_2}CH{=}CH_2$), 4.31–4.43 (2H, m, Fmoc-$C\mathit{H}C\mathit{H_2}$), 4.21 (2H, m, Fmoc-$CHC\mathit{H_2}$ and Pro α-H), 4.11 (1H, d, $J = 16.3$ Hz, $C\mathit{H_2}COOtBu$), 4.02 (1H, d, $J = 16.3$ Hz, $C\mathit{H_2}COOtBu$), 3.87 (1H, ABX type dd, $J = 5.0$, 10.0 Hz, CH_2OSi), 3.72 (1H, ABX type dd, $J = 2.8$, 10.1 Hz, CH_2OSi), 3.65 (1H, m, CH_2N), 3.56 (1H, m, CHN_3), 3.49 (2H, m, Pro δ-H), 3.43 (2H, m, CH_2N and CHO), 3.07–3.17 (3H, m, Orn δ-H and Tyr β-H), 2.81 (1H, ABX type dd, $J = 9.6$, 14.0 Hz, Tyr β-H), 1.85–2.10 (6H, m, Pro β-H, Pro γ-H, $C\mathit{H_2}CHO$ and Orn β-H), 1.58–1.82 (4H, m, Pro γ-H, Orn β-H, Leu β-H, Leu γ-H), 1.45 (9H, s, $COOtBu$), 1.28–1.36 (3H, m, Orn γ-H and Leu β-H), 1.05 (9H, s, $OSitBu$), 0.98 (6H, d, J = 6.3 Hz, Leu γ-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 169.3, 168.6, 157.7, 156.2, 156.1, 143.8, 143.7, 141.3, 137.0, 135.7, 135.5, 135.5, 133.4, 133.0, 130.3, 130.1, 129.7, 129.7, 128.5, 127.9, 127.7, 127.5, 127.0, 125.2, 125.1, 120.0, 119.9, 117.4, 115.0, 81.6, 79.3, 70.0, 67.7, 67.0, 65.3, 64.7, 63.2, 58.7, 54.6, 49.9, 47.3, 47.1, 42.3, 40.5, 40.4, 34.9, 31.7, 28.1, 27.2, 27.0, 26.9, 26.2, 24.6, 24.4, 23.5, 21.3, 19.3; IR (neat) 3301, 2108, 1720, 1635, 1511 cm^{-1}; ESMS calcd for $C_{75}H_{94}N_7O_{11}Si$ 1297.6 $(M+H^+)$, found 1297.4.

4.1.13. Compound 19. Compound **18** (0.240 mg, 0.185 mmol) was stirred in formic acid (6.0 mL) for 19 h, after which the solution was poured into H_2O (15 mL) and extracted with EtOAc (5×5 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered and concentrated. Flash column chromatography (toluene/ethanol, 30:1 → 10:1) of the residue gave **19** as a white amorphous solid (124 mg, 65%): $[\alpha]_D^{20} = -53.5$ (*c* 0.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, rotamer ratio 4:1) δ (major rotamer) 8.05 (1H, s, HCOO), 7.75 (2H, d, $J = 7.5$ Hz, Ph), 7.56 (2H, d, $J = 7.5$ Hz, Ph), 7.28–7.43 (9H, m, Ph), 7.15 (2H, d, $J = 8.2$ Hz, Ph_{Tyr}), 6.91 (2H, d, $J = 8.2$ Hz, Ph_{Tyr}), 5.88 (1H, m, $C\mathit{H}{=}CH_2$), 5.54 (1H, d, $J = 9.1$ Hz, Leu-NH), 5.12–5.29 (3H, m, $CH{=}C\mathit{H_2}$ and Orn α-H), 5.01 (2H, s, $C\mathit{H_2}Ph$), 5.00 (1H, m, Orn δ-NH), 4.68 (1H, m, Leu α-H), 4.47–4.58 (2H, m, $C\mathit{H_2}CH{=}CH_2$), 4.16–4.43 (8H, m, $C\mathit{H_2}COOH$, Fmoc-$C\mathit{H}C\mathit{H_2}$, Pro α-H, and $C\mathit{H_2}OCHO$), 3.80 (1H, m, CH_2N), 3.45–3.60 (5H, m, CHO, CHN_3, Pro δ-H and CH_2N), 3.17 (2H, m, Orn δ-H), 3.01 (1H, ABX type dd, $J = 3.2$, 13.4 Hz, Tyr β-H), 2.74 (1H, ABX type dd, $J = 9.6$, 13.4 Hz, Tyr β-H), 1.96–2.06 (3H, m, Pro β-H, Pro γ-H and $C\mathit{H_2}CHO$), 1.82–1.92 (4H, m, Pro β-H, Pro γ-H, $C\mathit{H_2}CHO$ and Orn β-H), 1.61–1.76 (3H, m, Orn β-H, Leu β-H), 1.41–1.51 (2H, m, Orn γ-H), 1.33 (1H, m, Leu γ-H), 1.01 (6H, d, $J = 5.3$ Hz, $CH(C\mathit{H_3})_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 171.1, 169.4, 160.8, 157.8, 156.2, 143.6, 141.2, 136.9, 132.8, 130.2, 129.3, 128.5, 127.9, 127.7, 127.5, 127.0, 125.1, 125.0, 120.0, 119.9, 117.6, 115.1, 79.6, 69.4, 67.9, 67.1, 65.5, 64.9, 62.9, 56.0, 54.1, 49.6, 47.2, 47.0, 42.4, 40.5, 40.2, 35.1, 32.2, 27.3, 26.9, 26.1, 24.6, 24.2, 23.4, 21.6; IR (neat) 3309, 2107, 1720, 1635, 1511 cm^{-1}; HRMS (FAB) calcd for $C_{56}H_{67}N_7O_{12}Na$ 1052.4698 $(M+Na^+)$, found 1052.4734; Anal. Calcd C, 65.2; H, 6.6; N, 9.5. Found C, 64.8; H, 6.6; N, 9.4.

4.1.14. Compound 21. Compound **19** (120 mg, 0.116 mmol) was activated by treatment with DIC (18.0 μL, 0.116 mmol) in ethyl acetate (0.8 mL) at 0 °C for 10 min, after which pentafluorophenol (32.2 mg, 0.175 mmol) was added. After stirring for 1 h the solvent was evaporated and the residue was purified by flash column chromatography (heptane/ethyl acetate, 5:1 → 1:1) to give the pentafluorophenyl ester corresponding to **19** as a white amorphous solid (115 mg). The pentafluorophenyl ester (115 mg) in dioxane (25 mL) was then added dropwise to a solution of DBU (43.9 mg, 0.288 mmol) in refluxing dioxane (25 mL) during 3 h. After adding all of the ester, the solution was allowed to reflux for a further 30 min and was then concentrated. Flash column chromatography (heptane/ethyl acetate, 2:1 then toluene/ethanol 25:1 → 5:1) of the residue gave **20** (39 mg) and **21** (5 mg) as white solids, as well as a mixture of **20** and **21** (7 mg). Then aq LiOH solution (1 M, 67.0 μL, 67.0 μmol) was added to a solution of **20** (39 mg) and the mixture of **20**

and **21** (7 mg) in THF, MeOH and H_2O (3:1:1, 2.0 mL) at 0 °C. After being stirred for 30 min the cooling bath was removed and the reaction was stirred for a further 3 h. The solution was acidified with aq HCl (1 M) (5 drops), then poured into EtOAc and H_2O (4:1, 10 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3×4 mL). The combined organic phases were then washed with brine, dried over Na_2SO_4, filtered and concentrated. Flash column chromatography (toluene/EtOH, 25:1 → 10:1) of the residue gave **21** as a white amorphous solid (40 mg in total, 51% from **19**): $[\alpha]_D^{20} = -69.7$ (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamer ratio 4:1) δ (major rotamer) 7.33–7.45 (5H, m, Ph), 7.14 (2H, d, $J = 8.3$ Hz, Ph_{Tyr}), 6.95 (2H, d, $J = 8.4$ Hz, Ph_{Tyr}), 6.50 (1H, d, $J = 8.4$ Hz, Leu NH), 5.90 (1H, m, $CH{=}CH_2$), 5.15–5.35 (3H, m, Orn α-H and $CH{=}CH_2$), 5.06 (2H, s, CH_2Ph), 4.93 (1H, m, Orn δ-NH), 4.71 (1H, m, Leu α-H), 4.54 (2H, m, $CH_2CH{=}CH_2$), 4.41 (1H, d, $J = 15.3$ Hz, OCH_2CON), 3.91 (1H, m, Pro α-H), 3.78 (1H, m, bridge CH_2N), 3.70 (1H, m, CH_2OH), 3.55 (1H, m, CH_2OH and Pro δ-H), 3.53 (1H, d, $J = 15.4$ Hz, OCH_2CON), 3.43 (3H, m, Pro δ-H, bridge CH_2N and CHN_3), 3.35 (1H, m, CHO), 3.21 (2H, m, Orn δ-H), 3.01 (1H, ABX type dd, $J = 7.1$, 13.8 Hz, Tyr β-H), 2.93 (1H, ABX type dd, $J = 7.5$, 13.9 Hz, Tyr β-H), 1.90–2.02 (4H, m, bridge CH_2, Orn β-H, Pro β-H and OH), 1.66–1.85 (5H, m, Pro γ-H, Leu γ-H and Leu β-H), 1.52–1.63 (3H, m, Orn β-H, bridge CH_2 and Pro β-H), 1.45 (2H, m, Orn γ-H), 1.03 (3H, t, $J = 5.7$ Hz, Leu δ-CH_3), 0.98 (3H, t, $J = 5.6$ Hz, Leu δ-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 171.2, 170.8, 158.0, 156.3, 136.8, 132.9, 130.3, 128.6, 128.6, 128.0, 127.5, 117.6, 115.2, 80.8, 70.2, 70.1, 66.5, 65.5, 65.0, 62.2, 54.6, 54.3, 47.9, 41.0, 40.5, 38.0, 36.2, 31.8, 28.3, 25.9, 25.9, 25.0, 24.2, 23.0, 21.2; IR (neat) 3334, 2106, 1701, 1679, 1624, 1512 cm^{-1}; HRMS (FAB) calcd for $C_{40}H_{55}N_7O_8Na$ 784.3998 ($M + Na^+$), found 784.3990.

4.1.15. Compound 2. Freshly prepared Jones reagent[39] (1 M, 0.257 mL, 0.257 mmol) was added to a solution of **21** (56.0 mg, 73.5 μmol) in acetone (1.8 mL) at 0 °C. After being stirred for 10 min the solution was allowed to reach room temperature and stirred for a further 3.5 h. Then the reaction was quenched with isopropanol (5 drops) and the solution was concentrated. The residue was partitioned between CH_2Cl_2 and H_2O (4:1, 5 mL). The aqueous layer was extracted with CH_2Cl_2 (4×5 mL) and the combined organic layers were dried with Na_2SO_4, filtered and concentrated. Flash column chromatography (toluene/ethanol, 25:1 → 5:1) of the residue gave **2** as a white amorphous solid (40 mg, 70%): $[\alpha]_D^{20} = -51.4$ (c 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD, rotamer ratio 3:1) δ (major rotamer) δ 7.33–7.47 (5H, m, Ph), 7.28 (2H, d, $J = 8.6$ Hz, Ph_{Tyr}), 7.01 (2H, d, $J = 8.6$ Hz, Ph_{Tyr}), 5.96 (1H, m, $CH{=}CH_2$), 5.43 (1H, t, $J = 7.4$ Hz, Orn α-H), 5.17–5.35 (2H, m, $CH{=}CH_2$), 5.12 (2H, s, CH_2Ph), 4.72 (1H, m, Leu α-H), 4.55 (2H, m, $CH_2CH{=}CH_2$), 4.46 (1H, m, OCH_2CON), 4.32 (1H, m, Pro α-H), 4.06 (1H, m, bridge CH_2N), 3.65–3.73 (3H, m, OCH_2CON, Pro δ-H and CHO), 3.55 (1H, m, Pro δ-H), 3.46 (1H, m, CHN_3), 3.35 (1H, m, bridge CH_2N), 3.17 (2H, m, Orn δ-H), 2.96 (1H, ABX type dd, $J = 4.0$, 14.3 Hz, Tyr β-H), 2.70 (1H, ABX type dd, $J = 9.2$, 14.3 Hz, Tyr β-H), 2.25 (1H, m, Pro β-H), 1.80–2.08 (7H, m, Pro β-H, Pro γ-H, Orn β-H, Leu β-H and bridge CH_2, Leu γ-H), 1.62–1.73 (3H, m, Orn β-H, Leu β-H, bridge CH_2), 1.46 (2H, m, Orn γ-H), 1.06 (3H, t, $J = 6.5$ Hz, Leu δ-CH_3), 1.00 (3H, t, $J = 6.5$ Hz, Leu δ-CH_3); ^{13}C NMR (100 MHz, CD_3OD) δ 173.9, 172.8, 169.4, 157.9, 157.4, 137.3, 133.1, 130.4, 130.2, 130.1, 129.7, 129.6, 128.2, 127.6, 127.3, 127.3, 116.4, 115.0, 114.9, 114.7, 82.3, 71.3, 70.9, 66.9, 66.2, 62.5, 56.0, 55.5, 48.4, 41.3, 41.2, 39.1, 37.1, 31.7, 30.5, 26.6, 26.5, 25.8, 25.5, 23.3, 21.3; IR (neat) 3325, 2106, 1714, 1624, 1512 cm^{-1}; HRMS (FAB) calcd for $C_{40}H_{54}N_7O_9$ 776.3979 ($M + H^+$), found 776.3999.

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References and notes

1. Amoss, M.; Burgus, R.; Blackwell, R.; Vale, W.; Fellows, R.; Guillemin, R. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 205–210.
2. Schally, A. V.; Arimura, A.; Kastin, A. J.; Matsuo, H.; Baba, Y.; Redding, T. W.; Nair, R. M. G.; Debeljuk, L.; White, W. F. *Science* **1971**, *173*, 1036–1038.
3. Matsuo, H.; Baba, Y.; Nair, R. M. G.; Arimura, A.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1971**, *43*, 1334–1339.
4. Baba, Y.; Matsuo, H.; Schally, A. V. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 459–463.
5. Conn, P. M.; Huckle, W. R.; Andrews, W. V.; McArdle, C. A. *Recent Prog. Horm. Res.* **1987**, *43*, 29–68.
6. Hazun, E.; Conn, P. M. *Endocr. Rev.* **1988**, *9*, 379–386.
7. Emons, G.; Schally, A. V. *Hum. Reprod.* **1994**, *9*, 1364–1379.
8. Kutscher, B.; Bernd, M.; Beckers, T.; Polymeropoulos, E. E.; Engel, J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2148–2161.
9. Ball, J. B.; Alewood, P. F. *J. Mol. Recog.* **1990**, *3*, 55–64.
10. Kahn, M. *Synlett* **1993**, 821–826.
11. Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244–1267.
12. Rizo, J.; Gierasch, L. M. *Annu. Rev. Biochem.* **1992**, *61*, 387–418.
13. Olson, G. L.; Bolin, D. R.; Bonner, M. P.; Bös, M.; Cook, C. M.; Fry, D. C.; Graves, B. J.; Hatada, M.; Hill, D. E.; Kahn, M.; Madison, V. S.; Rusiecki, V. K.; Sarabu, R.; Sepinwall, J.; Vincent, G. P.; Voss, M. E. *J. Med. Chem.* **1993**, *36*, 3039–3049.
14. Marshall, G. R. *Tetrahedron* **1993**, *49*, 3547–3558.
15. Smith, J. A.; Pease, L. G. *CRC Crit. Rev. Biochem.* **1980**, *8*, 315–399.
16. Rose, G. D.; Gierasch, L. M.; Smith, J. A. Advances in Protein Chemistry. In Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Turns in Peptides and Proteins; Academic: Orlando, FL, 1985; Vol. 37, pp 1–109.
17. Stanfield, R. L.; Fieser, T. M.; Lerner, R. A.; Wilson, I. A. *Science* **1990**, *248*, 712–719.
18. Marshall, G. R. *Curr. Opin. Struct. Biol.* **1992**, *2*, 904–919.
19. Hölzemann, G. *Kontakte (Darmstadt)* **1991**, 3–12 see also pages 55–63.
20. Momany, F. A. *J. Am. Chem. Soc.* **1976**, *98*, 2990–2996.

21. Karten, M. J.; Revier, J. E. *Endocr. Rev.* **1986**, *7*, 44–66.
22. Guarnieri, F.; Weinstein, H. *J. Am. Chem. Soc.* **1996**, *118*, 5580–5589.
23. Freidinger, R. M.; Veber, D. F.; Perlow, D. S. *Science* **1980**, 656–658.
24. Cho, N.; Harada, M.; Imaeda, T.; Imada, T.; Matsumoto, H.; Hayase, Y.; Sasaki, S. J. *Med. Chem.* **1998**, *41*, 4190–4195.
25. Kreye, P.; Kihlberg, J. *Tetrahedron Lett.* **1999**, *40*, 6113–6116.
26. Blomberg, D.; Hedenström, M.; Kreye, P.; Sethson, I.; Brickmann, K.; Kihlberg, J. *J. Org. Chem.* **2004**, *69*, 3500–3508.
27. Robinson, S.; Roskamp, E. J. *Tetrahedron* **1997**, *53*, 6697–6705.
28. McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 2562–2570.
29. Davies, J. S.; Mohammed, A. K. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2982.
30. Nahm, S. M.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815–3818.
31. Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029–6032.
32. Pietraszkiewicz, M.; Jurczak, J. *Tetrahedron* **1984**, *40*, 2967–2970.
33. Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201–203.
34. Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279–2282.
35. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.
36. Yuan, Z.; Blomberg, D.; Sethson, I.; Brickmann, K.; Ekholm, K.; Johansson, B.; Nilsson, A.; Kihlberg, J. *J. Med. Chem.* **2002**, *45*, 2512–2519.
37. Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*, 3rd ed.; Wiley: New York, 1999.
38. Jakubke, H.-D. *Peptide: Chemie und Biologie*; Spektrum Akad. Verl.: Heidelberg, 1996; pp 256–258.
39. Wellner, E.; Gustafsson, T.; Bäcklund, J.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2000**, *1*, 272–280.

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Investigations into the selective oxidation of vicinal diols to α-hydroxy ketones with the $NaBrO_3/NaHSO_3$ reagent: pH dependence, stoichiometry, substrates and origin of selectivity

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Abstract—The $NaBrO_3/NaHSO_3$ reagent is one of the few oxidizing agents that chemoselectively oxidizes vicinal diols to α-hydroxy ketones with little overoxidation to the corresponding vicinal-dione or dicarboxylic acid. Oxidation reactions performed with this reagent showed strong pH dependence. *cis*-Vicinal diols reacted faster than *trans*-vicinal diols to the α-hydroxy ketone product. Hydroxy functional groups at axial ring positions were more readily oxidized than equatorial hydroxy groups. The application of the $NaBrO_3/NaHSO_3$ reagent for the chemoselective oxidation of vicinal diols was limited to simple systems and failed with more complex monosaccharide compounds probably due to acid catalyzed dehydrogenation reactions. Despite the simple reaction set-up and good selectivity towards the α-hydroxy ketone product, the actual oxidation reaction mechanism is highly complex and postulated to involve at least six different equilibria with a plethora of bromine containing species. A possible oxidation reaction mechanism is discussed.

1. Introduction

α-Hydroxy ketone (α-ketol) is a functional group entity of natural products with widely studied chemistry.[1–3] The syntheses of α-hydroxy ketones have been achieved by oxidation of alkenes,[4,5] by acyloin condensation of diesters,[6] from enol ethers,[7] silylenol ethers[8,9] and epoxides[10] or by α-oxidation of ketones[11,12] and reduction of α-diketones[13] (Fig. 1). However, the synthetic route through selective oxidation of *sec.-sec.*-vicinal diols has only marginally been covered in the literature.[14–17] The secondary vicinal diol functionality is a key structural feature in sugars. Selective oxidation of one secondary hydroxyl to a carbonyl function opens the synthetic pathway to pyrano- or furanosiduloses also known as 'oxo'-sugars. To our knowledge, only two methods for this oxidation have been reported in the literature: an enzymatic process involving a rare fungal enzyme[18,19] and brominolysis of tin-alkyl sugar derivatives.[20,21] The difficulty in the selective oxidation of a vicinal diol to the corresponding α-hydroxy ketone is the prevention of overoxidation to a vicinal dione or—after C–C bond cleavage—dicarboxylic acid (Scheme 1). Assuming a first order reaction rate of the bimolecular reaction between the OH-compound, i.e. vicinal-diol or α-hydroxy ketone, and oxidizing species the relative ratio of the reaction rate constants k_1 (diol → α-OH-ketone)/k_2 (α-OH-ketone → dione) has to be $\geqq 10$ to support a synthetically useful selectivity discrimination between the

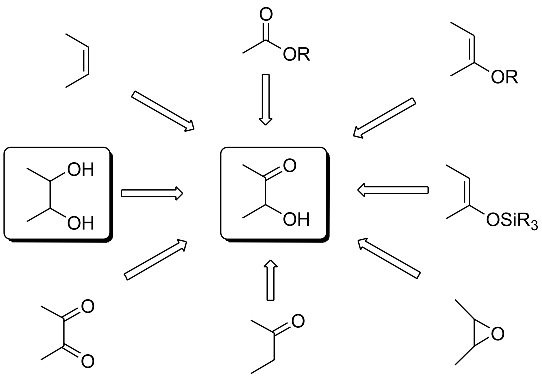
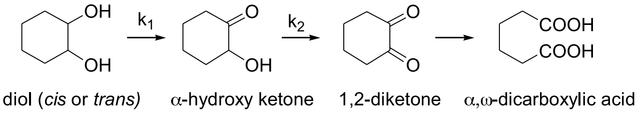


Figure 1. Synthetic routes to α-hydroxy ketones.



Scheme 1. Oxidation of vicinal diols.

Keywords: Chemoselective oxidation; Vicinal diols; α-Hydroxy ketone; Hypobromous acid.

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α-hydroxy ketone and the vicinal-dione compound.[22,23] This requirement is probably one of the reasons why the chemoselective oxidation of *sec.-sec.*-vicinal diols is limited to only few methods with Ishii's $NaBrO_3$/$NaHSO_3$ reagent being one of them.[24]

The simple oxidation reaction conditions for the $NaBrO_3$/$NaHSO_3$ reagent are particularly useful for organic syntheses as the chemicals are commercially available and inexpensive off-the-shelf compounds. Yet, the actual reaction mechanism is very intriguing and displays good chemoselectivity. In a remarkable methodology, a mild oxidizing agent is generated in situ by addition of both, an oxidant, sodium bromate, and a reductant, sodium bisulfite. Ishii et al. postulated that the actual oxidant was hypobromous acid, HOBr, as bromohydrins are formed in reactions involving C=C bonds.[24,25] The $NaBrO_3$/$NaHSO_3$ reagent has also been successfully employed in the γ-lactone formation of *o*-alkyl substituted benzoic acids.[26] Other reactions that make use of bromate together with reducing agents are the Belousov–Zhabotinskii class of oscillating reactions.[27,28] Metsger et al. reported that the oxidation of alkyl ethers occurred in α-position by Br_2, which is generated through the comproportionation reaction of bromate and bromide to provide a low but steady concentration of bromine.[29]

Here we report additional insights into the selective oxidation of vicinal diols to the corresponding α-hydroxy ketones using the $NaBrO_3$/$NaHSO_3$ reagent, specifically its scope and limitations with respect to pH, diol substrates, its reaction mechanism and the origin of the chemoselectivity.

2. Results

Oxidation reaction experiments were conducted on a small scale (4.2 mL, 0.24 M diol substrate) and monitored by quantitative gas chromatography calibrated against dimethyl sulfone as the internal standard. Oxidation products were identified by GC-MS and by comparison with authentic commercial samples when available. The oxidation experiments of the monosaccharide substrates were conducted on an NMR scale in D_2O (0.7 mL, 0.24 M substrate) and directly monitored by ^1H and ^{13}C NMR spectroscopy.

2.1. pH dependence

The oxidation reaction of *trans*-1,2-cyclohexanediol exhibited a strong pH dependence (Table 1), an important observation that has previously not been reported. The reaction stoichiometry of diol substrate, sodium bromate and sodium bisulfite was adopted from the established oxidation procedure published by Ishii et al.[24] Entry 2 corresponds to the reaction condition reported by Ishii et al., resulting in an initial pH of 1.7.[24] Changes of the pH of the reaction solution were effected by addition of sulfuric acid or Na_2HPO_4. The desired α-hydroxy cyclohexanone product was generated almost quantitatively within 1 h to 24 h while the overoxidation product, 1,2-cyclohexanedione, was only present in 0–5%. It was found that the oxidation reaction proceeded only in acidic solution at pH values <4 and that the oxidation reactions were faster at lower pH. When the oxidation was completed the final pH values of the reaction solutions were higher than the initial pH values by approximately 0.5 units. In a control experiment, Na_2SO_3—instead of $NaHSO_3$—was reacted with sodium bromate and no reaction occurred within 48 h (Entry 5). With *trans*-1,2-cyclohexanediol as the substrate, a reaction solution containing Na_2SO_3 and $NaBrO_3$ had an only slightly acidic pH of 5. When this reaction solution was subsequently acidified with 0.5 equiv of conc. sulfuric acid (0.6 mmol, 1.2 equiv of H^+) resulting in pH of 1.7, the oxidation reaction immediately proceeded as with the original $NaHSO_3$/$NaBrO_3$ reaction condition (Entry 2).

Table 1. pH dependence of the effectiveness of the $NaBrO_3$/$NaHSO_3$ reagent in the oxidation of *trans*-1,2-cyclohexanediol[a]

Entry	$pH_{initial}$	pH_{final}	α-Hydroxy cyclohexanone % (t)[b]
1	0[c]	0.5	95% (1 h)
2	1.7	2.3	74% (4 h) 97% (24 h)
3	2.3[d]	2.8	25% (4 h) 70% (24 h)
4	4.0[d]	4.4	No reaction (48 h)
5[e]	5	5	No reaction (48 h)
	1.7[f]	2.3	74% (4 h) 97% (24 h)

[a] Reaction conditions: 1.0 mmol diol, 1.2 mmol $NaBrO_3$, 1.2 mmol $NaHSO_3$ in 2.2 mL H_2O and 2.0 mL CH_3CN at 21 °C ± 1 °C.

[b] Determined by GC. Content of 1,2-cyclohexanedione was <5%.

[c] H_2SO_4 added until desired $pH_{initial}$ value reached.

[d] Na_2HPO_4 added until desired $pH_{initial}$ value reached.

[e] 1.2 mmol Na_2SO_3.

[f] Addition of 0.6 mmol H_2SO_4.

2.2. $NaBrO_3$/$NaHSO_3$ reagent stoichiometry

Various bromate and bisulfite amounts were added to *trans*-1,2-cyclohexanediol substrate in order to investigate the optimum oxidation reaction condition for the $NaBrO_3$/$NaHSO_3$ reagent (Table 2). Ishii et al. reportedly applied equal molar sodium bromate and sodium bisulfite quantities in slight excess with respect to the diol substrate (Entry 2).[24] Throughout all reactions (Entries 1–12), the initial concentration of *trans*-1,2-cyclohexanediol (0.24 M, 1.0 mmol) was held constant and yields of α-hydroxy cyclohexanone were determined by quantitative GC analysis. Generally, it was found that even with more equivalents of oxidant, the 1,2-cyclohexanedione yield was not significantly increased always remaining in the range of 0–5%. Addition of an equimolar ratio of $NaBrO_3$ and $NaHSO_3$ in half to three equivalents with respect to the diol substrate generated the α-hydroxy ketone quantitatively within 2–120 h (Entries 2–4). The 1,2-cyclohexanedione content was 15% at the extended reaction time of 120 h (Entry 1). No reaction—neither oxidation nor reduction—of *trans*-1,2-cyclohexanediol occurred when sodium bromate and sodium bisulfite were added individually (Entries 5 and 6). The oxidation reaction leveled off at 51% conversion after 48 h when the relative ratio of bromate and bisulfite was 0.6–1.2 mmol (Entry 7). However, addition of 1.2 mmol bromate and 2.4 mmol bisulfite to the diol substrate completed the oxidation reaction within 24 h (Entry 9). An oxidation reaction was not observed when the $NaBrO_3$: $NaHSO_3$ ratio was less than 1:3 (Entries 10–12). Quantitative oxidation

Table 2. *trans*-1,2-Cyclohexanediol oxidation with varying amounts of $NaBrO_3$ and $NaHSO_3$ reagent[a]

Entry	$NaBrO_3$ (mmol)	$NaHSO_3$ (mmol)	pH_{inital}	α-OH ketone % (time)[b]	Colour
1	0.6	0.6	2.0	20% (1 h) 55% (24 h) 70% (48 h) 85% (120 h)[c]	Yellow
2	1.2	1.2	1.7	74% (4 h) 97% (24 h)	Yellow
3	2.4	2.4	1.5	60% (1 h) 96% (4 h)	Yellow
4	3.6	3.6	1.3	73% (0.5 h) 100% (2 h)	Yellow
5	1.2	0	5.0	No reaction	Colourless
6	0	1.2	5.0	No reaction	Colourless
7	0.6	1.2	1.8	15% (1 h) 48% (24 h) 51% (48 h)	Yellow, colourless (48 h)
8	1.2	0.6	2.0	30% (1 h) 54% (6 h) 87% (24 h) 98% (48 h)	Light yellow
9	1.2	2.4	1.6	34% (1 h) 54% (4 h) 96% (24 h)	Orange
10	1.2	3.6	1.0	No reaction	Colourless
11	1.2	4.8	1.4	No reaction	Colourless
12	0.6	1.8	1.3	No reaction	Colourless

[a] Reaction conditions: 1.0 mmol diol in 2.2 mL H_2O and 2.0 mL CH_3CN at 21 °C ± 1 °C.

[b] Determined by GC based on α-hydroxy ketone. Content of dione was $<5\%$.

[c] Final 1,2-cyclohexanedione content was 15%.

was observed with 1.2 mmol of $NaBrO_3$ and 0.6 mmol of $NaHSO_3$ within 48 h (Entry 8).

The colour of the $NaBrO_3$/$NaHSO_3$ oxidation solution is an excellent indicator for oxidation activity. The oxidation reaction proceeded only when the solution was yellow-orange coloured. No reaction occurred when the solution was colourless. The initial yellow colour of the oxidation reaction of entry 7 disappeared when the α-hydroxy-cyclohexanone content leveled at 51%. In a control experiment, it was found that the colour change was very sensitive to the amount of bromate, bisulfite, sulfite and sulfuric acid. Adjusting the pH of the reaction solution with sulfuric acid (1.2 N) or NaOH (1.2 N) through a burette or Pasteur pipette changed the colour of the solution with one extra drop. The yellow colour/colourless sequence occurred fully reversibly when base and acid were alternatively added. When neutral or basic sulfite solutions were acidified quickly by addition of conc. sulfuric acid brown vapours of bromine were generated. The addition order of $NaBrO_3$, $NaHSO_3$ or Na_2SO_3 and H_2SO_4 is irrelevant. Each different order of addition produced the same yellow coloured oxidizing reagent solution with the same activity towards the *trans*-1,2-cyclohexanediol substrate.[30]

To explore the possibility of deactivation or formation of other species originating from the oxidizing agent, four reagent solutions containing sodium bromate (1.2 mmol) and sodium bisulfite (1.2 mmol) were prepared and stirred for 24 h and 7 days at room temperature and 60 °C, respectively. These prepared reagent solutions were then added to *trans*-1,2-cyclohexanediol (1.0 mmol) solutions at room temperature.[31] In each of the four experiments, the oxidation reaction proceeded quantitatively to the α-hydroxy cyclohexanone and was identical to the reaction condition when the diol substrate and the $NaBrO_3$/$NaHSO_3$ reagent solution were combined immediately upon preparation.

2.3. Solvent and temperature

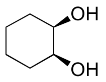
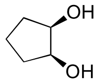
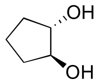
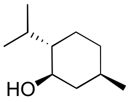
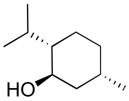
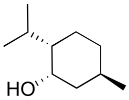
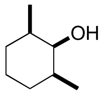
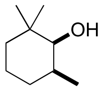
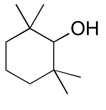
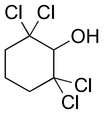
The $NaBrO_3$/$NaHSO_3$ reagent was active in various solvents such as pure water, aqueous acetonitrile and aqueous *N*,*N*-dimethylacetamide. The *trans*-1,2-cyclohexanediol oxidation reactions proceeded in these three solvent systems with similar reaction times, conversions and selectivities comparable with Ishii's published conditions using aqueous-acetonitrile solution.[24] An increase of reaction rates without the formation of side products or degradation of substrate or oxidant was found when the temperature was increased from ambient temperature to 45 and 60 °C. The conversions of *trans*-1,2-cyclohexanediol to α-hydroxy cyclohexanone were quantitative after 24, 6 and 2 h, respectively. The contents of the overoxidation product, 1,2-cyclohexane-dione, were not significantly different and again remained within $\leq 5\%$.

2.4. Substrates

In order to investigate the reactivity and selectivity of the oxidation of sugar substrates, we focused on relative reactivity of *cis* and *trans* vicinal diols. Using Ishii's standard reaction conditions, the oxidation results for the diol substrates showed that the *cis* compounds were slightly more reactive than their corresponding *trans* isomers (Table 3, entries 1–3; Table 2, entry 2). The cyclopentane-diol compounds were oxidized marginally slower than their cyclohexanediol homologues.

The influence of steric hindrance on the reactivity of

Table 3. Oxidation of various diols and monoalcohols with $NaBrO_3$/$NaHSO_3$ reagent[a]

Entry	Diol/monoalcohol substrate	Conversion % (t)[b]
1		64% (1 h) 99% (6 h)
2		64% (1 h) 98% (24 h)
3		50% (1 h) 89% (24 h)
4	 (−)-Menthol	85% (1 h) 100% (2 h)
5	 (+)-Isomenthol	34% (1 h) 100% (8 h)
6	 (+)-Neomenthol	100% (<15 min)
7		100% (1 h)
8		98% (1.5 h)
9		100% (2 h)
10		0% (24 h)

21 °C ± 1 °C.

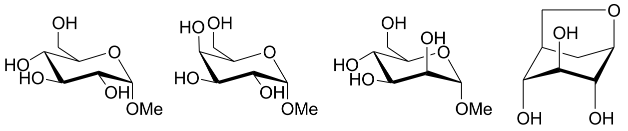
[a] Reaction conditions: 1.0 mmol diol, 1.2 mmol $NaBrO_3$, 1.2 mmol $NaHSO_3$ in 2.2 mL H_2O and 2.0 mL CH_3CN at rt and pH 1.7.

[b] Determined by GC based on α-hydroxy ketone or ketone. Content of dione was <5%.

secondary alcohols was explored by the oxidation of a series of monoalcohols (Table 3, entries 4–10). The steric positioning of the hydroxyl function in cyclic systems with respect to an alkyl substituent in axial or equatorial position was investigated using menthol isomers and cyclohexanol derivatives. In solution, the hydroxyl functionality is predominantly axially oriented in (+)-neomenthol and equatorially in (+)-isomenthol. It has an intermediate axial–equatorial position in (−)-menthol. (−)-Menthol was oxidized in 2 h while its hydroxyl epimer, (+)-neomenthol, required less than 15 min to produce (−)-menthone. (+)-Isomenthol was oxidized to (+)-isomethone in 8 h.

Additional methyl groups alpha to the secondary alcohol functionality decreased the reactivity to the corresponding ketone compound only marginally (Entries 7–9). While the 2,2,6,6-tetramethylcyclohexanol was oxidized within 2 h, the oxidation of 2,2,6,6-tetrachlorocyclohexanol failed (Entries 9 and 10).

The intrinsic challenges associated with the quantitative and qualitative detection of monosaccharide substrates and their reaction products by GC or GC-MS[32] lead us to conduct ^1H and ^{13}C NMR experiments in D_2O instead. As demonstrated above, the reactivity of the $NaBrO_3$/$NaHSO_3$ reagent in aqueous-acetonitrile solution was similar to water solvent systems. Methyl α-D-glucopyranoside (**1**), methyl α-D-galactopyranoside (**2**), methyl α-D-mannopyranoside (**3**) and 1,6-anhydro-D-glucopyranoside (**4**) were examined under the $NaBrO_3$/$NaHSO_3$ oxidation conditions (Fig. 2). No reaction occurred after 24 h at room temperature and thus the four samples were heated to 60 °C. A slow oxidation process then occurred but after 5 d the sugar substrates had reacted unselectively to complex intractable product mixtures.



1 **2** **3** **4**

Figure 2. Investigated monosaccharide substrates.

2.5. Other oxidizing conditions

Control experiments were conducted in order to determine the oxidation mechanism and active oxidizing species. As elemental bromine could be a possible oxidant, 1 equiv of Br_2 was added to an aqueous-acetonitrile solution containing *trans*-1,2-cyclohexanediol. The reaction solution was yellow coloured and produced a complex product mixture, which was analyzed by GC-MS and contained α-hydroxy cyclohexanone, various brominated species and other undetermined compounds. To simulate a low but steady Br_2 concentration in the reaction solution, a diluted Br_2 solution (1 equiv) was added with a syringe pump over a 6 h time period. Again, a complex product mixture was obtained containing α-hydroxy cyclohexanone along with a complex mixture of other products, albeit, less side products were formed. In the $NaBrO_3$/$NaHSO_3$ oxidation condition, no brominated side products were detected by GC-MS. Hypobromous acid, HOBr, was produced by mixing equal amounts of aqueous equimolar $AgNO_3$ and Br_2 solutions.[33] The *trans*-1,2-cyclohexanediol oxidation with in situ generated HOBr yielded a complex mixture of products after 6 h in stark contrast to chemoselective oxidation with the $NaBrO_3$/$NaHSO_3$ reagent.

3. Discussion

In consideration of above results, the nature of the active oxidant, the role of protons and the overall mechanism of the diol oxidation by the $NaBrO_3$/$NaHSO_3$ system remains elusive, however, a few clear features of the apparently complex system emerge and certain conclusion can be made.

3.1. Equilibria and HOBr

Ishii suggested that hypobromous acid, HOBr, is the actual oxidizing species as bromohydrins are synthesized when the $NaBrO_3/NaHSO_3$ reagent reacts with C=C double bonds.[24,34] It is, however, not immediately obvious that HOBr, is the actual oxidizing species, considering that a 1:1 $NaBrO_3$:$NaHSO_3$ reagent would generate 1 equiv of bromous acid, HOBrO, as bisulfite is oxidized to sulfate through a formal transfer of oxygen from bromate (Eq. 1). Disproportionation of HOBrO could then generate HOBr, but also reform bromate (Eq. 2), ultimately requiring an excess of bisulfite, but as our stoichiometry study showed the ratio of $NaHSO_3$ to $NaBrO_3$ does only influence the rate of reaction but not the selectivity of the oxidation. Therefore, the actual oxidant is probably not directly generated by $NaHSO_3$ and $NaBrO_3$ but through a cascade of intricate equilibria involving at least all of the steps listed in Equations 1–6. Thus the different bromo-species and potential oxidants $HOBrO_2$, HOBrO, HOBr, Br_2, Br_3^- and potentially the Br radical can be produced in the reaction solution in various concentrations.[27,28,35–38] The $NaBrO_3$/$NaHSO_3$ reagent is not deactivated when kept at 60 °C for 7 days. Thus the actual oxidant must be fairly stable and does not undergo side reactions once the equilibrium has been established.

$$BrO_3^- + HSO_3^- \rightleftharpoons HOBrO + SO_4^{2-} \qquad (1)$$

$$2\ HOBrO \rightleftharpoons HOBr + BrO_3^- + H^+ \qquad (2)$$

$$BrO_3^- + 5Br^- + 6H^+ \rightleftharpoons 3Br_2 + 3H_2O \qquad (3)$$

$$Br_2 + H_2O \rightleftharpoons HOBr + Br^- + H^+ \qquad (4)$$

$$HOBr + H^+ \rightleftharpoons [H_2O - Br]^+ \rightleftharpoons H_2O + Br^+ \qquad (5)$$

$$Br_2 + Br^- \rightleftharpoons Br_3^- \qquad (6)$$

3.2. pH dependence

Protons play a key role in the oxidation mechanism. The increased reaction rate at lower $pH_{initial}$ can be explained if Br^+—or $[Br–OH_2]^+$—were the actual oxidizing agent. Br^+ formation is favoured at higher H^+ concentration because the dehydration of HOBr is proton dependent (Eq. 5). The comproportionation and disproportionation reactions (Eq. 2–4) are known to proceed at pH 1.5–2.5,[38] which asserts our experimental work as yellow colour and oxidation activity was only detected when the solution pH was <4.

Furthermore, during the oxidation process the pH of the reaction solution increased, suggesting that protons were consumed. This is on first sight contradictory as the oxidation of a secondary alcohol with HOBr generates a proton along with bromide and carbonyl compound (Eq. 7). Another equilibrium must be responsible for the overall consumption of protons. As bromide ions are continuously produced, the comproportionation reaction of bromate and bromide to hypobromous acid will require a total of three protons (Eq. 3 and 4). The reaction is autocatalytic with respect of Br^- and a continuous low and steady regeneration of HOBr concentration is therefore established.

$$HOBr + RCH(OH)R' \rightarrow RC(O)R' + H_2O + Br^- + H^+ \qquad (7)$$

3.3. Control experiments

The yellow colour—an indicator for oxidation activity—can be associated with HOBr and Br_2 but not with bromide, bromate, sulfite and sulfate anions which are all colourless.[35] Br_2, a potential active oxidant proposed by Metsger et al.,[29] is probably not the active oxidant, as a very slow addition of diluted Br_2 solution to the diol substrate to produce a low and steady Br_2 concentration generated many side products of which some were bromo-species, while under our standard experimental $NaBrO_3$/$NaHSO_3$ reaction conditions no carbon–bromine species have been detected. Ishii was able to isolate bromo-species only if a C=C double bond was present in the substrate.[24] This is an indicator that the mechanism is probably ionic rather than radical. In the control experiment when HOBr was generated in situ from bromine and silver nitrate (Eq. 8),[33] the reaction products contained brominated species. However, the HOBr concentration in the control experiment may still have been relatively high so that HOBr can undergo bromination side reactions in contrast to a low and steady concentration generated by the $NaBrO_3$/$NaHSO_3$ reagent.

$$AgNO_3 + Br_2 + H_2O \rightarrow AgBr_{(s)} + HNO_3 + HOBr \qquad (8)$$

3.4. Oxidation mechanism and selectivity

The actual oxidation can in principle either occur directly at the hydroxyl function or at the alpha-position through an enol type intermediate. An oxidation mechanism that proceeds through an enol of the organic compound can, however, be excluded, as the complete oxidation of 2,2,6,6-tetramethylcyclohexanol, which contains no alpha-hydrogen, proceeded at similar reaction rates as the di- and trimethylated cyclohexanol derivatives.

Based on the observed pH dependence, we now postulate that a small but persistent and continuously replenished concentration of Br^+ or possibly $[H_2O–Br]^+$ rather than neutral HOBr as proposed by Ishii is the actual oxidizing agent. It is then logical to expect it to preferentially attack the least acidic hydrogens geminal to the OH functional group.

Once the first oxidation to the α-hydroxy ketone has occurred, the polarization of the remaining geminal hydrogen is slightly less negative than that in a vicinal diol as the carbonyl group withdraws electron density (Fig. 3) resulting in the observed chemoselectivity of diol oxidation.

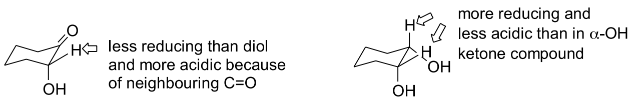


Figure 3. Geminal hydrogen polarization in α-hydroxy ketone and diol.

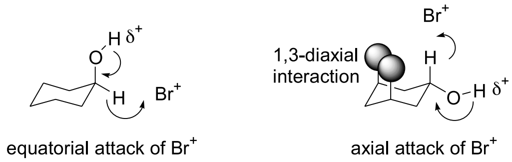


Figure 4. Axial and equatorial attach of Br^+ on cyclohexanol.

The preferential selectivity of axial vs. equatorial OH functions studied in the menthol derivatives series, can then also be rationalized by postulating Br^+ as the actual oxidant which attacks the geminal hydrogen of the hydroxyl group (Fig. 4). An equatorial attack by the oxidant is more preferred over an axial attack, which would experience more shielding due to 1,3-diaxial interactions. In addition moving the oxygen from the axial position towards a more carbonyl-like transition state during the oxidation reaction should also lead to a release of ring strain induced by the 1,3-interactions of the axial hydroxyl. The situation is slightly different for *cis*-diols which were oxidized faster than the *trans*-diol as there is always one geminal hydrogen in axial position while in the *trans*-diol isomer both OH functions occupy the equatorial position (Fig. 5). The oxidation of 2,2,6,6-tetrachlorocyclohexanol failed probably due to the high steric shielding and/or the lowest negative polarization of the geminal hydrogen due to the four electron withdrawing chlorine atoms present.

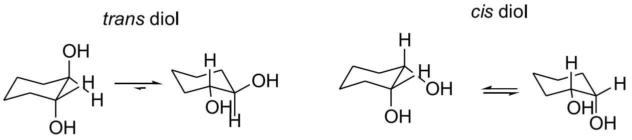


Figure 5. Relative conformations of *trans*- and *cis*-1,2-cyclohexanediols.

The chemoselective oxidation of polyhydroxy substrates, e.g. monosaccharides failed or yielded only complex intractable reaction mixtures, probably because these substrates can undergo acid catalyzed condensation reactions.

4. Concluding remarks

The $NaBrO_3/NaHSO_3$ reagent establishes an intricate network of equilibria in which HOBr acts as the source of but probably is not the actual oxidizing agent. The low and constantly replenished concentration of the actual oxidant, possibly $[H_2O–Br]^+$ or Br^+, generates a constant redox potential of the reagent solution. We propose that the redox potential of the reaction solution reaches a specific value which is sufficient enough for the first oxidation step of the diol but does not allow—or only very little—of the second oxidation step of α-hydroxy ketone to the dione to occur,[22] i.e. the $NaBrO_3/NaHSO_3$ reagent combination effectively acts as redox buffer.

In order to further elucidate the reaction mechanism a full quantitative analysis of each of the bromine containing species in the reaction solution in real time would be necessary, which constitutes a formidable challenge.

5. Experimental

trans-1,2-Cyclohexanediol, *cis*-1,2-cyclohexanediol, *trans*-1,2-cyclopentanediol, *cis*-1,2-cyclopentanediol, α-hydroxy cyclohexanone, cyclohexane-1,2-dione, cyclohexanone, acetonitrile, 2,2,6,6-tetrachlorocyclohexanol, sodium bromate, sodium bisulfite and sodium sulfite are commercially available and were used as received. *cis*,*cis*-2,6-Dimethylcyclohexanol [39170-84-8], *cis*-2,2,6-trimethylcyclohexanol [20580-68-1] and 2,2,6,6-tetramethylcyclohexanol [6948-41-0] were obtained by methylation of cyclohexanone with MeI and subsequent reduction with $LiAlH_4$ according to literature procedures.[39] The identities of these compounds as well as those of the corresponding oxidation products *cis*,*cis*-2,6-dimethylcyclohexanone [471256-22-1], *cis*-2,2,6-trimethylcyclohexanone [106036-02-6]/106033-56-1] (enantiomers) and 2,2,6,6-tetramethylcyclohexanol [1195-93-3] were established by ^1H NMR and MS. The pH values were measured with a Fisher Accumet® pH meter Model 620 and a calomel electrode. The NMR experiments were conducted on a Varian Avance 400 MHz spectrometer. GC and GC-MS experiments were conducted on a Varian CP-3800 and Varian CP-3800-Saturn 2000. Environmental note: residual solutions of the oxidation reactions should be quenched with a saturated $Na_2S_2O_3$ solution and properly discarded.

5.1. General reaction conditions

trans-1,2-Cyclohexanediol (116 mg, 1.0 mmol) and sodium bromate (181 mg, 1.2 mmol) were dissolved in 1 mL water and 2 mL acetonitrile. A solution of sodium bisulfite (124 mg, 1.2 mmol) in 1.2 mL water was then added via syringe. The pH was measured with a pH meter, the reaction stirred at room temperature (21 °C ± 1 °C) and monitored by quantitative GC.

5.2. NMR scale experiments

Methly α-D-glucopyranoside (33 mg, 0.17 mmol) was reacted with $NaBrO_3$ (30 mg, 0.20 mmol) and $NaHSO_3$ (20 mg, 0.20 mmol) in 0.7 mL D_2O at room temperature for 24 h, then stirred at 60 °C for 5 days and the reaction monitored by ^1H and ^{13}C NMR.

Acknowledgements

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Supplementary data

Supplementary material for this article detailing a generic kinetic model for the oxidation of *vic*-diols as well as an exemplary reaction protocol for larger scale applications of the $NaHSO_3/NaBrO_3$ method can be found, in the online version, at doi:10.1016/j.tet.2005.03.056.

References and notes

1. The Merck Index, 13th ed.; Merck: Whitehouse Station, NJ, 2001.
2. Larock, R. C. *Comprehensive Organic Transformation*, 2nd ed.; Wiley-VCH: New York, 1999.
3. Hudlicky, M. *Oxidations in Organic Chemistry*; ACS Monograph; American Chemical Society: Washington, DC, 1990.
4. Sakata, Y.; Katayama, Y.; Ishii, Y. *Chem. Lett.* **1992**, 671–674.
5. Murahashi, S.-I.; Saito, T.; Hanaoka, H.; Murakami, Y.; Naota, T.; Kumobayashi, H.; Akutagawa, S. *J. Org. Chem.* **1993**, *58*, 2929–2930.
6. Finley, K. T. *Chem. Rev.* **1964**, *64*, 573–589.
7. Hashiyama, T.; Morikawa, K.; Sharpless, K. B. *J. Org. Chem.* **1992**, *57*, 5067–5068.
8. Rubottom, G. M.; Vazquez, M. A.; Pelegrina, D. R. *Tetrahedron Lett.* **1974**, 4319–4322.
9. Takai, T.; Yamada, T.; Rhode, O.; Mukaiyama, T. *Chem. Lett.* **1991**, 281–284.
10. Tsuji, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 645–647.
11. Moriarty, R. M.; Hu, H.; Gupta, S. C. *Tetrahedron Lett.* **1981**, *22*, 1283–1286.
12. El-Qisairi, A. K.; Qaseer, H. A. *J. Organometal. Chem.* **2002**, *659*, 50–55.
13. Hekmatshoar, R.; Heravi, M. M.; Beheshtiha, Y. S. *Monatsh. f. Chem.* **2002**, *133*, 195–197.
14. Sakata, Y.; Ishii, Y. *J. Org. Chem.* **1991**, *56*, 6233–6235.
15. Curci, R.; D'Accolti, L.; Detomaso, A.; Fusco, C.; Takeuchi, K.; Ohga, Y.; Eaton, P. E.; Yip, Y. C. *Tetrahedron Lett.* **1993**, *34*, 4559–4562.
16. Bovicelli, P.; Lupattelli, P.; Sanetti, A.; Minicione, E. *Tetrahedron Lett.* **1994**, *35*, 8477–8480.
17. Bovicelli, P.; Lupattelli, P.; Sanetti, A. *Tetrahedron Lett.* **1995**, *36*, 3031–3034.
18. Giffhorn, F.; Koepper, S.; Huwig, A.; Freimund, S. *Enzymol. Micro. Tech.* **2000**, *27*, 734–742.
19. Freimund, S.; Huwig, A.; Giffhorn, F.; Koepper, S. *Chem. Eur. J.* **1998**, *4*, 2442–2455.
20. Liu, H.-M.; Sato, Y.; Tsuda, Y. *Chem. Pharm. Bull.* **1993**, *41*, 491–501.
21. Tsuda, Y.; Hanajima, M.; Mastuhira, N.; Okuno, Y.; Kanemitsu, K. *Chem. Pharm. Bull.* **1989**, *39*, 2344–2350.
22. See Supplementary Material.
23. Atkins, P.; de Paula, J. *Physical Chemistry*; Freeman: New York, 2002.
24. Sakaguchi, S.; Kikuchi, D.; Ishii, Y. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 2561–2566.
25. Kikuchi, D.; Sakaguchi, S.; Ishii, Y. *J. Org. Chem.* **1998**, *63*, 6023–6026.
26. Hayat, S.; Atta-ur-Rahman; Choudhary, I. M.; Khan, K. M.; Bayer, E. *Tetrahedron Lett.* **2001**, *42*, 1647–1649.
27. Field, R. J.; Koros, E.; Noyes, R. M. *J. Am. Chem. Soc.* **1972**, *94*, 8649–8664.
28. Noyes, R. M. *J. Phys. Chem.* **1990**, *94*, 4404–4412.
29. Metsger, L.; Bittner, S. *Tetrahedron Lett.* **2000**, *56*, 1905–1910.
30. All possible reagent combinations of *trans*-1,2-cyclohexanediol, $NaBrO_3$, $NaHSO_3$, Na_2SO_3 and H_2SO_4 were permutated yielding quantitative α-hydroxy cyclohexanone within 24 h. The 1,2-cyclohexanedione content was less than 5%.
31. The solutions were allowed to cool to room temperature.
32. Derivation of these compounds, e.g. by exhaustive silylation would be required to make them analyzable by GC. Given that the reaction mixtures are aqueous and that the addition of silylation agents such as Me_3Si-imidazolium chloride might influence the reaction equilibria, this is not practical.
33. Derbyshire, D. H.; Waters, W. A. *Nature* **1949**, *164*, 446–447.
34. Takase, K.; Masuda, H.; Kai, O.; Nishiyama, Y.; Sakaguchi, S.; Ishii, Y. *Chem. Lett.* **1995**, 871–872.
35. Toth, Z.; Fabian, I. *Inorg. Chem.* **2004**, *43*, 2717–2723.
36. Toth, Z.; Fabian, I. *Inorg. Chem.* **2000**, *39*, 4608–4614.
37. Beckwith, R. C.; Wang, T. X.; Margerum, D. W. *Inorg. Chem.* **1996**, *35*, 995–1000.
38. Greenwood, N. N.; Earnshaw, A., 2nd ed. In *Chemistry of the Elements*; Butterworth Heinemann: Oxford, 1997.
39. (a) Lissel, M.; Neumann, B.; Schmidt, S. *Liebigs Ann. Chem.* **1986**, 263–264. (b) Becker, H. *Organicum*; Addison-Wesley: Reading, MA, 1973; pp 521–524.

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Novel photo-induced oxidative cyclization of 1,3-dimethyl-5-(1-arylmethylidene)pyrimidine-2,4,6(1,3,5*H*)-triones: synthesis and properties of areno[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dionylium ions and their photo-induced autorecycling oxidizing reaction toward some amines

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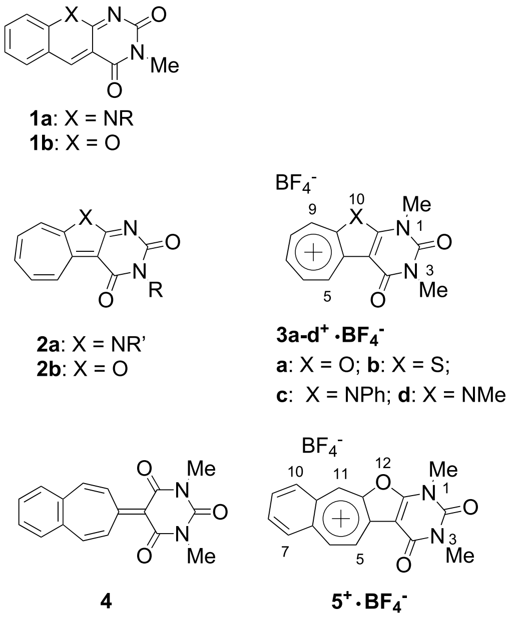
Abstract—Novel photo-induced oxidative cyclization was accomplished to synthesize areno[*b*]pyrimido[5,4-*e*]pyran-2,4(1,3*H*)-dionylium ions **13a–c**$^{+}\cdot$**ClO$_4^-$**. Furthermore, **13a–c**$^{+}\cdot$**BF$_4^-$** and their phenyl-substituted derivatives **19a**,**b**$^{+}\cdot$**BF$_4^-$** were alternatively synthesized by the reaction of salicylaldehyde and its naphthyl derivatives with barbituric acids and subsequent treatment with aq. HBF_4. Structural characteristics of **13a–c**$^{+}$ and **19a**,**b**$^{+}$ were clarified on inspection of the UV–vis and NMR spectral data as well as X-ray crystal analyses. The electrochemical properties were studied by the CV measurement. In a search for reactivity, reactions of **13a–c**$^{+}\cdot$**BF$_4^-$** with some nucleophiles, hydride, benzylamine, and H_2O, were also carried out. The photo-induced autorecycling oxidation reactions of **13a–c**$^{+}\cdot$**BF$_4^-$** toward some amines under aerobic conditions were carried out to give the corresponding imines (isolated by converting to the corresponding 2,4-dinitrophenylhydrazones) in 643–3600% yield (recycling number of **13a–c**$^{+}\cdot$**BF$_4^-$**: 6.4–36.0).

1. Introduction

Flavins and flavinium ions are known to play an important role as cofactors in a wide variety of biological redox reactions.[1,2] The flavin–redox systems have been investigated extensively through synthetic model systems and theoretical calculations.[3] Among these, 5-deazaflavin **1a** (Fig. 1) has been studied extensively in both enzymatic[4] and model systems,[5] in the hope of gaining mechanistic insight into flavin-catalyzed reactions. In this relation, 5-deaza-10-oxaflavin **1b** (2*H*-chromeno[2,3-*d*]pyrimidine-2,4(3*H*)-dione), in which the nitrogen atom is replaced by an oxygen, has also been synthesized and found to possess a strong function to oxidize alcohols to the corresponding carbonyl compounds.[6] On the basis of the above observations, we have previously studied the preparation of 3,10-disubstituted cyclohepta[4,5]pyrro[2,3-*d*]pyrimidin-2,4(3*H*)-diones (**2a**)[7] and 3-substituted cyclohepta[4,5]-furo[2,3-*d*]pyrimidin-2,4(3*H*)-diones (**2b**),[8,9] which are



1a: X = NR
1b: X = O

2a: X = NR’
2b: X = O

3a-d$^{+}\cdot$**BF$_4^-$**
a: X = O; **b**: X = S;
c: X = NPh; **d**: X = NMe

4

5$^{+}\cdot$**BF$_4^-$**

Figure 1.

Keywords: Benzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dionylium tetrafluoroborate; Photo-induced oxidative cyclization; X-ray analysis; Photo-induced autorecycling oxidation.

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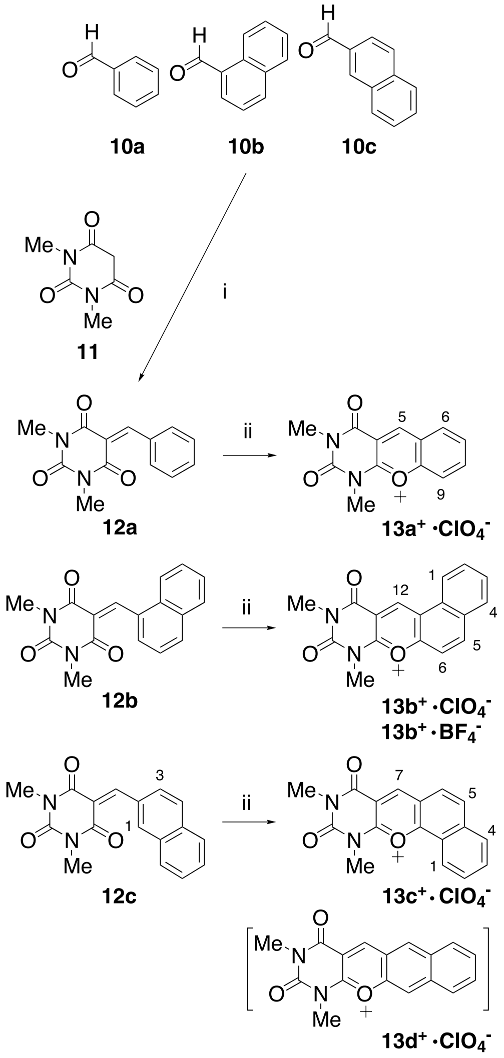


Scheme 1. Reagents and conditions: (i) H_2SO_4, EtOH, reflux, 1 h; (ii) *hν*, Ac_2O, 60% aq. $HClO_4$ or 42% aq. HBF_4, aerobic, rt.

structural isomers of **1a** and **1b**, respectively. In this context, 1,3-disubstituted cyclohepta[4,5]furo[2,3-*d*]pyrimidin-2,4(1,3*H*)-dionylium tetrafluoroborate ($\mathbf{3a^+\cdot BF_4^-}$)[10,11] and its sulfur and nitrogen analogues $\mathbf{3b^+\cdot BF_4^-}$ and $\mathbf{3c,d^+\cdot BF_4^-}$ have been synthesized,[12,13] and photo-induced autorecycling oxidizing reaction of $\mathbf{3a\text{–}d^+\cdot BF_4^-}$ toward some alcohols and amines was studied as well.[11–13] In addition, oxidative cyclization of heptafulvene (**4**) by using DDQ/Sc(OTf)$_3$ or photo-irradiation in acidic media to give 1,3-dimethylbenzocyclohepta[7′,6′:4,5]furo[2,3-*d*]pyrimidin-2,4(1,3*H*)-dionylium tetrafluoroborate ($\mathbf{5^+\cdot BF_4^-}$), which has also exhibited oxidizing ability toward some alcohols,[14] has been studied.

On the other hand, the photosensitizing ability of 2,4,6-triarylpyrylium ions has been widely applied in photo-induced electron transfer processes. Among these salts, the 2,4,6-tripheny-pyrylium ion is a strong oxidant in both the singlet and triplet excited states to oxidize several organic donors to the corresponding radical cations with concomitant formation of a triphenylpyranyl radical.[15] Since the 2,4,6-tripheny-pyrylium ion was regenerated through the reaction of the triphenylpyranyl radical with O_2, photooxidation processes can be usually carried out in air using catalytic amounts of the triphenylpyrylium ion. Thus, pyrylium ions are good oxidants in the photo-excited state and can act as efficient photosensitizers. Recently, a steady-state and laser flash photolysis studies of the pyrylium ion-photosensitized oxidation of benzyl alcohol have been reported.[16] Thus, the uracil-annulated pyrylium ions $\mathbf{13a\text{–}c^+\cdot BF_4^-}$ (Scheme 1) are interesting from the viewpoint of functions of both the pyrylium ions and the flavinium ions. From this viewpoint, we studied a novel photo-induced oxidative cyclization of 1,3-dimethyl-5-(1-arylmethylidene)pyrimidine-2,4,6(1,3,5*H*)-triones (**12a–c**), which derived from the reaction of barbituric acid with easily available aromatic aldehydes, to explore the methodology for synthesizing the benzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dionylium ion ($\mathbf{13a^+\cdot BF_4^-}$) and related compounds $\mathbf{13b,c^+\cdot BF_4^-}$ (Scheme 1). Furthermore, alternative synthesis of $\mathbf{13a\text{–}c^+\cdot BF_4^-}$ was also accomplished by the reaction of salicylaldehyde **14a** and its derivatives **14b**,**c** with dimethylbarbituric acid **11** and subsequent treatment with aq. HBF_4. The structural details and chemical properties as well as the photo-induced oxidizing reaction of $\mathbf{13a\text{–}c^+\cdot BF_4^-}$ toward some amines were investigated as well. We report herein the results in detail.

2. Results and discussion

2.1. Photo-induced oxidative cyclization

Since compound **12a** has been synthesized by the reaction of benzaldehyde (**10a**) with dimethylbarbituric acid (**11**),[17] the method was applied to prepare naphthyl derivatives **12b**,**c** (Scheme 1). The acid-catalyzed reactions of **10b**,**c** with **11** in EtOH were carried out to give **12b**,**c** in 94 and 95% yields, respectively. Compounds **12b**,**c** were fully characterized on the basis of the NMR, IR, UV–vis, mass spectral data, and elemental analyses. Unlike the case of **4**,[14] a thermal oxidative cyclization of compound **12a**,**b** by using DDQ and Sc(OTf)$_3$ in xylene did not afford $\mathbf{13a,b^+}$, and the reactions were complicated. We have reported that the photo-induced oxidative cyclization of **4** afforded $\mathbf{5^+\cdot BF_4^-}$ in better yield (100%) as compared with the thermal oxidative cyclization by using DDQ and Sc(OTf)$_3$ (20%).[14] Thus, photo-irradiation of **12a–c** was carried out under aerobic and acidic conditions. The results are summarized in Table 1 (Runs 1–4). While a trace amount of $\mathbf{13a^+\cdot ClO_4^-}$ was obtained in the photo-reaction of **12a** (Run 1), a solution of **12b** in Ac_2O in the presence of 42% aq. HBF_4 afforded $\mathbf{13b^+\cdot BF_4^-}$ in 47% yield (Run 2). A similar reaction by using 60% aq. $HClO_4$ as additive proceeded smoothly to give $\mathbf{13b^+\cdot ClO_4^-}$ in 90% yield (Run 3). The cyclization of **12c** proceeded at the C1 to give cation $\mathbf{13c^+\cdot ClO_4^-}$ as a single product in good yield (Run 4). Alternative formation of cation $\mathbf{13d^+\cdot ClO_4^-}$, which is expected from the cyclization at the C3 of **12c**, was not obtained. Compounds $\mathbf{13a\text{–}c^+\cdot ClO_4^-}$ and $\mathbf{13b^+\cdot BF_4^-}$ (vide infra) were fully characterized on the basis of the spectral data and mass spectral data as well as elemental analyses. During recrystallization, hydrolysis of $\mathbf{13c^+\cdot ClO_4^-}$ with stray water in the solvent proceeded gradually due to its lability. Thus, satisfactory elemental

Table 1. Results for the photo-induced oxidative cyclization of **12a–c**[a]

Run	12	Additive	Solvent	Time/h	Product	Yield/%
1	**12a**	$HClO_4$	Ac_2O	7	$\mathbf{13a^+ \cdot ClO_4^-}$	Trace
2	**12b**	HBF_4	Ac_2O	96	$\mathbf{13b^+ \cdot BF_4^-}$	47
3	**12b**	$HClO_4$	Ac_2O	2	$\mathbf{13b^+ \cdot ClO_4^-}$	90
4	**12c**	$HClO_4$	Ac_2O	2	$\mathbf{13c^+ \cdot ClO_4^-}$	99

[a] Reaction was carried out under photo-irradiation (RPR-100, 350 nm lamps).

Table 2. Redox potentials of **12a–c**, $\mathbf{13a\text{–}c^+ \cdot BF_4^-}$, and $\mathbf{19a,b^+ \cdot BF_4^-}$ and reference compounds **1b** and **4**

Compd.	Redox potential[a]		Compd.	Redox potential[a]	
	$E1_{red}$	$E1_{ox}$		$E1_{red}$	$E1_{ox}$
12a	−1.36	+1.91	$\mathbf{13a^+ \cdot BF_4^-}$	−0.49	—
12b	−1.29	+1.37	$\mathbf{13b^+ \cdot BF_4^-}$	−0.46	—
12c	−1.29	+1.37	$\mathbf{13c^+ \cdot BF_4^-}$	−0.44	—
4[b]	−1.11	+1.20	$\mathbf{19a^+ \cdot BF_4^-}$	−0.47	—
1b[c]	−1.11	—	$\mathbf{19b^+ \cdot BF_4^-}$	−0.47	—

[a] V vs $Ag/AgNO_3$; cathodic and anodic peak potentials.
[b] Ref. 14.
[c] This work.

analysis of $\mathbf{13c^+ \cdot ClO_4^-}$ was not obtained; however, satisfactory ^1H and ^{13}C NMR and HRMS data were obtained. In a search for details of the oxidative cyclization, the characteristics of **12a–c** were studied by an inspection of their redox potentials and UV–vis spectra. The redox property of **12a–c** was determined by cyclic voltammetry (CV) in CH_3CN. The oxidation and reduction waves of **12a–c** were irreversible under the conditions of the CV measurements, and thus, the peak potentials are summarized in Table 2, together with those of the reference compound **4**.[14] The first reduction potentials ($E1_{red}$) of **12a–c** are more negative than that of **4**,[14] and the order is **12a** > **12b** = **12c**, and the first oxidation potentials ($E1_{ox}$) are more positive in the order **12a** ≫ **12b** = **12c** > **4**.[14] Thus, the feature suggests the difficulty of oxidative cyclization of **12a**,**b** by using DDQ and $Sc(OTf)_3$ (vide supra). On the other hand, the UV–vis spectra of **12a–c** are shown in Figure 2, and the longest wavelength absorption maxima (λ_{max}) of **12b**,**c** (382 and 346 nm) appear at longer wavelength than that of **12a** (323 nm). The visible region of the spectra of **12a–c** was not changed by adding 60% aq. $HClO_4$. Thus, protonation of **12a–c** does not seem to occur in their ground state, and the postulated mechanistic pathway for the present photo-induced oxidative cyclization is depicted in Scheme 2. The photo-induced 6π-cyclization of **12a–c** gives intermediate **14a–c**, which would undergo 1,3-hydrogen shift in acidic media and oxidation under aerobic conditions to give $\mathbf{13a\text{–}c^+ \cdot ClO_4^-}$. Concerning the selectivity of cyclization of **12c**, the cyclization at the C1 giving **14c**, which retains a benzene ring and affords **13c**, would be preferable as compared with the cyclization at the 3-position giving **14d**, which has a quinoid structure. Although the $E1_{ox}$ of **12a** is more positive and photo-induced oxidative cyclization affords a trace of $\mathbf{13a^+ \cdot ClO_4^-}$, the feature would be attributable to an intrinsic inertness of the benzene ring.

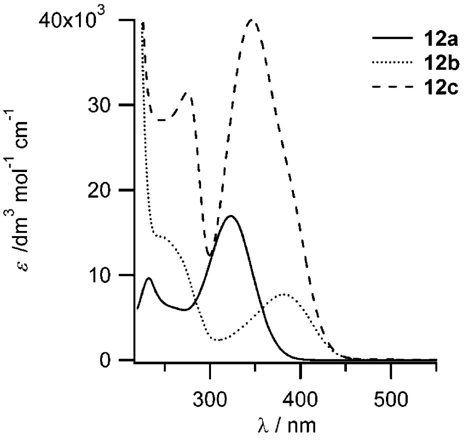
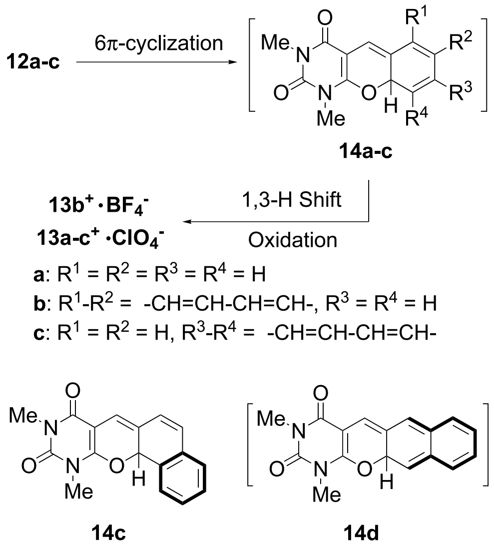


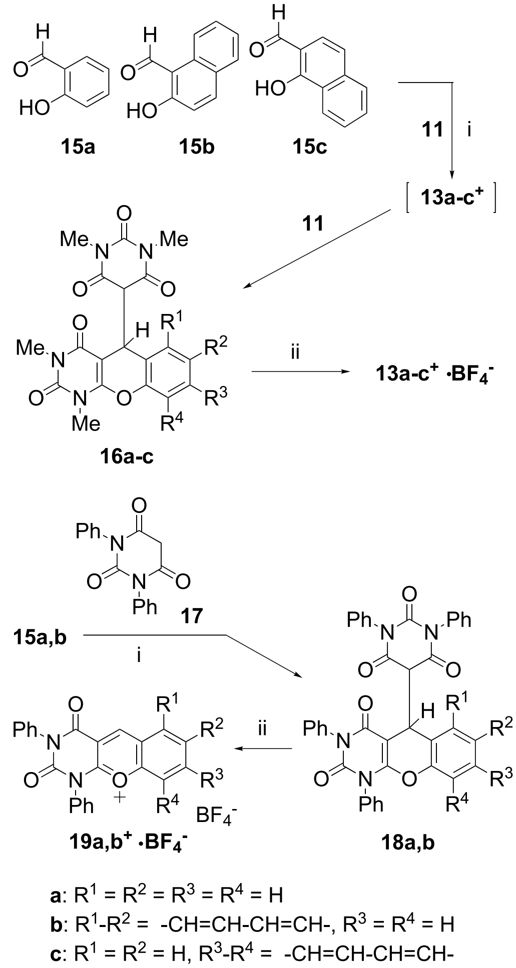
Figure 2. UV–vis spectra of **12a–c** in CH_3CN.



Scheme 2.

2.2. Alternative synthetic method

Although the reaction of salicylaldehyde (**15a**) with **11** has been shown to give **16a** in modest yield (Scheme 3),[18] a modified reaction of **15a–c** with 2 M equiv amounts of **11** afforded **16a–c** in good yields as summarized in Table 3 (Runs 1–3). Upon treatment with 42% aq. HBF_4, **16a–c**



Scheme 3. Reagents and conditions: (i) EtOH, reflux, 2 h; (ii) 42% aq. HBF$_4$, Ac$_2$O, 0 °C, 1 h.

underwent elimination reaction of barbituric acid to give **13a–c**$^+\cdot\mathbf{BF_4^-}$ in good yields. In order to obtain a single crystal for X-ray analysis, similar reactions of **15a**,**b** with diphenylbarbituric acid (**17**) were also carried out to give **18a**,**b**, respectively, which were converted to **19a**,**b**$^+\cdot\mathbf{BF_4^-}$ by treatment with 42% aq. HBF$_4$ (Table 3, Runs 4 and 5). Compound **16a** was identified on the basis of a comparison of the physical data with those reported in the literature.[18] New compounds **16b**,**c** and **18a**,**b** were fully characterized on the basis of the ^{1}H and ^{13}C NMR, IR, and mass spectral data as well as elemental analyses.

2.3. Properties of pyrylium ions 13a–c$^+\cdot\mathbf{BF_4^-}$ and 19a,b$^+\cdot\mathbf{BF_4^-}$

Compounds **13a–c**$^+\cdot\mathbf{BF_4^-}$ and **19a**,**b**$^+\cdot\mathbf{BF_4^-}$ were fully characterized on the basis of the ^{1}H and ^{13}C NMR, IR, UV–vis, and mass spectral data, as well as elemental analyses. Similarly to compound **13c**$^+\cdot\mathbf{ClO_4^-}$, satisfactory elemental analysis of **13c**$^+\cdot\mathbf{BF_4^-}$ was not obtained; however, satisfactory ^{1}H and ^{13}C NMR and HRMS data were obtained. In contrast, **13b**$^+\cdot\mathbf{BF_4^-}$ is relatively stable as compared with **13c**$^+\cdot\mathbf{BF_4^-}$. Mass spectra of **13a–c**$^+\cdot\mathbf{BF_4^-}$ and **19a**,**b**$^+\cdot\mathbf{BF_4^-}$ exhibited the correct M$^+-$BF$_4^-$ ion peak, which is indicative of the cationic structure of the compounds. The characteristic absorption band for the counter ion of BF$_4^-$ was observed at 1084 cm^{-1} in the IR spectra of **13a–c**$^+\cdot\mathbf{BF_4^-}$ and **19a**,**b**$^+\cdot\mathbf{BF_4^-}$. The UV–vis spectra of **13a–c**$^+\cdot\mathbf{BF_4^-}$ in CH$_3$CN are shown in Figure 3. The longest wavelength absorption maximum ($\lambda_{\max}$) of **13b**$^+\cdot\mathbf{BF_4^-}$ shows a red-shift by 70 nm as compared with that of **13a**$^+\cdot\mathbf{BF_4^-}$, suggesting the elongated π-conjugation of **13b**$^+\cdot\mathbf{BF_4^-}$. The spectrum of **13c**$^+\cdot\mathbf{BF_4^-}$ is similar to both **13a**$^+\cdot\mathbf{BF_4^-}$ and **13b**$^+\cdot\mathbf{BF_4^-}$, suggesting both contributions of the elongated π-conjugation similar to **13b**$^+\cdot\mathbf{BF_4^-}$ and the structural feature similar to **13a**$^+\cdot\mathbf{BF_4^-}$.

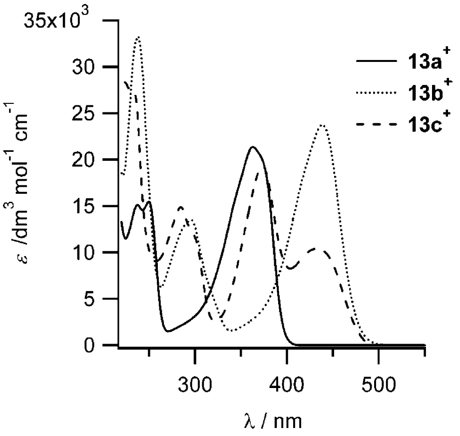


Figure 3. UV–vis spectra of **13a–c**$^+$ in CH$_3$CN.

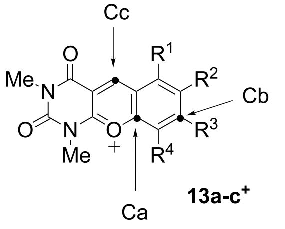
The ^{13}C NMR spectral data of **13a–c**$^+\cdot\mathbf{BF_4^-}$ were fully assigned by using the H–C Cosy spectra (HMQC and HMBC) as summarized in Table 4. Delocalization of the positive charge is affected by benzo-annulation. Concerning the benzene-ring of **13a**$^+\cdot\mathbf{BF_4^-}$ and the naphthalene-ring of **13b**,**c**$^+\cdot\mathbf{BF_4^-}$, the chemical shift of the labeled carbons (Figure 4. Ca and Cb) appears at lower field (**13a**$^+$: δ_C 154.7, 142.3; **13b**$^+$: δ_C 157.0, 145.7; **13c**$^+$: δ_C 154.6, 139.8) as compared with those of other carbons (**13a**$^+$: δ_C 119.4–134.1; **13b**$^+$: δ_C 117.4–132.8; **13c**$^+$: δ_C 119.8–135.2), suggesting that the positive charge is localized partially at these positions. The chemical shift of the Cc carbon of **13b**$^+\cdot\mathbf{BF_4^-}$ (Fig. 4) appears at higher field than those

Table 3. Results for the preparation of **17a–c** and **19b** and cation **13a–c**$^+\cdot\mathbf{BF_4^-}$ and **19a**,**b**$^+\cdot\mathbf{BF_4^-}$

Run	Compd.	Barbituric acid	Condensation		Cation	
			Product	Yield (%)	Product	Yield (%)
1	**15a**	**11**	**16a**	96	**13a**$^+\cdot\mathbf{BF_4^-}$	95
2	**15b**	**11**	**16b**	100	**13b**$^+\cdot\mathbf{BF_4^-}$	94
3	**15c**	**11**	**16c**	97	**13c**$^+\cdot\mathbf{BF_4^-}$	93
4	**15a**	**17**	**18a**	85	**19a**$^+\cdot\mathbf{BF_4^-}$	88
5	**15b**	**17**	**18b**	96	**19b**$^+\cdot\mathbf{BF_4^-}$	85

Table 4. ^{13}C NMR chemical shifts (δ) of **13a–c**$^{+}\cdot$**BF**$_4^-$

Compd.	Ca	Cb	Benzene and naphthalene ring	Cc	Remaining carbons
13a^{+}	154.7 (C9a)	142.3 (C8)	119.4 (C9), 121.5 (C5a), 131.1 (C7), 134.1 (C6)	156.8 (C5)	30.0 (N3Me), 32.0 (N1Me), 111.7 (C4a), 148.9 (C2), 157.7 (C4), 163.8 (C10a)
13b^{+}	157.0 (C6a)	145.7 (C5)	117.4 (C6), 119.2 (C12a), 125.0 (C1), 129.8 (C12b), 130.9 (C3), 131.3 (C4), 132.6 (C2), 132.8 (C4a)	151.2 (C12)	30.0 (N10Me), 31.7 (N8Me), 110.3 (C11a), 149.1 (C9), 157.9 (C11), 162.7 (C7a)
13c^{+}	154.6 (C11a)	139.8 (C4a)	119.8 (C6a), 122.6 (C12b), 124.8 (C1), 125.9 (C5), 130.3 (C4), 130.9 (C2), 131.5 (C6), 135.2 (C3)	155.9 (C7)	30.0 (N9Me), 31.9 (N11Me), 111.2 (C7a), 149.0 (C10), 157.8 (C8), 162.8 (C11a)



13a-c^{+}

a: R^1 = R^2 = R^3 = R^4 = H
b: R^1-R^2 = -CH=CH-CH=CH-, R^3 = R^4 = H
c: R^1 = R^2 = H, R^3-R^4 = -CH=CH-CH=CH-

Figure 4.

of **13a**,**c**$^{+}\cdot$**BF**$_4^-$, suggesting that the positive charge of **13b**$^{+}\cdot$**BF**$_4^-$ is delocalized more effectively as compared with those of **13a**,**c**$^{+}\cdot$**BF**$_4^-$. The feature supports the relatively larger stability of **13b**$^{+}\cdot$**BF**$_4^-$.

Single crystals of **19a**,**b**$^{+}\cdot$**BF**$_4^-$ were obtained by recrystallization from CH$_3$CN–AcOEt. Thus, in order to clarify the structural details, X-ray crystal analyses of **19a**,**b**$^{+}\cdot$**BF**$_4^-$ were performed and the ORTEP drawings are shown in Figure 5. The π-system of **19a**$^{+}\cdot$**BF**$_4^-$ has a nearly planar structure, and the planes of the N1- and N2-phenyl groups are twisted 65.6° and 69.1°, respectively, against the plane of the π-system. For compound **19b**$^{+}\cdot$**BF**$_4^-$, a small deformation of the π-system from planarity is observed, and the planes of N1 and N2-phenyl groups are twisted 73.7° and 72.4°, respectively, against the plane of the π-system. In both compounds **19a**,**b**$^{+}\cdot$**BF**$_4^-$, the bond length of O1–C11 is shorter than that of O1–C10, suggesting that the former bond has a larger bond order. In addition, the bond length of C9–C10 is shorter than those of C5–C6 and C7–C8. Furthermore, for compound **19a**$^{+}\cdot$**BF**$_4^-$, the bond length of C6–C7 is shorter than those of C8–C9. These facts suggest the existence of bond alternation as shown in the canonical structures of **19a**,**b**$^{+}$-**A**, **19a**,**b**$^{+}$-**B**, and **19a**,**b**$^{+}$-**C** (Fig. 6). Furthermore, contribution of the canonical structure **19a**,**b**$^{+}$-**D** seems to be less important.

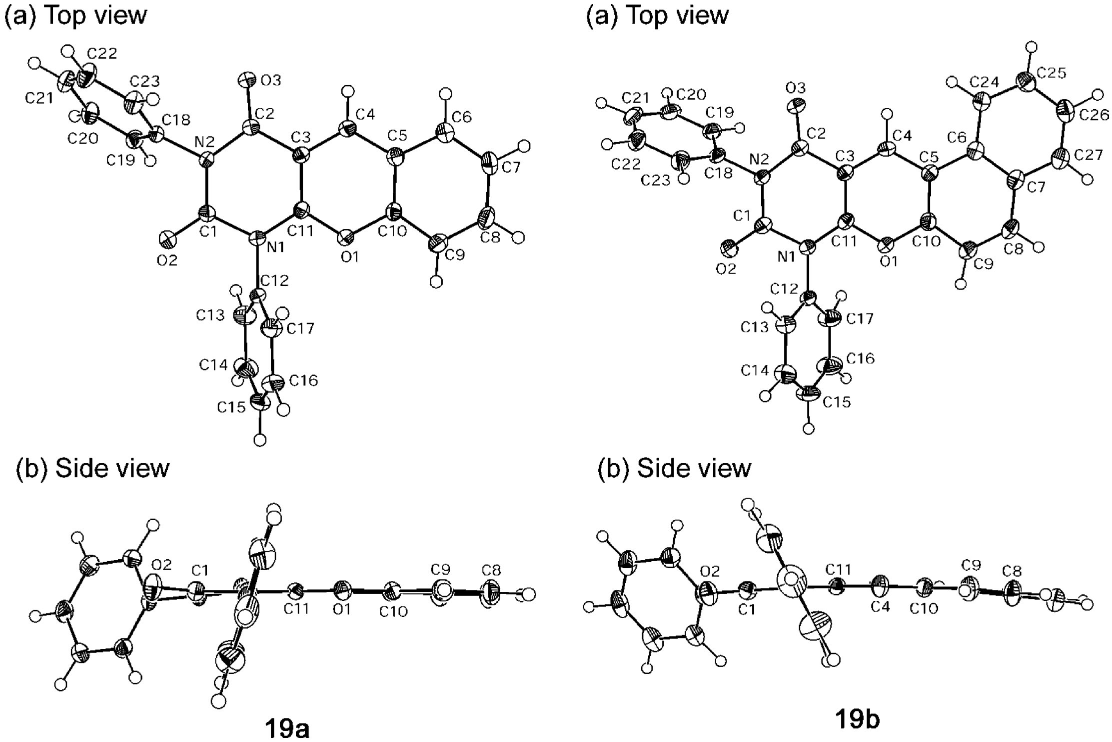


Figure 5. ORTEP drawing of **19a**,**b**$^{+}\cdot$**BF**$_4^-$ with thermal ellipsoid plot (50% probability). Selected bond lengths (Å) of **19a**$^{+}\cdot$**BF**$_4^-$; N1–C11 1.336(2), O1–C10 1.388(2), O1–C11 1.316(2), C3–C11 1.404(2), C3–C4 1.360(2), C4–C5 1.416(2), C5–C6 1.409(2), C5–C10 1.403(2), C6–C7 1.377(2), C7–C8 1.402(2), C8–C9 1.386(2), C9–C10 1.382 (2). Selected bond lengths (Å) of **19b**$^{+}\cdot$**BF**$_4^-$; N1–C11 1.336(2), O1–C10 1.383(2), O1–C11 1.325(2), C3–C11 1.395(3), C3–C4 1.363(2), C4–C5 1.409(2), C5–C6 1.448(2), C5–C10 1.388(3), C6–C7 1.418(3), C7–C8 1.418(3), C8–C9 1.355(3), C9–C10 1.397(3), C6–C24 1.408(3), C24–C25 1.372(3), C25–C26 1.403(3), C26–C27 1.363(3), C7–C27 1.413(3).

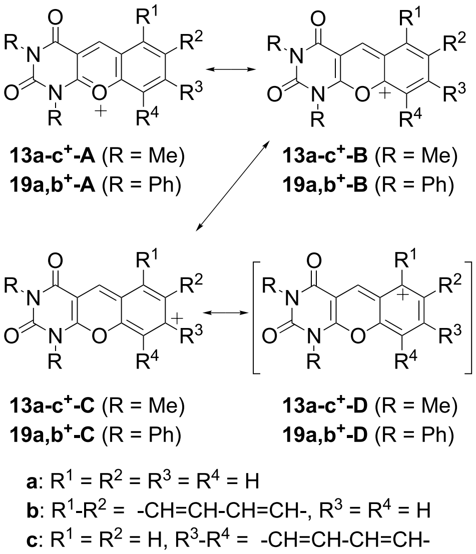
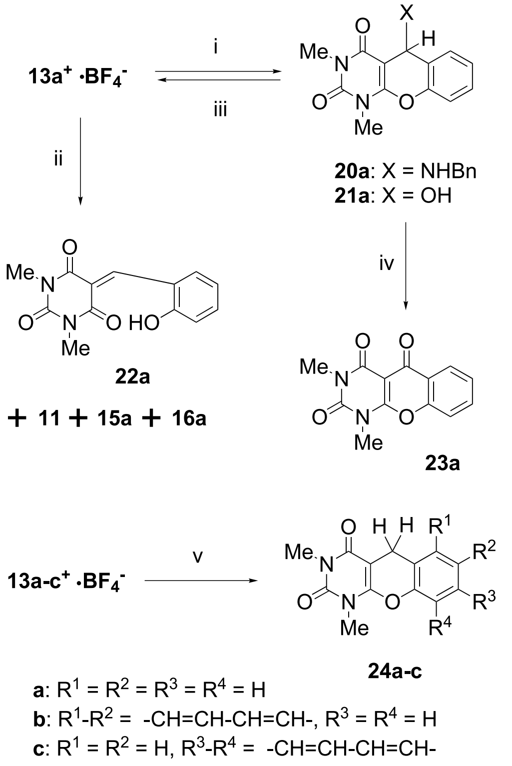


Figure 6.



Scheme 4. Reagents and conditions: (i) benzylamine or aq. NaHCO$_3$, CH$_3$CN, rt, 1 h; (ii) 3% HCl, CH$_3$CN, rt, 1 min; (iii) 42% aq. HBF$_4$, Ac$_2$O, 0 °C, 1 h; (iv) DDQ, CH$_2$Cl$_2$, rt, 1 h; (v) NaBH$_4$, CH$_3$CN, rt, 1 h.

Although the pK_{R+} values of **13a–c**$^+$ and **19a**,**b**$^+$ could not be determined due to irreversible ring-opening reaction (vide infra), the reduction potential of **13a–c**$^+$ and **19a**,**b**$^+$ was determined by cyclic voltammentry (CV) in CH$_3$CN. The reduction wave was irreversible under the conditions of the CV measurements; the peak potential is summarized in Table 2, together with that of the reference compound **1b**. The values of **13a–c**$^+$ and **19a**,**b**$^+$ are less negative than that of **1b**, suggesting that **13a–c**$^+$ and **19a**,**b**$^+$ have lower LUMO, and thus, they are expected to have strong oxidizing ability. Furthermore, the first reduction potentials ($E1_{\mathrm{red}}$) are less negative in the order **13a**$^+$ > **13b**$^+$ > **13c**$^+$. The feature is rationalized on the basis of the elongated conjugation of **13b**,**c**$^+$. We have recently clarified that perturbations derived from benzo-annulation onto the cyclohepta[*a*]azulenylium ion are highly dependent on the annulating positions.[19] While the benzo-annulation on the double bond having a large bond order of the parent cation causes smaller perturbation, the benzo-annulation on the double bond having a small bond order significantly perturbs both the original π-system and the annulating benzene ring. Regarding cation **19a**$^+$, the X-ray crystal analysis revealed that the bond length of the C8–C9 (1.386 Å) is longer than that of the C6–C7 (1.377 Å) (vide supra), suggesting that the former bond has a smaller bond order. Thus, the benzo-annulation onto **13a**$^+$ at the C8–C9 giving **13c**$^+$ would cause a larger perturbation than the benzo-annulation onto **13a**$^+$ at the C6–C7 giving **13b**$^+$. Thus, the cation **13c**$^+$ would exhibit a less negative $E1_{\mathrm{red}}$ as compared with that of **13b**$^+$.

2.4. Reactivity

In order to clarify the reactivity of the pyrylium cation, several reactions of **13a–c**$^+\cdot$**BF**$_4^-$ were carried out. The reaction of **13a**$^+\cdot$**BF**$_4^-$ with benzylamine proceeded at the C5 to afford a single isomer **20a** in 97% yield (Scheme 4). Similarly, a reaction of **13a**$^+\cdot$**BF**$_4^-$ with H$_2$O under basic conditions (aq. NaHCO$_3$) proceeded at the C5 to give **21a** in 92% yield. In contrast, a reaction of **13a**$^+\cdot$**BF**$_4^-$ with H$_2$O under acidic conditions (0.6% HCl) afforded a mixture of **11**, **15a**, **16a**, and **22a**. The retro-aldol reaction of **22a** afforded compounds **15a** and **11**, which would react with remaining **13a**$^+\cdot$**BF**$_4^-$ to afford **16a**. Thus, under acidic conditions, the addition reaction of **13a**$^+\cdot$**BF**$_4^-$ with H$_2$O afforded C9a-adduct, which underwent a ring-opening reaction to give **22a**. Since the ring-opening reaction was irreversible, the pK_{R+} values of **13a–c**$^+\cdot$**BF**$_4^-$ could not be determined (vide supra). Upon treatment with 42% aq. HBF$_4$ in Ac$_2$O, compounds **20a** and **21a** regenerated **13a**$^+\cdot$**BF**$_4^-$ in quantitative yields, respectively. Furthermore, an oxidizing reaction of **21a** by using DDQ afforded **23a** in 86% yield. On the other hand, the reaction of **13a–c**$^+\cdot$**BF**$_4^-$ with NaBH$_4$ afforded single isomers **20a–23a** and **24a–c** in good yields (Scheme 4). Compounds **24a–c** were fully characterized on the basis of the ^{1}H and ^{13}C NMR, IR, and mass spectral data, as well as elemental analyses. Furthermore, the DDQ-oxidation reaction of **24a** in CH$_2$Cl$_2$ afforded **23a** in quantitative yield: the intermediate **13a**$^+$, generated by the oxidative hydrogen abstraction of **24a** with DDQ, reacts with contaminated H$_2$O to give **21a**, hydrogen abstraction of which with another DDQ gives **23a**.

2.5. Autorecycling oxidation

The photo-induced oxidizing reaction of benzyl alcohol by using pyrylium salts has been reported.[15] Furthermore, we have previously reported that compounds **3a–d**$^+\cdot$**BF**$_4^-$ undergo autorecycling oxidation of some alcohols to give the corresponding carbonyl compounds under photo-irradiation.[11–13] In addition, compounds **2a** and **3a–d**$^+\cdot$**BF**$_4^-$ have also been reported to undergo

Table 5. Autorecycling oxidation of some amines by $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b** under photo-irradiation[a]

Run	Compd.	Amine	Yield[b]/%	Recycling no.[c]	Run	Compd.	Amine	Yield[b]/%	Recycling no.[c]
1	$\mathbf{13a^+ \cdot BF_4^-}$	$PhCH_2NH_2$	909	9.1	9	$\mathbf{13c^+ \cdot BF_4^-}$	$PhCH_2NH_2$	1189	11.9
2	$\mathbf{13a^+ \cdot BF_4^-}$	$PhCH(Me)NH_2$	1133	11.3	10	$\mathbf{13c^+ \cdot BF_4^-}$	$PhCH(Me)NH_2$	2067	20.7
3	$\mathbf{13a^+ \cdot BF_4^-}$	Hexylamine	643	6.4	11	$\mathbf{13c^+ \cdot BF_4^-}$	Hexylamine	3357	33.6
4	$\mathbf{13a^+ \cdot BF_4^-}$	Cyclohexylamine	0[d]	0	12	$\mathbf{13c^+ \cdot BF_4^-}$	Cyclohexylamine	647	6.5
5	$\mathbf{13b^+ \cdot BF_4^-}$	$PhCH_2NH_2$	2098	21.0	13	**1b**	$PhCH_2NH_2$	699	7.0
6	$\mathbf{13b^+ \cdot BF_4^-}$	$PhCH(Me)NH_2$	3600	36.0	14	**1b**	$PhCH(Me)NH_2$	67	0.7
7	$\mathbf{13b^+ \cdot BF_4^-}$	Hexylamine	2857	28.6	15	**1b**	Hexylamine	929	9.3
8	$\mathbf{13b^+ \cdot BF_4^-}$	Cyclohexylamine	791	7.9	16	**1b**	Cyclohexylamine	72	0.7

[a] CH_3CN solution was irradiated by RPR-100, 350 nm lamps under aerobic conditions.

[b] Based on $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b** used; the yield is calculated by subtraction of the 'blank' yield from the total yield of carbonyl compound in the presence of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b**.

[c] Isolated by converting to the corresponding 2,4-dinitrophenylhydrazone.

[d] The 'blank' yield was higher than the yield in the presence of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b**.

autorecycling oxidation toward some amines under photo-irradiation.[9,20] In this context, we examined the oxidation of some amines by using $\mathbf{13a{-}c^+ \cdot BF_4^-}$ as well as reference compound **1b** under aerobic and photo-irradiation conditions (RPR-100, 350 nm lamps). We found that compounds $\mathbf{13a{-}c^+ \cdot BF_4^-}$ have an oxidizing ability toward benzylamine, 1-phenylethylamine, hexylamine, and cyclohexylamine to give the corresponding imines. Imine $R^1R^2C{=}NH$ is produced at first; however, it reacts with another amine to result in the formation of $R^1R^2C{=}N{-}CHR^1R^2$. The results are summarized in Table 5. Direct irradiation of the amines in the absence of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b** (named 'blank') gives the imines in low to modest yields. Thus, the yields of imines are calculated by subtraction of the 'blank' yield from the yields obtained in the presence of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b**. More than 100% yields are obtained (based on compounds $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and **1b**) (Table 5), and thus, autorecycling oxidation clearly proceeds; however, cyclohexylamine was not oxidized effectively by $\mathbf{13a^+ \cdot BF_4^-}$ and **1b** (Table 5, Runs 4 and 16). In order to clarify the time dependency of the oxidizing reaction, ^1H NMR monitoring of the photo-oxidation reactions of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ toward benzylamine was carried out. By using mesitylene as the internal standard, the yield of imine is recorded by using the ^1H spectra at selected intervals. The results are summarized in Figure 7, and the yield of benzaldimine was increased linearly as the irradiation time was prolonged to 16 h. Concerning the photo-oxidation reaction by using $\mathbf{13b^+ \cdot BF_4^-}$, ^1H NMR monitoring was continued to 54 h, and the yield of benzaldimine was increased linearly to be 20057%. These features suggest that $\mathbf{13a{-}c^+ \cdot BF_4^-}$ would be stable under these conditions. Attempted detection of the intermediate such as a reduced radical species and **24a–c**, in the oxidation reaction of amines is unsuccessful at this stage. We propose that the present autorecycling oxidation proceeds via a radical species derived from the electron-transfer from amines to the excited cations $\mathbf{13a{-}c^+ \cdot BF_4^-}$ or from the homolysis of amine-adduct such as **20a** by photo-irradiation.[9,15,20]

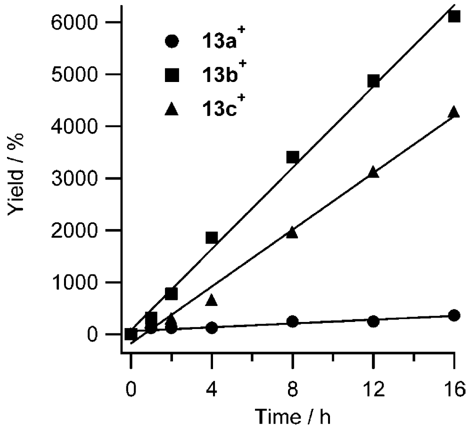


Figure 7. Time dependency of autorecycling oxidation of benzylamine by using $\mathbf{13a{-}c^+ \cdot BF_4^-}$.

3. Conclusion

Novel photo-induced oxidative cyclization of **12a–c**, which derived from the reaction of barbituric acid with easily available aromatic aldehydes, was accomplished to synthesize areno[*b*]pyrimido[5,4-*e*]pyran-2,4(1,3*H*)-dionylium ions ($\mathbf{13a{-}c^+ \cdot ClO_4^-}$). Furthermore, $\mathbf{13a{-}c^+ \cdot BF_4^-}$ and $\mathbf{19a,b^+ \cdot BF_4^-}$ were also synthesized by the reaction of salicylaldehyde **14a** and related naphthyl derivative **14b,c** with dimethylbarbituric acid and subsequent treatment with aq. HBF_4. Structural characteristics of $\mathbf{13a{-}c^+ \cdot ClO_4^-}$, $\mathbf{13a{-}c^+ \cdot BF_4^-}$, and $\mathbf{19a,b^+ \cdot BF_4^-}$ were investigated on the basis of the inspection of the UV–vis and NMR spectral data as well as X-ray crystal analyses. The electrochemical properties were also clarified by the CV measurement. In a search for reactivity, reactions of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ with some nucleophiles, hydride, benzylamine, and H_2O, were carried out. The photo-induced autorecycling oxidation reactions of $\mathbf{13a{-}c^+ \cdot BF_4^-}$ toward some amines under aerobic conditions were carried out to give the corresponding imines (isolated by converting to the corresponding 2,4-dinitrophenylhydrazones) in 643–3600% yield (recycling number of $\mathbf{13a{-}c^+ \cdot BF_4^-}$: 6.4–36.0).

4. Experimental

4.1. General

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

AVANCE600 spectrometers using CD$_3$CN as a solvent, and the chemical shifts are given relative to internal SiMe$_4$ standard; *J*-values are given in Hz. Mps were recorded on a Yamato MP-21 apparatus and were uncorrected.

4.2. Preparation of 12a–c

A solution of **10a–c** (10 mmol) and **11** (1.56 g, 10 mmol) in EtOH (50 mL) in the presence of H$_2$SO$_4$ (three drops) was heated under reflux for 1 h. After the reaction was completed, the resulting mixture was cooled to rt, and the precipitates were collected by filtration to give products **12a–c** (**12a**: 2.20 g, 84%, **12b**: 2.76 g, 94%, **12c**: 2.79 g, 95%). Compound **12a** was identified on the basis of a comparison of the physical data with those reported in the literature.[17]

4.2.1. 1,3-Dimethyl-5-(1-naphthylmethylidene)pyrimidine-2,4,6(1,3,5*H*)-trione (12b). Yellow needles; mp 195–196 °C (from AcOEt); ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.32 (3H, s, NMe), 3.48 (3H, s, NMe), 7.51–7.60 (3H, m), 7.84–7.94 (2H, m), 7.96 (1H, d, $J=7.3$ Hz), 7.99 (1H, d, $J=8.3$ Hz), 9.24 (1H, s); ^{13}C NMR (150.9 MHz, CDCl$_3$) δ 28.4, 29.0, 119.7, 124.1, 124.7, 126.4, 127.3, 129.1, 129.2, 130.3, 131.5, 132.2, 133.1, 151.3, 157.7, 159.9, 162.1; IR (CHCl$_3$) ν 1738, 1684, 1676, 1458, 1420, 1376 cm^{-1}; MS (FAB) *m/z* 295 (M$^+$+H); HRMS calcd for C$_{17}$H$_{14}$N$_2$O$_3$: 295.1082 (M+H). Found: 295.1105 (M$^+$+H). Anal. Calcd for C$_{17}$H$_{14}$N$_2$O$_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.25; H, 4.84; N, 9.60.

4.2.2. 1,3-Dimethyl-5-(2-naphthylmethylidene)pyrimidine-2,4,6(1,3,5*H*)-trione (12c). Yellow needles; mp 201–202 °C (from AcOEt); ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.41 (3H, s, NMe), 3.45 (3H, s, NMe), 7.54 (1H, dd, $J=8.1$, 7.1 Hz, H-6), 7.61 (1H, dd, $J=8.1$, 7.1 Hz, H-7), 7.86 (1H, d, $J=8.1$ Hz, H-8), 7.87 (1H, d, $J=8.8$ Hz, H-3), 7.95 (1H, d, $J=8.1$ Hz, H-4), 8.15 (1H, d, $J=8.8$ Hz, H-5), 8.61 (1H, s, H-1), 8.74 (1H, s, C=CH); ^{13}C NMR (150,9 MHz, CDCl$_3$) δ 28.5, 29.1, 117.3, 126.8, 127.7, 127.7, 128.7, 129.0, 129.7, 130.4, 132.6, 135.4, 136.4, 151.3, 159.3, 160.5, 162.6; IR (CHCl$_3$) ν 1733, 1674, 1580, 1380, 1215, 1087 cm^{-1}; MS (FAB) *m/z* 295 (M$^+$+H); HRMS calcd for C$_{17}$H$_{14}$N$_2$O$_3$: 295.1082 (M+H). Found: 295.1071 (M$^+$+H). Anal. Calcd for C$_{17}$H$_{14}$N$_2$O$_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.16; H, 4.68; N, 9.51.

4.3. Attempted oxidative cyclization of 12a,b with DDQ and Sc(OTf)$_3$

To stirred solutions of **12a**,**b** (0.2 mmol) in xylene (10 mL) was added DDQ (150 mg, 0.66 mmol) and Sc(OTf)$_3$ (324 mg, 0.66 mmol) and the mixtures were heated under reflux for 72 h; however, the reactions were complicated.

4.4. Photo-induced oxidative cyclization of 12a–c

A solution of **12a–c** (0.25 mmol) and 60% aq. HClO$_4$ (1 mL) or 42% aq. HBF$_4$ (1 mL) or NaBF$_4$ (28 mg, 0.25 mmol) in a solvent (8 mL) in a Pyrex tube was irradiated by RPR-100, 350 nm lamps under aerobic condition until the reaction completed (Table 1). To the mixture was added Et$_2$O (50 mL) and the precipitates were collected by filtration to give **13a–c$^+\cdot$ClO$_4^-$** or **13b$^+\cdot$BF$_4^-$**. The results are summarized in Table 1.

4.4.1. 1,3-Dimethylbenzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dionylium perchlorate (13a–c$^+\cdot$ClO$_4^-$). Colorless powder; mp 269–272 °C dec (from CH$_3$CN–AcOEt); ^{1}H NMR (400 MHz, CD$_3$CN) δ 3.46 (3H, s, N3Me), 3.85 (3H, s, N1Me), 7.97 (1H, dd, $J=7.9$, 7.3 Hz, H-7), 8.16 (1H, d, $J=8.6$ Hz, H-9), 8.34 (1H, dd, $J=8.6$, 7.3 Hz, H-8), 8.37 (1H, d, $J=7.9$ Hz, H-6), 9.70 (1H, s, H-5); IR (KBr) ν 1754, 1694, 1637, 1251, 1229, 1106, 1087 cm^{-1}; MS (FAB) *m/z* 243 (M$^+-$ClO$_4$); HRMS calcd for C$_{13}$H$_{11}$N$_2$O$_7$Cl: 243.0770 (M$-$ClO$_4$). Found: 243.0761 (M$^+-$ClO$_4$). Anal. Calcd for C$_{13}$H$_{11}$N$_2$O$_7$Cl: C, 45.56; H, 3.24; N, 8.17. Found: C, 45.45; H, 3.31; N, 8.00.

4.4.2. 8,10-Dimethylnaphto[1′,2′:5,6]pyrano[2,3-*d*]pyrimidine-9,11(8,10*H*)-dionylium perchlorate (13b$^+\cdot$ClO$_4^-$). Orange needles; mp 281–284 °C dec (from CH$_3$CN–AcOEt); ^{1}H NMR (400 MHz, CD$_3$CN) δ 3.50 (3H, s, N10Me), 3.89 (3H, s, N8Me), 7.98 (1H, dd, $J=8.1$, 7.1 Hz, H-3), 8.09 (1H, dd, $J=8.5$, 7.1 Hz, H-2), 8.18 (1H, d, $J=9.3$ Hz, H-6), 8.32 (1H, d, $J=8.1$ Hz, H-4), 8.86 (1H, d, $J=9.3$ Hz, H-5), 8.92 (1H, d, $J=8.5$ Hz, H-1), 10.30 (1H, s, H-12); IR (KBr) ν 1744, 1695, 1629, 1289, 1209, 1091 cm^{-1}; MS (FAB) *m/z* 293 (M$^+-$ClO$_4$); HRMS calcd for C$_{17}$H$_{13}$N$_2$ O$_7$Cl: 293.0926 (M$-$ClO$_4$). Found: 293.0957 (M$^+-$ClO$_4$). Anal. Calcd for C$_{17}$H$_{13}$N$_2$O$_7$Cl: C, 51.99; H, 3.34; N, 7.13. Found: C, 51.92; H, 3.40; N, 6.99.

4.4.3. 9,11-Dimethylnaphto[1′,2′:6,5]pyrano[2,3-*d*]pyrimidine-8,10(9,11*H*)-dionylium perchlorate (13c$^+\cdot$ClO$_4^-$). Yellow needles; mp 291–294 °C dec (from CH$_3$CN–Et$_2$O); ^{1}H NMR (400 MHz, CD$_3$CN) δ 3.49 (3H, s, N9Me), 4.01 (3H, s, N11Me), 8.03 (1H, dd, $J=8.4$, 6.9 Hz, H-2), 8.12 (1H, dd, $J=8.2$, 6.9 Hz, H-3), 8.15 (1H, d, $J=8.7$ Hz, H-5), 8.29 (1H, d, $J=8.2$ Hz, H-4), 8.32 (1H, d, $J=8.7$ Hz, H-6), 8.83 (1H, d, $J=8.4$ Hz, H-1), 9.77 (1H, s, H-7); IR (KBr) ν 1751, 1700, 1653, 1294, 1249, 1091 cm^{-1}; MS (FAB) *m/z* 293 (M$^+-$ClO$_4$); HRMS calcd for C$_{17}$H$_{13}$N$_2$O$_7$Cl: 293.0926 (M$-$ClO$_4$). Found: 293.0954 (M$^+-$ClO$_4$).

4.5. Preparation of 16a–c and 18a,b

A solution of **15a–c** (10 mmol) and **11** (3.12 g, 20 mmol) or **17** (5.64 g, 20 mmol) in EtOH (50 mL) was heated under reflux for 2 h. After the reaction was completed, the resulting mixture was cooled to rt, and the precipitates were collected by filtration to give products **16a–c** and **18a**,**b**. The results are summarized in Table 3. Compound **16a** was identified on the basis of the comparison of the physical data with those reported in the literature.[18]

4.5.1. 8,10-Dimethyl-12-(1,3-dimethyl-2,4,6(1,3,5*H*)-trioxopyrimidin-5-yl)naphto[1′,2′:5,6]pyrano[2,3-*d*]-pyrimidine-9,11(8,10*H*)-dione (16b). Yellow prisms; mp 244–247 °C dec (from CHCl$_3$–Et$_2$O); ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.05 (3H, s, NMe), 3.34 (6H, s, NMe), 3.59 (3H, s, NMe), 3.95 (1H, d, $J=1.2$ Hz, CH), 5.67 (1H, d, $J=1.2$ Hz, H-12), 7.33 (1H, d, $J=9.0$ Hz, H-6), 7.55 (1H, dd, $J=8.1$, 6.8 Hz, H-3), 7.71 (1H, dd, $J=8.5$, 6.8 Hz, H-2), 7.87 (1H, d, $J=9.0$ Hz, H-5), 7.91 (1H, d, $J=8.1$ Hz, H-4), 8.55 (1H, d, $J=8.5$ Hz, H-1); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 28.1, 28.2, 28.7, 29.2, 35.5, 54.0, 85.6, 114.5, 116.2, 122.8, 125.9, 128.4, 129.2, 130.1, 130.4, 131.9, 147.9, 150.4, 151.3, 154.7, 162.3, 167.4, 167.7; IR (KBr) ν 1712, 1691, 1672, 1662, 1637, 1491, 1454, 1375, 1240 cm^{-1}; MS (FAB) *m/z* 449 ($M^+ + H$); HRMS calcd for $C_{23}H_{20}N_4O_6$: 449.1461 ($M+H$). Found: 449.1448 ($M^+ + H$). Anal. Calcd for $C_{23}H_{20}N_4O_6$: C, 61.60; H, 4.50; N, 12.49. Found: C, 61.32; H, 4.43; N, 12.48.

4.5.2. 9,11-Dimethyl-7-(1,3-dimethyl-2,4,6(1,3,5*H*)-trioxopyrimidin-5-yl)naphto[1′,2′:6,5]pyrano[2,3-*d*]pyrimidine-8,10(9,11*H*)-dione (16c). Colorless needles; mp 256–259 °C dec (from $CHCl_3$–Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (3H, s, NMe), 3.28 (3H, s, NMe), 3.40 (3H, s, NMe), 3.74 (3H, s, NMe), 4.17 (1H, d, $J=2.2$ Hz, CH), 5.27 (1H, d, $J=2.2$ Hz, H-7), 7.21 (1H, d, $J=8.5$ Hz, H-6), 7.55–7.67 (2H, m, H-2 and H-3), 7.66 (1H, d, $J=8.5$ Hz, H-5), 7.85 (1H, d, $J=7.6$ Hz, H-4), 8.14 (1H, d, $J=8.1$ Hz, H-1); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 28.2, 28.3, 28.5, 29.3, 37.0, 54.1, 86.5, 115.1, 120.6, 123.2, 124.0, 126.0, 127.4, 127.4, 128.0, 133.7, 145.0, 150.6, 151.2, 154.6, 162.1, 167.0, 167.1; IR (KBr) ν 1701, 1687, 1678, 1668, 1637, 1491, 1458, 1383, 1244, 1213 cm^{-1}; MS (FAB) *m/z* 449 ($M^+ + H$); HRMS calcd for $C_{23}H_{20}N_4O_6$: 449.1461 ($M+H$). Found: 449.1473 ($M^+ + H$). Anal. Calcd for $C_{23}H_{20}N_4O_6$: C, 61.60; H, 4.50; N, 12.49. Found: C, 61.42; H, 4.45; N, 12.48.

4.5.3. 1,3-Diphenyl-5-(1,3-diphenyl-2,4,6(1,3,5*H*)-trioxopyrimidin-5-yl)benzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dione (18a). Colorless needles; mp 189–192 °C dec (from EtOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.47 (1H, d, $J=2.7$ Hz, CH), 5.35 (1H, d, $J=2.7$ Hz, H-5), 6.82–6.85 (1H, m), 7.01 (2H, bs), 7.17 (2H, d), 7.28–7.32 (4H, m), 7.37–7.41 (3H, m), 7.41–7.51 (12H, m); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 37.5, 55.0, 86.5, 117.1, 120.3, 126.4, 128.1, 128.2, 128.4, 128.8, 128.9, 129.0, 129.1, 129.1, 129.2, 129.3, 129.3, 129.4, 129.7, 129.7, 133.2, 133.9, 134.0, 134.5, 149.7, 150.0, 150.5, 155.0, 162.3, 166.7, 166.8; IR (KBr) ν 1707, 1693, 1655, 1491, 1448, 1396, 1367, 1273 cm^{-1}; MS (FAB) *m/z* 647 ($M^+ + H$); HRMS calcd for $C_{39}H_{26}N_4O_6$: 647.1895 ($M+H$). Found: 647.1913 ($M^+ + H$). Anal. Calcd for $C_{39}H_{26}N_4O_6 + EtOH$: C, 71.09; H, 4.66; N, 8.09. Found: C, 70.68; H, 4.69; N, 8.12.

4.5.4. 8,10-Diphenyl-12-(1,3-diphenyl-2,4,6(1,3,5*H*)-trioxopyrimidin-5-yl)naphto[1′,2′:5,6]pyrano[2,3-*d*]pyrimidine-9,11(8,10*H*)-dione (18b). Colorless needles; mp 272–275 °C dec (from $CHCl_3$–Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (1H, d, $J=1.2$ Hz, CH), 5.88 (1H, d, $J=1.2$ Hz, H-12), 6.88 (1H, d, $J=8.8$ Hz, H-5), 7.11 (2H, d, $J=6.8$ Hz, NPh-*o*), 7.28–7.57 (19H, m, H-8 and NPh-*o*, *m*, *p*), 7.68 (1H, dd, $J=7.0$, 8.4 Hz, H-2), 7.75 (1H, d, $J=8.8$ Hz, H-5), 7.87 (1H, d, $J=8.0$ Hz, H-4), 8.68 (1H, d, $J=8.4$ Hz, H-1); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 36.5, 54.7, 85.9, 114.4, 116.3, 122.9, 126.0, 128.1, 128.4, 128.4, 128.5, 128.6, 128.7, 128.7, 128.9, 129.0, 129.2, 129.2, 129.3, 129.3, 129.4, 129.5, 129.7, 129.9, 130.4, 132.0, 133.1, 134.1, 134.4, 134.5, 147.9, 149.9, 150.8, 155.3, 162.7, 167.6, 167.7; IR (KBr) ν 1697, 1662, 1446, 1390, 1363, 1240 cm^{-1}; MS (FAB) *m/z* 697 ($M^+ + H$); HRMS calcd for $C_{43}H_{28}N_4O_6$: 697.2087 ($M+H$). Found: 697.2104 ($M^+ + H$). Anal. Calcd for $C_{43}H_{28}N_4O_6 + 1/4CHCl_3$: C, 71.50; H, 3.92; N, 7.71. Found: C, 71.59; H, 4.09; N, 7.74.

4.6. Preparation of 13a–c$^+ \cdot BF_4^-$ and 19a,b$^+ \cdot BF_4^-$

A solution of **16a–c** (1 mmol) or **18a**,**b** (1 mmol) in Ac_2O (10 mL) and 42% aq. HBF_4 (2 mL) was stirred at 0 °C for 1 h. To the mixture was added Et_2O (50 mL) and the precipitates were collected by filtration to give **13a–c**$^+ \cdot$**BF**$_4^-$ or **19a**,**b**$^+ \cdot$**BF**$_4^-$. The results are summarized in Table 3.

4.6.1. 1,3-Dimethylbenzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dionylium tetrafluoroborate (13a$^+ \cdot BF_4^-$). Colorless powder; mp 225–228 °C dec (from CH_3CN–AcOEt); ^{1}H NMR (500 MHz, CD_3CN) δ 3.46 (3H, s, N3Me), 3.85 (3H, s, N1Me), 7.97 (1H, dd, $J=7.9$, 7.3 Hz, H-7), 8.16 (1H, d, $J=8.6$ Hz, H-9), 8.34 (1H, dd, $J=8.6$, 7.3 Hz, H-8), 8.37 (1H, d, $J=7.9$ Hz, H-6), 9.70 (1H, s, H-5); ^{13}C NMR (150.9 MHz, CD_3CN) δ 30.0, 32.0, 111.7, 119.4, 121.5, 131.1, 134.1, 142.3, 148.9, 154.7, 156.8, 157.7, 163.8; IR (KBr) ν 1752, 1696, 1640, 1250, 1084 cm^{-1}; MS (FAB) *m/z* 243 ($M^+ - BF_4$); HRMS calcd for $C_{13}H_{11}BF_4N_2O_3$: 243.0770 ($M-BF_4$). Found: 243.0790 ($M^+ - BF_4$). Anal. Calcd for $C_{13}H_{11}BF_4N_2O_3$: C, 47.31; H, 3.36; N, 8.49. Found: C, 47.30; H, 3.44; N, 8.48.

4.6.2. 8,10-Dimethylnaphto[1′,2′:5,6]pyrano[2,3-*d*]pyrimidine-9,11(8,10*H*)-dionylium tetrafluoroborate (13b$^+ \cdot BF_4^-$). Yellow plates; mp 239–241 °C dec (from CH_3CN–AcOEt); ^{1}H NMR (500 MHz, CD_3CN) δ 3.50 (3H, s, N10Me), 3.89 (3H, s, N8Me), 7.98 (1H, dd, $J=8.1$, 7.1 Hz, H-3), 8.09 (1H, dd, $J=8.5$, 7.1 Hz, H-2), 8.18 (1H, d, $J=9.3$ Hz, H-6), 8.32 (1H, d, $J=8.1$ Hz, H-4), 8.86 (1H, d, $J=9.3$ Hz, H-5), 8.93 (1H, d, $J=8.5$ Hz, H-1), 10.31 (1H, s, H-12); ^{13}C NMR (150.9 MHz, CD_3CN) δ 30.0, 31.7, 110.3, 117.4, 119.2, 125.0, 129.8, 130.9, 131.3, 132.6, 132.8, 145.7, 149.1, 151.2, 157.0, 157.9, 162.7; IR (KBr) ν 1700, 1684, 1654, 1214, 1084 cm^{-1}; MS (FAB) *m/z* 293 ($M^+ - BF_4$); HRMS calcd for $C_{17}H_{13}BF_4N_2O_3$: 293.0950 ($M-BF_4$). Found: 293.0938 ($M^+ - BF_4$). Anal. Calcd for $C_{17}H_{13}BF_4N_2O_3$: C, 53.72; H, 3.45; N, 7.37. Found: C, 53.66; H, 3.50; N, 7.21.

4.6.3. 9,11-Dimethylnaphto[1′,2′:6,5]pyrano[2,3-*d*]pyrimidine-8,10(9,11*H*)-dionylium tetrafluoroborate (13c$^+ \cdot BF_4^-$). Yellow plates; mp 233–236 °C dec (from CH_3CN–Et_2O); ^{1}H NMR (600 MHz, CD_3CN) δ 3.49 (3H, s, N9Me), 4.00 (3H, s, N11Me), 8.03 (1H, dd, $J=8.4$, 6.9 Hz, H-2), 8.12 (1H, dd, $J=8.2$, 6.9 Hz, H-3), 8.15 (1H, d, $J=8.7$ Hz, H-5), 8.29 (1H, d, $J=8.2$ Hz, H-4), 8.32 (1H, d, $J=8.7$ Hz, H-6), 8.83 (1H, d, $J=8.4$ Hz, H-1), 9.77 (1H, s, H-7); ^{13}C NMR (150.9 MHz, CD_3CN) δ 30.0, 31.9, 111.2, 119.8, 122.6, 124.8, 125.9, 130.3, 130.9, 131.5, 135.2, 139.8, 149.0, 154.6, 155.9, 157.8, 162.8; IR (KBr) ν 1774, 1700, 1648, 1250, 1084 cm^{-1}; MS (FAB) *m/z* 293 ($M^+ - BF_4$); HRMS calcd for $C_{17}H_{13}BF_4N_2O_3$: 293.0950 ($M-BF_4$). Found: 293.0938 ($M^+ - BF_4$).

4.6.4. 1,3-Diphenylbenzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dionylium tetrafluoroborate (19a$^+ \cdot BF_4^-$). Pale yellow prisms; mp 256–259 °C dec (from CH_3CN–

AcOEt); ^{1}H NMR (400 MHz, CD_3CN) δ 7.44–7.49 (2H, m, NPh-*o*), 7.57–7.68 (5H, m, NPh-*m* and *p* and *o*), 7.72 (1H, d, $J=8.8$ Hz, H-9), 7.72–7.78 (3H, m, NPh-*m* and *p*), 7.96 (1H, dd, $J=8.1$, 7.3 Hz, H-7), 8.26 (1H, dd, $J=8.8$, 7.3 Hz, H-8), 8.41 (1H, d, $J=8.1$ Hz, H-6), 9.83 (1H, s, H-5); ^{13}C NMR (150, 9 MHz, CD_3CN) δ 112.2, 116.9, 119.4, 121.9, 128.7, 129.1, 129.8, 130.0, 130.2, 130.9, 131.0, 131.3, 131.4, 132.1, 132.5, 134.1, 134.8, 142.7, 148.9, 154.8, 157.9, 158.1, 163.9; IR (KBr) ν 1761, 1709, 1606, 1548, 1387, 1261, 1084 cm^{-1}; MS (FAB) *m/z* 367 (M^+-BF_4); HRMS calcd for $C_{23}H_{15}BF_4N_2O_3$: 367.1083 ($M-BF_4$). Found: 367.1060 (M^+-BF_4). Anal. Calcd for $C_{23}H_{15}BF_4N_2O_3$: C: 60.82; H: 3.33;N: 6.17. Found: C: 60.66; H: 3.29; N: 6.09.

4.6.5. 8,10-Diphenylnaphto[1′,2′:5,6]pyrano[2,3-*d*]pyrimidine-9,11(8,10*H*)-dionylium tetrafluoroborate ($19b^+\cdot BF_4^-$). Yellow prisms; mp 231–234 °C dec (from CH_3CN–AcOEt); ^{1}H NMR (500 MHz, CD_3CN) δ 7.47–7.51 (2H, m, NPh-*o*), 7.57–7.65 (3H, m, NPh-*m* and *p*), 7.68–7.72 (3H, m, H-6 and NPh-*o*), 7.75–7.79 (3H, m, NPh-*m* and *p*), 7.99 (1H, dd, $J=8.1$, 7.1 Hz, H-3), 8.11 (1H, dd, $J=8.5$, 7.1 Hz, H-2), 8.30 (1H, d, $J=8.1$ Hz, H-4), 8.78 (1H, d, $J=9.3$ Hz, H-5), 8.95 (1H, d, $J=8.5$ Hz, H-1), 10.44 (1H, s, H-12); ^{13}C NMR (150,9 MHz, CD_3CN) δ 110.9, 117.3, 119.9, 125.1, 129.0, 129.2, 129.7, 129.9, 130.9, 131.0, 131.1, 131.4, 131.5, 132.3, 132.5, 132.8, 132.8, 135.1, 146.3, 149.2, 152.2, 152.2, 157.4, 158.3, 162.8; IR (KBr) ν 1715, 1705, 1626, 1204, 1084 cm^{-1}; MS (FAB) *m/z* 417 (M^+-BF_4); HRMS calcd for $C_{27}H_{17}BF_4N_2O_3$: 417.1239 ($M-BF_4$). Found: 417.1260 (M^+-BF_4). Anal. Calcd for $C_{27}H_{17}BF_4N_2O_3$: C, 64.31; H, 3.40; N, 5.56. Found: C, 64.09; H, 3.24; N, 5.50.

4.7. Cyclic voltammetry of 12a–c, $13a–c^+\cdot BF_4^-$, and $19a,b^+\cdot BF_4^-$

The reduction potential of **12a–c**, $\mathbf{13a–c^+\cdot BF_4^-}$, and $\mathbf{19a,b^+\cdot BF_4^-}$ was determined by means of CV-27 voltammetry controller (BAS Co). A three-electrode cell was used, consisting of Pt working and counter electrodes and a reference $Ag/AgNO_3$ electrode. Nitrogen was bubbled through an acetonitrile solution (4 mL) of **12a–c**, $\mathbf{13a–c^+\cdot BF_4^-}$, and $\mathbf{19a,b^+\cdot BF_4^-}$ (0.5 mmol dm^{-3}) and Bu_4NClO_4 (0.1 mol dm^{-3}) to deaerate it. The measurements were made at a scan rate of 0.1 V s^{-1} and the voltammograms were recorded on a WX-1000-UM-019 (Graphtec Co) X-Y recorder. Immediately after the measurements, ferrocene (0.1 mmol) ($E_{1/2}=+0.083$) was added as the internal standard, and the observed peak potential was corrected with reference to this standard. Compounds **12a–c** exhibited one irreversible oxidation wave and one irreversible reduction wave. Compounds $\mathbf{13a–c^+\cdot BF_4^-}$ and $\mathbf{19a,b^+\cdot BF_4^-}$ exhibited one irreversible reduction wave. The results are summarized in Table 2.

4.8. X-ray structure determination of $19a^+\cdot BF_4^-$ †

Yellow prisms, $C_{23}H_{15}N_2O_3BF_4$, $M=454.19$, monoclinic, space group $P2_1/c$, $a=11.1093(4)$, $b=9.7258(3)$, $c=19.1523(7)$ Å, $\beta=100.998(2)°$, $V=2031.3(1)$ Å^3, $Z=4$, $Dc=1.485$ g mL^{-1}, crystal dimensions $0.80\times0.40\times0.30$ mm. Data were measured on a Rigaku RAXIS-RAPID radiation diffractomater with graphite monochromated Mo Kα radiation. Total 19,651 reflections were collected, using the ω–2θ scan technique to a maximum 2θ value of 55.0°. The structure was solved by direct methods and refined by a full-matrix least-squares method using SIR92 structure analysis software,[21] with 313 variables and 3309 observed reflections [$I>3.00\sigma(I)$]. The non-hydrogen atoms were refined anisotropically. The weighting scheme $w=[0.5000\sigma_c(F_0^2)+0.0030F_0^2]^{-1}$ gave satisfactory agreement analysis. The final *R* and *Rw* values were 0.0390 and 0.0620. The maximum peak and minimum peak in the final difference map were 0.34 and -0.18 e$^-$/Å^3.

4.9. X-ray structure determination of $19b^+\cdot BF_4^-$ ‡

Yellow prisms, $C_{27}H_{17}N_2O_3BF_4$, $M=504.25$, monoclinic, space group $P2_1/c$, $a=10.78(1)$, $b=18.02(1)$, $c=11.96(1)$ Å, $\beta=100.83(2)°$, $V=2281(3)$ Å^3, $Z=4$, $Dc=1.468$ g mL^{-1}, crystal dimensions $0.80\times0.40\times0.20$ mm. Data were measured on a Rigaku RAXIS-RAPID radiation diffractometer with graphite monochromated Mo Kα radiation. Total 21,709 reflections were collected, using the ω–2θ scan technique to a maximum 2θ value of 55.0°. The structure was solved by direct methods and refined by a full-matrix least-squares method using SIR92 structure analysis software,[21] with 351 variables and 2741 observed reflections [$I>3.00\sigma(I)$]. The non-hydrogen atoms were refined anisotropically. The weighting scheme $w=[0.9000\sigma_c(F_0^2)+0.0005F_0^2]^{-1}$ gave satisfactory agreement analysis. The final *R* and *Rw* values were 0.0330 and 0.0380. The maximum peak and minimum peak in the final difference map were 0.32 and -0.23 e$^-$/Å^3.

4.10. Reaction of $13a^+\cdot BF_4^-$ with benzylamine

To a solution of $\mathbf{13a^+\cdot BF_4^-}$ (33 mg, 0.1 mmol) in CH_3CN (5 mL) was added benzylamine (21.4 mg, 0.20 mmol), and the mixture was stirred at rt for 1 h. To the mixture was added saturated aq. NH_4Cl solution, and the mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo to give **20a** (34 mg, 97%).

4.10.1. 5-Benzylamino-1,3-dimethylbenzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4(1,3*H*)-dione (20a). Colorless prisms; mp 192–194 °C (from $CHCl_3$–Et_2O); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.33 (3H, s, NMe), 3.40 (2H, s, $PhCH_2$), 3.49 (3H, s, NMe), 5.27 (1H, s, H-5), 7.12–7.24 (6H, m, H-9 and Ph), 7.28 (1H, t, $J=7.5$ Hz, H-8), 7.34 (1H, dd, $J=7.7$, 7.5 Hz, H-7), 7.64 (1H, d, $J=7.7$ Hz, H-6); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 28.0, 28.9, 47.6, 48.1, 88.2, 116.1, 121.8, 125.9, 126.6, 127.8, 128.0, 128.9, 130.4, 140.7, 150.0, 150.6, 153.3, 162.6; IR (KBr) ν 3417, 1709, 1664, 1645, 1581, 1479, 1456, 1240 cm^{-1}; MS (FAB) *m/z* 350 (M^++H); HRMS calcd for $C_{20}H_{19}N_3O_3$: 350.1515 ($M+H$). Found: 350.1528 (M^++H). Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 69.87; H, 5.39; N, 12.11.

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† CCDC reference number 262897.

‡ CCDC reference number 262898.

4.11. Reaction of 13a$^{+}\cdot$BF$_4^{-}$ with OH^{-}

To a solution of **13a**$^{+}\cdot$**BF**$_4^{-}$ (33 mg, 0.1 mmol) in CH_3CN (5 mL) was added saturated aq. $NaHCO_3$ (1 mL), and the mixture was stirred at rt for 1 h. The mixture was extracted with CH_2Cl_2 and the extract was dried over Na_2SO_4. The filtrate was concentrated in vacuo to give **21a** (24 mg, 92%).

4.11.1. 1,3-Dimethyl-5-hydroxybenzo[5,6]pyrano[2,3-*d*]-pyrimidine-2,4(1,3*H*)-dione (21a). Colorless prisms; mp 105–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 (3H, s, NMe), 3.57 (3H, s, NMe), 3.81 (1H, d, $J=2.4$ Hz, OH), 5.97 (1H, d, $J=2.4$ Hz, H-5), 7.20 (1H, d, $J=8.4$ Hz, H-9), 7.32 (1H, dd, $J=7.6$, 7.2 Hz, H-7), 7.40 (1H, dd, $J=8.4$, 7.2 Hz, H-8), 7.60 (1H, d, $J=7.6$ Hz, H-6); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 28.0, 29.1, 59.4, 89.7, 116.4, 121.5, 126.2, 129.8, 130.3, 148.4, 150.6, 152.7, 163.4; IR ($CHCl_3$) ν 3566, 3330, 1700, 1663, 1639, 1486, 1464, 1280 cm^{-1}; MS (FAB) *m/z* 259 ($M^{+}-H$); HRMS calcd for $C_{13}H_{12}N_2O_4$: 259.0719 ($M^{+}-H$). Found: 259.0694 ($M^{+}-H$).

4.12. Reaction of 13a$^{+}\cdot$BF$_4^{-}$ with H$_3$O^{+}

To a solution of **13a**$^{+}\cdot$**BF**$_4^{-}$ (16.5 mg, 0.05 mmol) in CH_3CN (5 mL) was added 0.6% HCl (5 mL), and the mixture was stirred at rt for 1 min. To the mixture was added H_2O, and the mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo to give a mixture of **22a**, **11**, **15a**, and **16a** (12 mg, the ratio of **22a**, **11**, **15a**, and **16a** was determined to be 9:2:2:1 by ^{1}H NMR).

4.12.1. 1,3-Dimethyl-5-[(2′-hydroxyphenyl)methylidene]pyrimidine-2,4,6(1,3,5*H*)-trione (22a). Yellow prisms; mp 184–185 °C (from AcOEt); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 (3H, s, NMe), 3.45 (3H, s, NMe), 7.04 (1H, dd, $J=7.8$, 7.3 Hz, H-4′), 7.08 (1H, d, $J=8.3$ Hz, H-6′), 7.49 (1H, dd, $J=8.3$, 7.3 Hz, H-5′), 7.60 (1H, d, $J=7.8$ Hz, H-3′), 8.13 (1H, s, OH), 8.75 (1H, d, C=CH); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 28.8, 29.2, 115.9, 120.3, 121.0, 122.9, 135.5, 135.9, 150.7, 157.1, 158.9, 162.0, 163.9; IR (KBr) ν 3282, 1727, 1672, 1645, 1603, 1396, 1384, 1259, 1159 cm^{-1}; MS (FAB) *m/z* 261 ($M^{+}+H$); HRMS calcd for $C_{13}H_{12}N_2O_3$: 261.0875 ($M+H$). Found: 261.0849 ($M^{+}+H$). Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 60.00; H, 4.65; N, 10.76. Found: C, 59.42; H, 4.49; N, 10.57.

4.13. Reactions of 20a or 21a with HBF$_4$

To a solution of **20a** (35 mg, 0.1 mmol) or **21a** (24 mg, 0.1 mmol) in Ac_2O (2 mL) was added 42% aq. HBF_4 (0.4 mL) at 0 °C. The mixture was stirred for 1 h. To the mixture was added Et_2O (10 mL) and the precipitate was collected by filtration to give **13a**$^{+}\cdot$**BF**$_4^{-}$ (33 mg, 100% from **20a** and 33 mg, 100% from **21a**).

4.14. Reaction of 21a with DDQ

To a stirred solution of **21a** (13 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) was added DDQ (11.4 mg, 0.05 mmol) and the mixture was stirred at rt for 1 h. After evaporation of the solvent, the residue was dissolved in aq. $NaHCO_3$ solution, and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on SiO_2 using AcOEt as the eluent to give **23a** (11 mg, 86%).

4.14.1. 1,3-Dimethylbenzo[5,6]pyrano[2,3-*d*]pyrimidine-2,4,5(1,3*H*)-trione (23a). Colorless needles; mp 290–293 °C dec (from $CHCl_3$–Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 (3H, s, NMe), 3.72 (3H, s, NMe), 7.42–7.53 (2H, m, H-7 and H-9), 7.72 (1H, dd, $J=8.3$, 7.3 Hz, H-8), 8.31 (1H, d, $J=7.8$ Hz, H-6); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 28.3, 29.7, 96.0, 116.9, 123.2, 126.8, 127.2, 134.2, 149.6, 152.5, 158.1, 160.9, 172.2; IR (KBr) ν 1727, 1719, 1677, 1673, 1617, 1566, 1270, 1226, 774, 754 cm^{-1}; MS (FAB) *m/z* 259 ($M^{+}+H$); HRMS calcd for $C_{13}H_{10}N_2O_4$: 259.0719 ($M+H$). Found: 259.0709 ($M^{+}+H$). Anal. Calcd for $C_{13}H_{10}N_2O_4$: C, 60.47; H, 3.90; N, 10.85. Found: C, 60.06; H, 3.76; N, 10.73.

4.15. Reaction of 13a–c$^{+}\cdot$BF$_4^{-}$ with NaBH$_4$

A solution of **13a–c**$^{+}\cdot$**BF**$_4^{-}$ (0.3 mmol) and $NaBH_4$ (11 mg, 0.3 mmol) in CH_3CN (15 mL) was stirred at rt for 1 h. To the mixture was added saturated aq. NH_4Cl solution, and the mixture was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo to give **24a–c** (**24a**: 73 mg, 100%, **24b**: 85 mg, 96%, **24c**: 88 mg, 100%).

4.15.1. 1,5-Dihydro-1,3-dimethylbenzo[5,6]pyrano[2,3-*d*]-pyrimidine-2,4(1,3*H*)-dione (24a). Colorless prisms; mp 190–192 °C (from $CHCl_3$–Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.39 (3H, s, NMe), 3.53 (3H, s, NMe), 3.74 (2H, s, H-5), 7.07 (1H, d, $J=8.2$ Hz, H-9), 7.16 (1H, dd, $J=8.4$, 7.4 Hz, H-7), 7.22–7.27 (2H, m, H-6 and H-8); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 21.8, 28.2, 29.0, 85.5, 116.5, 120.0, 125.7, 128.0, 130.0, 149.2, 150.9, 152.7, 162.7; IR ($CHCl_3$) ν 1705, 1665, 1637, 1490, 1450, 1244 cm^{-1}; MS (FAB) *m/z* 245 ($M^{+}+H$); HRMS calcd for $C_{13}H_{12}N_2O_3$: 245.0926 ($M+H$). Found: 245.0969 ($M^{+}+H$). Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.85; H, 4.80; N, 11.48.

4.15.2. 8,12-Dihydro-8,10-dimethylnaphto[1′,2′:5,6]-pyrano[2,3-*d*]pyrimidine-9,11(8,10*H*)-dione (24b). Colorless needles; mp 279–282 °C dec (from $CHCl_3$–Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.42 (3H, s, NMe), 3.56 (3H, s, NMe), 4.00 (2H, s, H-12), 7.24 (1H, d, $J=8.9$ Hz, H-6), 7.53 (1H, dd, $J=8.0$, 6.9 Hz, H-3), 7.63 (1H, dd, $J=8.5$, 6.9 Hz, H-2), 7.77 (1H, d, $J=8.9$ Hz, H-5), 7.85 (1H, d, $J=8.0$ Hz, H-4), 7.94 (1H, d, $J=8.5$ Hz, H-1); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 19.9, 28.2, 29.0, 85.5, 113.1, 116.4, 123.3, 125.7, 127.5, 128.4, 128.9, 131.1, 131.7, 146.3, 150.8, 152.5, 162.9; IR ($CHCl_3$) ν 1704, 1674, 1638, 1490, 1465, 1233, 1213 cm^{-1}; MS (FAB) *m/z* 295 ($M^{+}+H$); HRMS calcd for $C_{17}H_{14}N_2O_3$: 295.1083 ($M+H$). Found: 295.1096 ($M^{+}+H$). Anal. Calcd for $C_{17}H_{14}N_2O_3+1/2H_2O$: C, 67.32; H, 4.98; N, 9.24. Found: C, 66.94; H, 4.80; N, 9.43.

4.15.3. 7,11-Dihyro-9,11-dimethylnaphto[1′,2′:6,5]-pyrano[2,3-*d*]pyrimidine-8,10(9,11*H*)-dione (24c). Colorless needles; mp 218–221 °C dec (from $CHCl_3$–Et_2O); ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.42 (3H, s, NMe), 3.69 (3H, s, NMe), 3.86 (2H, s, H-7), 7.29 (1H, d, $J=8.4$ Hz, H-6), 7.54

(1H, dd, $J=7.7, 6.9$ Hz, H-3), 7.58 (1H, dd, $J=8.2, 6.9$ Hz, H-2), 7.65 (1H, d, $J=8.4$ Hz, H-5), 7.84 (1H, d, $J=7.7$ Hz, H-4), 8.07 (1H, d, $J=8.2$ Hz, H-1); ^{13}C NMR (150,9 MHz, $CDCl_3$) δ 22.4, 28.2, 29.1, 85.6, 115.1, 120.2, 123.5, 125.2, 126.4, 126.6, 127.0, 128.0, 133.4, 143.8, 150.9, 152.8, 162.7; IR ($CHCl_3$) ν 1704, 1674, 1639, 1490, 1440, 1387, 1326, 1247, 1229 cm^{-1}; MS (FAB) *m/z* 295 ($M^{+}+H$); HRMS calcd for $C_{17}H_{14}N_2O_3$: 295.1083 (M+H). Found: 295.1068 ($M^{+}+H$). Anal. Calcd for $C_{17}H_{14}N_2O_3+1/4H_2O$: C, 68.33; H, 4.89; N, 9.38. Found: C, 68.51; H, 4.71; N, 9.39.

4.16. Reaction of 24a with DDQ

To a stirred solution of **24a** (24.4 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) was added DDQ (56.8 mg, 0.25 mmol) and the mixture was stirred at rt for 1 h. After evaporation of the solvent, the residue was dissolved in aq. $NaHCO_3$ solution, and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on SiO_2 using AcOEt as the eluent to give **23a** (26 mg, 100%).

4.17. General procedure for the autorecycling oxidation of amines catalyzed by 13a–c$^{+}\cdot BF_4^{-}$ and 1b under photo-irradiation

A CH_3CN (16 mL) solution each of compounds **13a–c**$^{+}\cdot$**BF**$_4^{-}$ and **1b** (0.005 mmol) and amines (2.5 mmol, 500 equiv) in a Pyrex tube was irradiated by RPR-100, 350 nm lamps under aerobic conditions for the period indicated in Table 5. The reaction mixture was concentrated in vacuo and diluted with Et_2O and filtered. The ^{1}H NMR spectra of the filtrates revealed the formation of the imines (Table 5). The filtrate was treated with a saturated solution of 2,4-dinitrophenylhydrazine in 6% HCl to give 2,4-dinitrophenylhydrazone. The results are summarized in Table 5.

4.18. ^{1}H NMR monitoring of the photo-oxidation of benzylamine by using 13a–c$^{+}\cdot BF_4^{-}$

A CD_3CN (0.5 mL) solution of each compound **13a–c**$^{+}\cdot$**BF**$_4^{-}$ (0.0005 mmol), benzylamine (18.73 mg, 0.175 mmol), and mesitylene (internal standard; 3.6 mg, 0.03 mmol) in an NMR tube irradiated by RPR-100, 350 nm lamps under aerobic conditions. The NMR measurement was carried out at selected intervals, and the yield of benzaldimine calculated by using the internal standard was plotted against irradiated time (Fig. 7).

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References and notes

1. Muller, F. In Muller, F., Ed.; Chemistry and Biochemistry of Flavoenzymes; CRC: Boca Raton, 1991; Vol. 1, pp 1–71; and references cited therein.
2. Hamilton, G. A. In Kaiser, E. T., Kezdy, F. J., Eds.; Progress in Bioorganic Chemistry; Wiley: New York, 1971; Vol. 1, p 83.
3. (a) Chiu, C. C.; Pan, K.; Jordan, F. *J. Am. Chem. Soc.* **1995**, *117*, 7027–7028. (b) Kim, J.; Hoegy, S. E.; Mariano, P. S. *J. Am. Chem. Soc.* **1995**, *117*, 100–105. (c) Murahashi, S.; Ono, S.; Imada, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 2366–2368. (d) Bergstad, K.; Jonsson, S.; Bächvall, J. *J. Am. Chem. Soc.* **1999**, *121*, 10424–10425. (e) Van Houten, K. A.; Kim, J.; Bogdan, M. A.; Ferri, D. C.; Mariano, P. S. *J. Am. Chem. Soc.* **1998**, *120*, 5864–5872. (f) Zheng, Y.; Ornstein, R. L. *J. Am. Chem. Soc.* **1996**, *118*, 9402–9408. (g) Breinlinger, E. C.; Keenan, C. J.; Rotello, V. M. *J. Am. Chem. Soc.* **1998**, *120*, 8606–8609. (h) Hasford, J. J.; Rizzo, C. J. *J. Am. Chem. Soc.* **1998**, *120*, 2251–2255. (i) Antony, J.; Medvedev, D. M.; Stuchebrukhov, A. A. *J. Am. Chem. Soc.* **2000**, *122*, 1057–1065.
4. Walsh, C. *Acc. Chem. Res.* **1986**, *19*, 216–221 and references cited therein.
5. Yoneda, F.; Tanaka, K. *Med. Res. Rev.* **1987**, *4*, 477–506 and references cited therein.
6. Yoneda, F.; Hirayama, R.; Yamashita, M. *Chem. Lett.* **1980**, 1157–1160.
7. Nitta, M.; Tajima, Y. *Synthesis* **2000**, 651–654.
8. Takayasu, T.; Mizuta, Y.; Nitta, M. *Heterocycles* **2001**, *54*, 601–606.
9. Naya, S.; Iida, Y.; Nitta, M. *Tetrahedron* **2004**, *60*, 459–467.
10. Naya, S.; Nitta, M. *Tetrahedron* **2003**, *59*, 3709–3718.
11. Naya, S.; Miyama, H.; Yasu, K.; Takayasu, T.; Nitta, M. *Tetrahedron* **2003**, *59*, 1811–1821.
12. Naya, S.; Miyama, H.; Yasu, K.; Takayasu, T.; Nitta, M. *Tetrahedron* **2003**, *59*, 4929–4938.
13. Naya, S.; Nitta, M. *Tetrahedron* **2003**, *59*, 7291–7299.
14. Naya, S.; Tokunaka, T.; Nitta, M. *J. Org. Chem.* **2003**, *68*, 9317–9321.
15. (a) Miranda, M. A.; Garcia, H. *Chem. Rev.* **1994**, *94*, 1063–1089. (b) Akaba, R.; Kamata, M.; Sakuragi, H.; Tokumaru, K. *Tetrahedron Lett.* **1992**, *33*, 8105–8108. (c) Akaba, R.; Sakuragi, H.; Tokumaru, K. *J. Chem. Soc., Perkin Trans. 2* **1992**, 291–297.
16. Branchi, B.; Bietti, M.; Ercolani, G.; Izquierdo, M. A.; Miranda, M. A.; Stella, L. *J. Org. Chem.* **2004**, *69*, 8874–8885.
17. Sekiya, M.; Yanaihara, C. *Chem. Pharm. Bull.* **1969**, *17*, 747–751.
18. Akabori, S. *Chem. Ber.* **1933**, *66B*, 139.
19. Yamane, K.; Yamamoto, H.; Nitta, M. *J. Org. Chem.* **2002**, *67*, 8114–8122.
20. Naya, S.; Nitta, M. *Tetrahedron* **2004**, *60*, 9139–9148.
21. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A.; Polidori, G. *J. Appl. Crystallogr.* **1994**, *27*, 435.

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Macrocyclization studies and total synthesis of cyclomarin C, an anti-inflammatory marine cyclopeptide

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Abstract—The studies on macrocyclization at possible sites toward the total synthesis of cyclomarin C are described. The results showed that both Trp and Phe derivatives involved in the target could not be the terminals of the final linear peptide precursors. Additionally, preparation of corresponding dipeptides with an *N*-methyl amide bond is not favorable in the synthesis of linear precursors. Site d was finally proved a proper site for the cyclopeptide formation, and the corresponding head-to-tail macrocyclization was achieved under mild conditions and gave repeatable and satisfactory yields.

1. Introduction

Cyclomarins A–C (**1**–**3**), three novel bioactive metabolites, were recently isolated and characterized from a marine bacterium collected in the vicinity of San Diego by Clardy et al.[1] The major constituent (95% of the total cyclomarin mixture), cyclomarin A displays significant anti-inflammatory activities both in vitro and in vivo assays, as well as anti-cancer activity. In the phorbol ester (PMA)-induced mouse ear edema assay, this compound shows 92% inhibition of edema at the standard testing dose of 50 μg/ear. In the same assay, cyclomarin A also shows promising in vivo activity (45% reduction in edema at 30 mg/kg ip administration), indicating it may be a potential drug candidate. Structurally, all three natural products are cyclic heptapeptides, and contain four noncoded amino acids in each. Their structures show remarkable resemblance to each other (Fig. 1). Cyclomarin A and C each contain three common amino acids (alanine, valine, and *N*-methylleucine), two uncommon amino acids (β-methoxyphenylalanine, *N*-methyl-5-hydroxyleucine), and two novel amino acids (2-amino-3,5-dimethylhex-4-enoic acids for both, *N*′-prenyl-β-hydroxytryptophan for cyclomarin C, and *N*′-(1,1-dimethyl-2,3- expoxypropyl)-β-hydroxytryptophan for cyclomarin A).

Cyclomarins have attracted much attention within the synthetic community due to their potent bioactivities and the unique structural complexicities. In the past several years, many efforts and achievements were reported about the elaboration of key amino acids,[2] while the first total synthesis of cyclomarin C was demonstrated by us very recently.[3] Due to its limited availability (1 mg, 3% of cyclomarins from nature), the biological activities of cyclomarin C could not be studied intensively. Therefore, the synthetic efforts toward cyclomarin C will definitely illustrate two sides of importance. In chemistry side, because of the structural similarity between cyclomarins, the methodologies and strategies developed during its total synthesis will benefit those efforts toward other members of cyclomarins. The other income will be on the biology side, because the total synthesis of cyclomarin C will be able to offer more quantity of sample to meet the requirements of biological studies, as well as possible derivatives. In our recent preliminary communication,[3] we reported the results on the total synthesis of cyclomarin C, including methods to

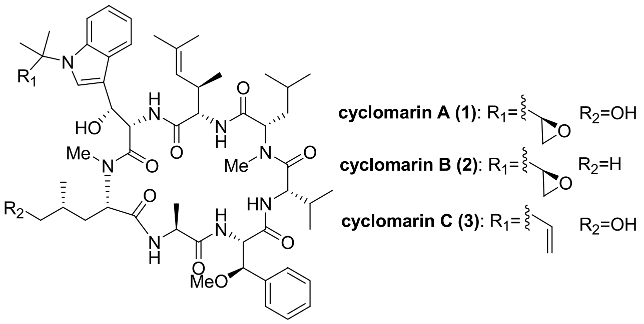

Figure 1. The chemical structures of cyclomarins A–C.

Keywords: Macrocyclization; Dipeptides; Cyclomarin C.

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elaborate those noncoded amino acids,[4] convergent assembly of linear peptide, and final successful macrocyclization at the optimized site. In this paper, we would like to address the details of our efforts to search a proper site for the final macrocyclization, which have played a key role in the total synthesis of cyclomarin C (**3**).

As mentioned, seven components (single amino acid derivatives) appear in cyclomarin C were synthesized by various fashions, and modified as the proper derivatives. With those fragments in hand, the following major task is how to link them together, affording the corresponding linear heptapeptide precursors for the final macrocyclization. In order to enhance the synthetic efficiency, two key points were initially considered in our retrosynthetic analysis for this stage of total synthesis. First, a convergent peptide-segment assembly strategy rather than a stepwise one should be more preferable. Second, because the noncoded amino acid, N'-prenyltryptophan, was highly sensitive to various acidic conditions, it would be favorable to incorporate this amino acid at the last stage of the synthesis of linear heptapeptide. Figure 2 illustrates all of our trials for a proper macrocyclization site and the corresponding linear precursors.

2. Synthesis and discussion

Disconnection of the amide bond at site a afforded the first route (see Fig. 2), in which Trp derivative **22** was incorporated at the last step of the linear precursor **4**, and a [3+3+1] peptide-segment-assembly strategy was adopted. The first tripeptide **13** was prepared by the coupling of noncoded amino acid derivative (amine) **12** and dipeptide (acid) **10**,[5] using EDCI-HOBt-based conditions (Scheme 1). Similarly, the other tripeptide **18** was synthesized starting from **14** via two successive coupling reactions. The active ester of N-Boc-Ala-OH generated in situ was treated with β-methoxyphenylalanine derivative (amine) **15** to afford dipeptide **16** (88%), whose N-Boc protecting group could be removed by 10% TFA in dichloromethane quantitatively. Another noncoded amino acid derivative (acid) **17** was activated by EDCI and HOBt and then allowed to react with the amine derived from dipeptide **16**, giving the desired tripeptide **18** in 80% yield.

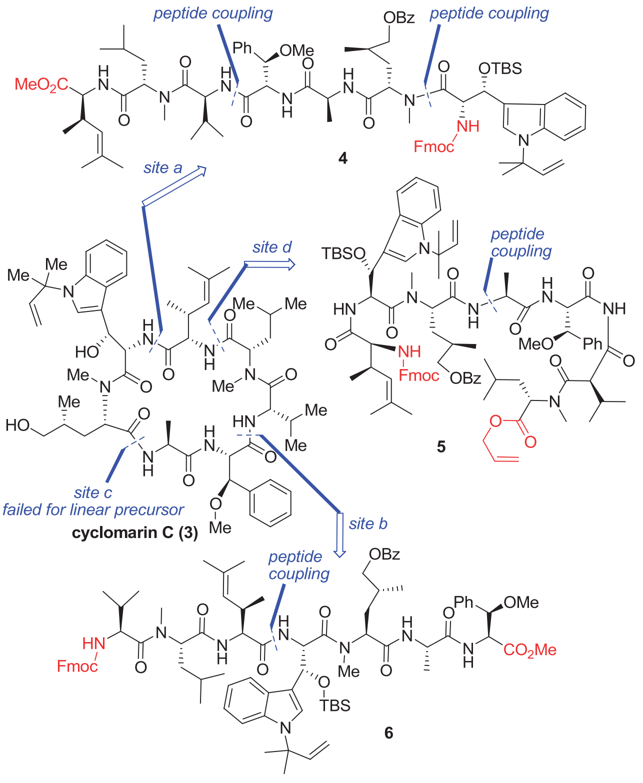
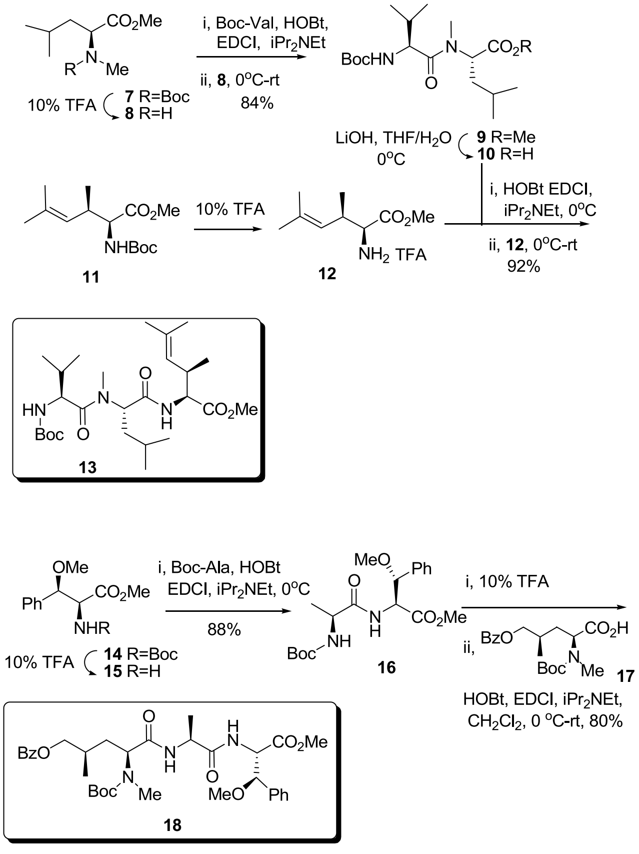
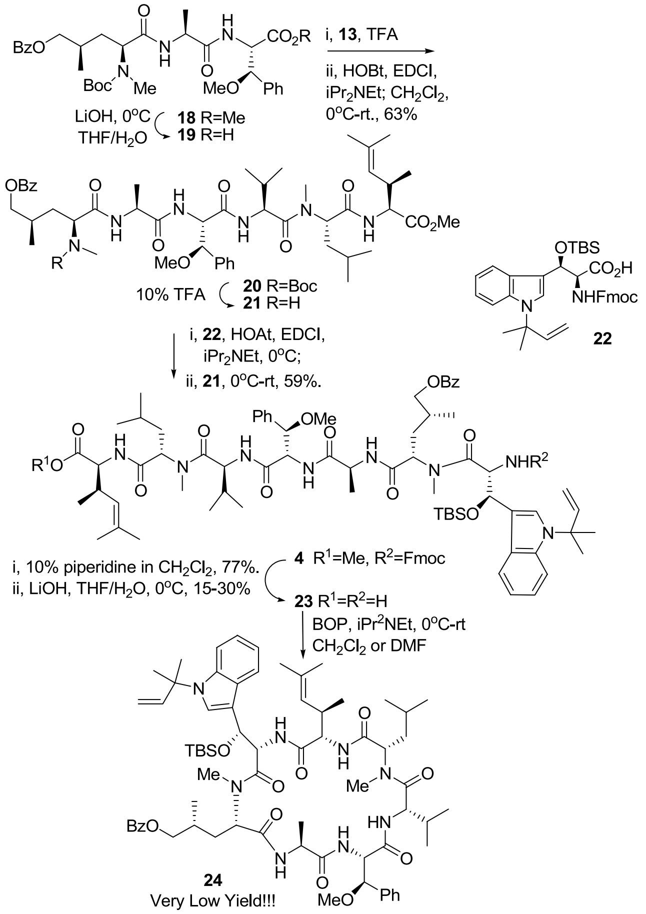


Figure 2. Outline of tested macrocyclization sites and their corresponding linear precursors.

Coupling between the above two tripetides was executed as Scheme 2. At first, methyl ester **18** and N-Boc derivative **13** were treated with LiOH in water–THF and 10% TFA in dichloromethane, respectively, affording the corresponding acid and amine. The amide bond formation between these two intermediates was achieved under EDCI and HOBt conditions, and the linear hexapeptide **20** was obtained in 63% yield. After removal of N-Boc functionality of **20**, the resulting amine reacted with tryptophan derivative **22** in the presence of EDCI and HOAt[6] to give heptapeptide **4** in reasonable yield. Treatment of heptapeptide **4** with 10% piperidine in dichloromethane followed by LiOH in THF–water afforded macrocyclization precursor **23** with both amino and carboxylic acid functionalities. It is noteworthy that the hydrolysis of methyl ester was a low-yield reaction and generated many undesired by-products. To make matters worse, macrocyclization of **23** turned out to be troublesome. A variety of condensation conditions were



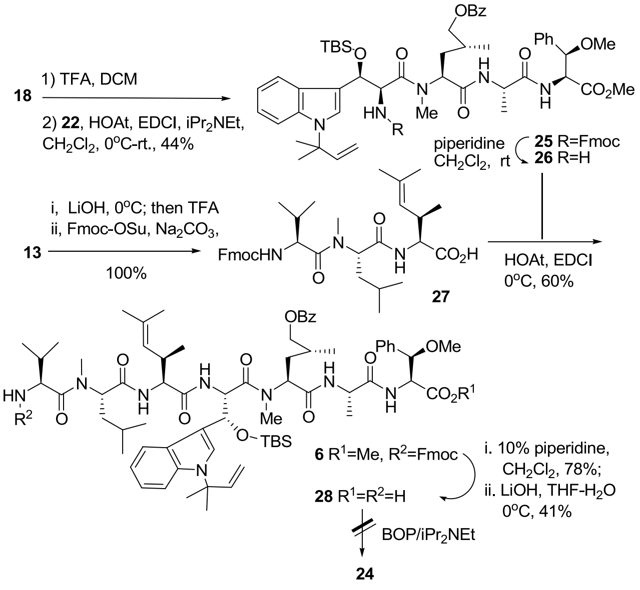
Scheme 1. Synthesis of two tripeptides, **13** and **18**.



Scheme 2. Coupling between tripeptides **13** and **18**, and trials for the macrocyclization.

examined for this cylcization, but none led to satisfactory results. For example, in the presence of BOP and diisopropylethylamine in DMF or dichloromethane, only trace of macrocycle **24** (~2–4%) was formed as detected by mass spectrum and the linear precursor was decomposed during the reaction (Scheme 2). Based on this failure and our experience on the preparation of **22**, we realized that not only the free form of **22** (nonprotected form) is unstable, the linear peptides with it as the terminal moiety, like **23**, were not stable either. So, in the alternative macrocyclization precursors, the unstable component **22** should be placed in the middle region of the final precursor (with two amide bond in both C and N terminals of this amino acid, as it is in natural product) for macrocyclization.

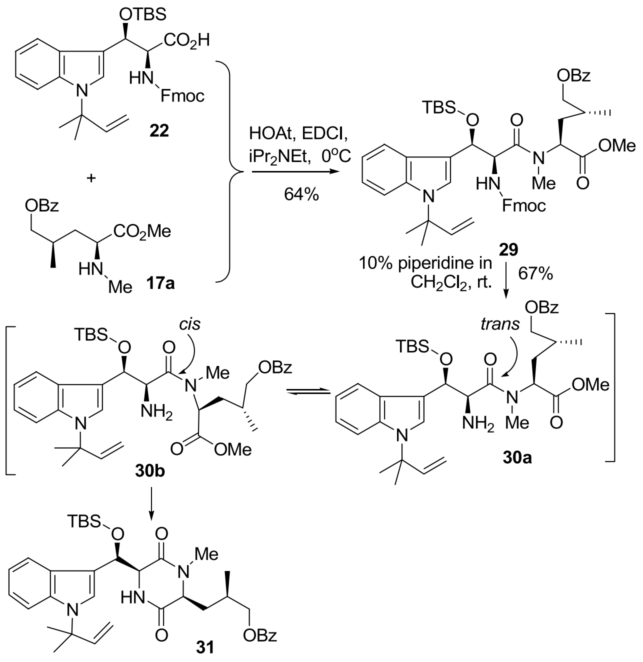
With the above considerations in mind, the second route (b) was designed and heptapeptide 6 was envisioned to be the cyclization precursor (Fig. 2), in which the β-hydroxyl Trp derivative **22** was at the middle of the molecule to avoid the involvement in final cyclization reaction. Synthesis of the linear peptide 6 adopted a [4+3] peptide assembly strategy (Scheme 3). Tripeptide **13** was saponified with LiOH in water, and then treated with 10% TFA in dichloromethane and FmocOSu in the presence of Na_2CO_3 sequentially to give peptide acid **27** quantitatively. The tetrapeptide segment (amine) **26** was prepared by coupling of tripeptide **18** derived amine and β-hydroxyl Trp derivative **22** (44%), and *N*-Fmoc removal by 10% piperidine in dichloromethane. Linkage of segments **26** with **27** was achieved through amide bond formation in the presence of EDCI and HOAt (60% yield) to give heptapeptide **6**, whose *N*-Fmoc group was removed smoothly (78%) using 10% piperidine in dichloromethane. Subsequent saponification of methyl ester gave 41% yield of precursor **28**. The lower yield of ester hydrolysis may be partially due to the elimination of C-terminal residue, β-methoxylphenylalanine methyl ester. To our disappointment, all efforts towards macrocyclization of precursor **28** again failed. Similar to route a, the last step resulted in complex mixtures under a variety of conditions. These results indicate β-methoxyphenylalanine residue is not a suitable C-terminal for the cyclization precursor.



Scheme 3. Synthetic efforts via route b and its [4+3] peptide assembly strategy.

The third approach to cyclomarin C (**3**) through bond disconnection at site c (Fig. 2) also encountered problems at the early stage of total synthesis, and failed to prepare the designed heptapeptide (Scheme 4). Dipeptide **29** with an *N*-methyl amide functionality was found to easily cyclize intramolecularly[7] when it was treated with 10% piperidine in dichloromethane, giving 2,5-piperazinedione derivative **31** (67%). An explanation for this result is the conformational equilibrium (*trans* and *cis*) of *N*-methyl amide bond, in which its *cis* conformation favors the observed cyclization. Because of the severe side reactions in preparing the dipeptides, further investigations were not explored.

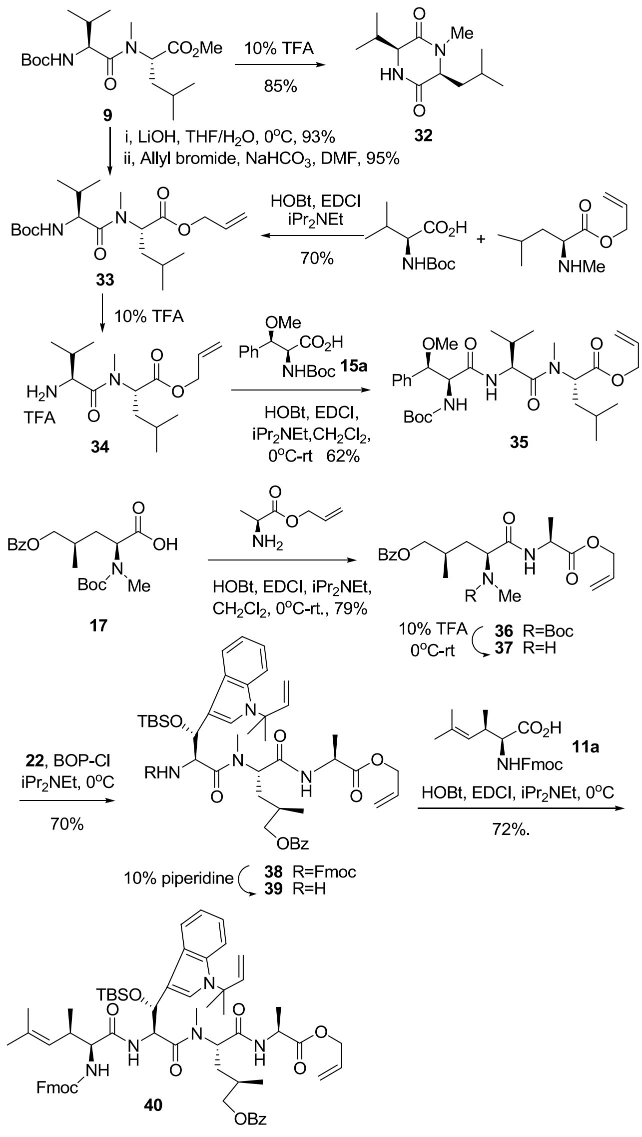
Analyzing the above unsatisfactory results, we concluded that the following situations should be avoided during the total synthesis: (1) the Trp and Phe derivatives involved should not be the terminal of the final linear peptide precursors; (2) preparations of dipeptides with an *N*-methyl amide bond is not favorable (see also the conversion of **9** to **32**, Scheme 5). With those principles, only limited sites could be chosen for the macrocylization. Path d was the final and successful one we tried to elaborate the whole molecule. In this route, cyclomarin C was disconnected at site d and a [4+3] peptide assembly strategy was employed (Fig. 2). In order to avoid the complexity caused by saponifications at



Scheme 4. 2,5-Piperazinedione derivative formation at the early stage of route c.

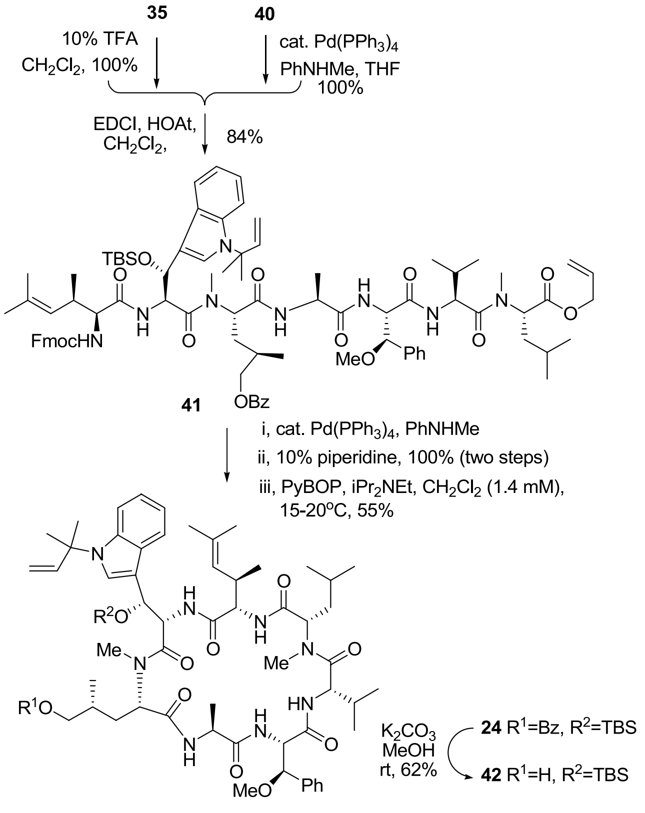
C-terminals, route d adapted allyl ester as the acid protecting form, because it could be easily removed by Pd(0) or Rh(I)-catalyzed isomerization protocols[8–9] in mild conditions (Scheme 5). Dipeptide **33** derived from *N*-Boc Val-OH and *N*-Me Leu(Oallyl) was treated with 10% TFA, giving the dipeptide TFA salt **34**. The *N*-Boc amino acid **15a** was then allowed to couple with **34** in the presence of EDCI and HOBt to afford tripeptide **35** (62%). Preparation of the other segment, tetrapeptide **40** was started from the coupling of *N*-Boc amino acid **17** and Ala(Oallyl) using EDCI and HOBt. The resultant dipeptide **36** was first treated with 10% TFA and then coupled with *N*-Fmoc amino acid **22** using BOPCl as the carboxyl activation reagent to afford tripeptide **38** (70%). The fourth amino acid derivative **11a** was finally incorporated into amino-liberated tripeptide **39**, affording tetrapeptide **40** (72%). Parallel removals of *N*-Boc protecting group of **35** (10% TFA in dichloromethane) and *O*-allyl ester of **40** ($Pd(PPh_3)_4$, PhNHMe, THF)[10] afforded the both precursors essential for the followed coupling to generate the desired heptapeptide **41** (Scheme 6).

This coupling reaction was performed very well under the conditions of EDCI and HOAt.[11] It is noteworthy here that replacement of HOAt with HOBt resulted in much lower efficiency. Upon stable heptapeptide **41**, both the N- and C-terminal protections were similarly removed by the above methods, Pd(0)-catalyzed deprotection of allyl ester and 10% piperidine for *N*-Fmoc removal. Under the presence of PyBOP[12] and diisopropylethylamine, the resulting precursor was cyclized smoothly by head to tail in dichloromethane at a diluted concentration (1.4 mM), affording macrocycle **24** in 55% yield. Deprotection of benzoate at one of the side chains of **24** was achieved by K_2CO_3 in methanol at rt, giving **42** in 62% yield. Unfortunately, the followed removal of *O*-TBS ether existing in **42** using TBAF failed. No reactions were observed at rt, while decomposition happened when the temperature was raised up to 60–70 °C. Spatially hindrance enhancement after macrocycle formation may be a reason for the difficulty to remove *O*-TBS ether of **42**. Therefore, we decided to remove *O*-TBS ether before the cyclization step, that is, at the linear stage (Scheme 7). The subtle change of the reaction sequence proved very successful. Removal of caps at C- and N-terminals of **41** followed by TBS-deprotection with TBAF gave a satisfactory yield of **43** (98%). Macrocylclization was again accomplished under similar conditions and afforded macrocycle **44** (63%). Finally, treatment of **44** with K_2CO_3 in methanol at rt afforded cyclomarin C (**3**, 38 mg, 80% yield), and reproducibility of this reaction was perfectly high.



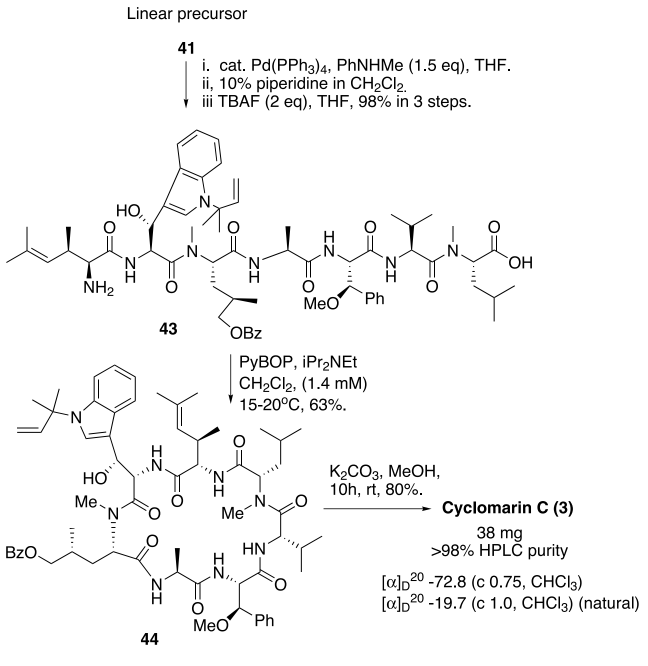
Scheme 5. Synthesis of tetrapeptide **40** and tripeptide **35**.

The synthetic sample of **3** purified by silica gel chromatography showed high purity (>98%, by HPLC analysis using same conditions described in Ref. 1). However, significant difference was observed in comparison of specific optical rotation of synthetic sample ($[\alpha]_D^{20} = -72.8$ (*c* 0.75, $CHCl_3$)) with that reported for natural product ($[\alpha]_D^{20} = -19.7$ (*c* 1.0, $CHCl_3$)). Because of unavailability



Scheme 6. Macrocyclization of linear heptapeptide.

of natural product sample for a CD spectrum, peak-to-peak comparisons[4] of both ^{1}H NMR and ^{13}C NMR spectra were adapted. These results show the synthetic sample is exactly same as the natural one, while the reported ^{1}H NMR spectrum of natural product (in supporting information of Ref. 1) shows that it contains small amount of impurities.



Scheme 7. Completion of total synthesis of cyclomarin C.

3. Conclusion

In summary, our efforts to search for the suitable macrocylization sites and the first total synthesis of cyclomarin C were described in details. These studies showed that the Trp and Phe derivatives involved in the target could not be the terminals of the final linear peptide precursors, and preparations of the corresponding dipeptides with an *N*-methyl amide bond is not favorable. Site d finally proved to be a proper site for macrocyclization, and the head-to-tail macrocyle formation was realized under the dilute conditions at ambient temperature in repeatable and satisfactory yields. The first total synthesis of cyclomarin C was thus achieved based on these explorations. All these will be helpful for the synthesis of other members of cyclomarins. Further applications of the developed methods and strategies to cyclomarins A and B, as well as the evaluation of activity contribution caused by each amino acid component in cyclomarins are under investigation in our laboratory.

4. Experimental

4.1. General

Physical data of compounds in rout d are available in supporting information of Ref. 3.

4.1.1. Dipeptide 9. To a solution of *N*-Boc, *N*-methyl-L-Leu-OMe (3.8 g, 14.7 mmol) in dichloromethane (60 mL) cooled with ice-water bath was added dropwise TFA (9 mL). After stirred at room temperature for about 4.5 h, the reaction mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (30 mL) and basified to pH 9 with sat. aq. $NaHCO_3$ solution at 0 °C. The aqueous phase was separated and extracted with ethyl acetate (20 mL×3). The organic layers were dried with anhydrous Na_2SO_4 and concentrated to give compound **8** (2.42 g) as a yellow oil, which was used in the next step without further purification.

To a solution of *N*-Boc-L-valine (3.25 g, 15 mmol) in dichloromethane (60 mL) at 0 °C were added successively HOBt (2.18 g, 16 mmol), EDCI (3.13 g, 16 mmol) and diisopropylethylamine (2.85 mL, 16 mmol). The mixture was stirred for 20 min, and then a solution of compound **8** (14.7 mmol) in dichloromethane (60 mL) was added. The resulting mixture was stirred at room temperature until starting material disappeared, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane (60 mL×3). The organic phases were combined, washed with sat. aq. NH_4Cl solution (50 mL×3) and brine (50 mL) and dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by silica gel chromatography (PE/EA, 5/1, v/v) to give compound **9** (4.42 g, 84%) as an oil.[5] $[\alpha]_D^{20} = -26.8$ (*c* 1.05, $CHCl_3$). IR: ν_{max} 1014, 1176, 1270, 1367, 1496, 1648, 1709, 1745, 2961, 3327 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85–1.02 (m, 12H), 1.43 (s, 9H), 1.67–1.77 (m, 3H), 2.00 (m, 1H), 3.02 (s, 3H), 3.70 (s, 3H), 4.43 (dd, 1H, $J=6.9$, 9.3 Hz), 5.22 (d, 1H, $J=8.7$ Hz), 5.38

(dd, 1H, $J=6.9$, 9.0 Hz) ppm. EI-MS (*m/z*): 302 (M^+-56), 285 (M^+-100).

4.1.2. Tripeptide 13. To a solution of compound **9** (700 mg, 1.96 mmol) in THF/H_2O (10 mL, v/v 2:1) at 0 °C was added $LiOH\cdot H_2O$ (168 mg, 4 mmol). After 3 h, 4 N HCl was added to adjust the pH to 2–3. The mixture was extracted three times with ethyl acetate. The organic phases were dried with anhydrous Na_2SO_4 and then concentrated to give the crude product **10**.[5] The crude acid was dissolved in dichloromethane (5 mL) and cooled with ice-water bath. To it were added sequentially diisopropylethylamine (0.16 mL, 0.95 mmol), HOBt (83 mg, 0.61 mmol) and EDCI (119 mg, 0.61 mmol). After 15 min, a solution of **12** (0.47 mmol) in dichloromethane (2 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then allowed to warm to room temperature and stirred for additional 10 h, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The organic phases were combined, washed with sat. aq. NH_4Cl solution and brine (50 mL) and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 5/1, v/v) to give compound **13** (208 mg, 89%) as an oil. $[\alpha]_D^{20}=-56.7$ (*c* 1.1, $CHCl_3$). IR: ν_{max} 1015, 1174, 1367, 1518, 1630, 1685, 1743, 2933, 2964, 3315 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.82–1.02 (m, 15H), 1.43 (s, 9H), 1.52–1.70 (m, 3H), 1.65 (s, 6H), 1.92–2.05 (m, 1H), 2.75–2.85 (m, 1H), 3.01 (s, 3H), 3.71 (s, 3H), 4.39–4.45 (m, 2H), 4.86 (d, 1H, $J=9.6$ Hz), 5.15–5.22 (m, 2H), 6.34 (d, 1H, $J=8.1$ Hz) ppm. ESI-MS (*m/z*): 498.3 (MH^+).

4.1.3. Dipeptide 16. To a mixture of compound **15** (3.2 mmol) and *N*-Boc-L-Ala-OH (725 mg, 3.8 mmol) in dichloromethane (15 mL) at 0 °C were added sequentially diisopropylethylamine (0.75 mL, 4.18 mmol), HOBt (569 mg, 4.18 mmol) and EDCI (818 mg, 4.18 mmol). The reaction mixture was stirred at the same temperature for 2 h, additional 1 h at room temperature, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane (10 mL × 3). The combined organic phase was washed with sat. aq. NH_4Cl solution (7 mL × 3) and brine (7 mL), and dried with anhydrous Na_2SO_4. After filtration, the filtrate was concentrated and the residue was purified by silica gel chromatography (PE/EA, 4/1, v/v) to give compound **16** (1.07 g, 88%) as an oil. $[\alpha]_D^{20}=-52.2$ (*c* 1.4, $CHCl_3$). IR: ν_{max} 716, 1098, 1178, 1555, 1676, 1749, 2933, 3271, 3364 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (m, 3H), 1.45 (s, 9H), 3.28 (s, 3H), 3.76 (s, 3H), 4.13 (m, 1H), 4.73–4.85 (m, 3H), 6.70 (d, 1H, $J=8.7$ Hz), 7.25–7.37 (m, 5H) ppm. ESI-MS (*m/z*): 403.2 ($M+Na^+$).

4.1.4. Tripeptide 18. To a solution of **17** (438 mg, 1.2 mmol) in dichloromethane (5 mL) at 0 °C were added successively HOBt (177 mg, 16 mmol), EDCI (254 mg, 1.3 mmol), and diisopropylethylamine (0.80 mL, 4.4 mmol) and then a solution of dipeptide **16** derived amine (1 mmol) in dichloromethane (5 mL). After stirred at 0 °C for 2 h and then room temperature 2 h, the reaction mixture was quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phase was washed with sat. aq. NH_4Cl solution and brine, and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (PE/EA, 2/1, v/v) to give compound **18** (500 mg, 80%) as white solid. IR: ν_{max} 714, 1142, 1274, 1509, 1721, 1752, 2977, 3322 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.11 (d, 3H, $J=6.6$ Hz), 1.20 (d, 3H, $J=7.2$ Hz), 1.47 (s, 9H), 1.70–2.18 (m, 3H), 2.77 (s, 3H), 3.26 (s, 3H), 3.75 (s, 3H), 4.20–4.30 (m, 2H), 4.40–4.60 (m, 2H), 4.70–4.82 (m, 2H), 6.37–6.74 (m, 2H), 7.20–7.40 (m, 5H), 7.40–7.50 (m, 2H), 7.54–7.60 (m, 1H), 8.04 (d, $J=7.8$ Hz, m, 2H) ppm. ESI-MS (*m/z*): 628.3 (MH^+). Anal. Calcd for $C_{33}H_{45}N_3O_9$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.08; H, 7.24; N, 6.55.

4.1.5. Hexapeptide 20. Acid **19** (0.30 mmol) derived from ester **18** by saponification was dissolved in dichloromethane (3 mL) and cooled with ice-water bath. To it were added sequentially diisopropylethylamine (52 μL, 0.30 mmol), HOBt (49 mg, 0.36 mmol) and EDCI (70 mg, 0.36 mmol). After 15 min, a solution of an amine (0.30 mmol, prepared from **13** by treatment with TFA) in dichloromethane (3 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then at room temperature overnight, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phase was washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 1/1, v/v) to give compound **20** (186 mg, 63%) as a solid. $[\alpha]_D^{20}=-75.9$ (*c* 0.7, $CHCl_3$). IR: ν_{max} 713, 1100, 1274, 1522, 1627, 1655, 1689, 1723, 1745, 2933, 2966, 3310 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.90–1.00 (m, 18H), 1.13 (d, 3H, $J=6.3$ Hz), 1.50 (s, 9H), 1.58–1.78 (m, 4H), 1.66 (s, 6H), 1.88–2.16 (m, 3H), 2.80 (s, 3H), 2.78–2.82 (m, 1H), 2.99 (s, 3H), 3.31 (s, 3H), 3.70 (s, 3H), 4.26 (d, 2H, $J=4.5$ Hz), 4.08–4.30 (m, 2H), 4.60–4.92 (m, 6H), 5.18–5.25 (m, 1H), 6.34 (d, 1H, $J=9.3$ Hz), 6.70–6.79 (m, 2H), 7.13–7.38 (m, 5H), 7.42–7.50 (m, 2H), 7.55–7.60 (m, 1H), 8.08 (d, 2H, $J=7.8$ Hz) ppm. HR-MS (ESI, *m/z*) for $C_{53}H_{80}N_6O_{12}Na^+$: 1015.5726, found: 1015.5719.

4.1.6. Heptapeptide 4. To a solution of acid **22** (94 mg, 102 μmol) in dichloromethane (2 mL) were added sequentially diisopropylethylamine (36 μL, 0.20 mmol), HOBt (28 mg, 0.20 mmol) and EDCI (40 mg, 0.20 mmol). After 15 min, a solution of compound **21** (70 μmol, prepared from **20** by treatment with TFA) in dichloromethane (2 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then at room temperature overnight, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (CH_2Cl_2/MeOH, 100/1, v/v) to give compound **4** (62 mg, 59%) as a solid. IR: ν_{max} 742, 1099, 1211, 1453, 1529, 1631, 1686, 1722, 2928, 2960, 3310 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ −0.24 (m, 3H), 0.15 (s, 3H), 0.78–1.00 (m, 27H), 1.15 (d, 3H, $J=6.9$ Hz), 1.52–1.74 (m, 16H), 1.90–2.10 (m, 3H), 2.64 (s, 3H), 2.80 (m, 1H), 2.91 (s, 3H), 3.10 (s, 3H), 3.70 (s, 3H), 4.22–4.47 (m, 5H), 4.50–4.92 (m, 8H), 5.00–5.35 (m, 5H), 5.80–6.12 (m, 2H), 6.38 (d, 1H, $J=8.7$ Hz), 6.62 (d, 1H, $J=7.2$ Hz), 7.00–7.66 (m, 18H), 7.72–7.82 (m, 2H), 7.90 (d, 1H, $J=9.0$ Hz), 8.00 (t, 2H, $J=7.5$ Hz), 8.13

(m, 1H) ppm. HR-MS (ESI, *m/z*) for $C_{85}H_{114}N_8O_{14}SiNa^+$: 1521.8116, found: 1521.8107.

4.1.7. Compound 24. To a solution of **4** (100 mg, 67 μmol) in dichloromethane (5 mL) was added piperidine (0.5 mL) at 0 °C. The mixture was stirred for 2 h and then concentrated. The residue was purified by flash chromatography (PE/EA, 2:1 then CH_2Cl_2/MeOH, 20/1) to give the de-Fmoc product (66 mg, 77%). The product obtained above was dissolved in THF/H_2O (8 mL, 3:1) and cooled with ice-water bath. To it was added LiOH·H_2O (9 mg, 0.21 mmol), and the resulting mixture was stirred for 9 h, neutralized with 1 N HCl to pH 7 and then extracted with ethyl acetate. The combined organic phases were dried with anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by chromatography (CH_2Cl_2/MeOH, 100/1, 40/1 and then 10/1) to give **23** (16 mg, 25%) as a solid. **23** (15 mg, 12 μmol) was dissolved in dichloromethane (10 mL), and was added slowly via syringe pump to a mixture of BOP (16 mg, 36 μmol) and diisopropylethylamine (7 μL, 40 μmol) in dichloromethane (10 mL) over 12 h. After complete addition, the reaction mixture was stirred for additional 4 h and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA 4/1, 2/1, 1/1) to give compound **24** (4 mg). ESIMS (*m/z*): 1263.7 (MH^+).

4.1.8. Heptapeptide 6. To a solution of compound **13** (30 mg, 60 μmol) in THF/H_2O (2.5 mL, v/v 4:1) at 0 °C was added LiOH·H_2O (5 mg, 120 μmol). After 4 h, 4 N HCl was added to adjust the pH to 3–4. The mixture was extracted three times with ethyl acetate. The organic phases were combined, dried with anhydrous Na_2SO_4, and then concentrated to give the crude product. The crude acid (29 mg) was dissolved in dichloromethane (2 mL) and cooled with ice-water bath. To it was added TFA (0.5 mL), and the resulting mixture was stirred for 2 h, and then concentrated. The residue was dissolved in THF (2 mL) and neutralized with sat. aq. $NaHCO_3$ at 0 °C, then solid sodium carbonate (13 mg, 120 μmol) and Fmoc-*O*su (24 mg, 63 μmol) were added. After 3 h, 0.5 N HCl was added to adjust the pH to 2–3. The aqueous phase was extracted with ethyl acetate (10 mL×3). The organic phases were combined, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 4/1, then CH_2Cl_2/MeOH, 20/1) to give dipeptide-acid **27** (38 mg, 100%).

4.1.9. Compound 25. The title compound was prepared according to the procedure similar to compound **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.31 to −0.23 (m, 3H), 0.14 (d, 3H, J=5.1 Hz), 0.81 (s, 9H), 0.85 (d, 3H, J=6.6 Hz), 1.10 (d, 3H, J=7.2 Hz), 1.50–1.80 (m, 9H), 2.63 (d, 2H, J=4.8 Hz), 2.80 (d, 1H, J=3.0 Hz), 3.10 (s, 2H), 3.21 (s, 1H), 3.60 (s, 2H), 3.70 (s, 1H), 4.10–4.38 (m, 6H), 4.60–4.76 (m, 5H), 5.00–5.32 (m, 2H), 5.81 (d, 1H, J=4.5 Hz), 5.98–6.18 (m, 1H), 6.65–6.78 (m, 2H), 7.00–8.04 (m, 18H) ppm.

To a solution of acid **27** (19 mg, 31 μmol) in dichloromethane (1 mL) were added sequentially diisopropylethylamine (6 μL, 34 μmol), HOAt (4 mg, 29 μmol) and EDCI (6 mg, 31 μmol). After 15 min, a solution of compound **26** (24 μmol, prepared from **25** by treatment with piperidine) in dichloromethane (1 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, then at room temperature for 5 h, and then quenched with aq. NH_4Cl solution at 0 °C. The aqueous phase was extracted with dichloromethane. The combined organic phases were washed with sat. aq. NH_4Cl solution and brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography (PE/EA, 2/1, v/v) to give compound **6** (23 mg, 60%) as a solid. ^{1}H NMR (300 MHz, d^6-DMSO): δ −0.38 (s, 3H), −0.07 (s, 3H), 0.64 (s, 9H), 0.89–0.98 (m, 15H), 1.12 (d, 3H, J=7.5 Hz), 1.24 (m, 3H), 1.41 (s, 3H), 1.52 (s, 3H), 1.60 (s, 3H), 1.63 (s, 3H), 1.40–1.70 (m, 4H), 1.92–2.10 (m, 3H), 2.80 (m, 1H), 2.93 (s, 3H), 3.10 (s, 3H), 3.34 (s, 3H), 3.50 (s, 3H), 3.85–4.34 (m, 8H), 4.45 (dd, 2H, J=4.5, 9.0 Hz), 4.66 (d, 1H, J=4.8 Hz), 4.82–5.10 (m, 6H), 5.38–5.50 (m, 1H), 6.10 (dd, 1H, J=11.1, 18.6 Hz), 6.90–7.04 (m, 3H), 7.20–7.54 (m, 10H), 7.54–7.80 (m, 7H), 7.80–8.00 (m, 6H), 8.40–8.52 (m, 1H) ppm. ESI-MS (*m/z*): 1520.8 ($M+NH_4^+$), 1521.7 ($M+Na^+$).

4.1.10. Dipeptide 29. The title compound was prepared according to the procedure similar to compound **6**: $[\alpha]_D^{20}=-38.2$ (c 1.2, $CHCl_3$). IR: ν_{max} 741, 838, 1071, 1274, 1452, 1721, 2929, 2955, 3310 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.27 (s, 3H), 0.04 (s, 3H), 0.84 (s, 9H), 0.98 (d, 3H, J=6.6 Hz), 1.60–1.70 (m, 1H), 1.70 (d, 6H, J=3.9 Hz), 1.80–1.92 (m, 1H), 1.98–2.10 (m, 1H), 2.71 (s, 3H), 3.46 (s, 3H), 4.04–4.38 (m, 5H), 5.10 (d, 1H, J=17.1 Hz), 5.14–5.25 (m, 4H), 5.79 (d, 1H, J=8.1 Hz), 6.15 (dd, 1H, J=10.2, 17.1 Hz), 7.02–7.14 (m, 2H), 7.22–7.62 (m, 11H), 7.71–7.82 (m, 3H), 8.01 (d, 2H, J=6.9 Hz) ppm. ESI-MS (*m/z*): 908.4 ($M+Na^+$), 754.3 (M^+-131). Anal. Calcd for $C_{52}H_{63}N_3O_8Si$: C, 70.48; H, 7.17; N, 4.74. Found: C, 70.62; H, 7.42; N, 4.49.

4.1.11. Compound 31. To a solution of **29** (22 mg, 25 μmol) in dichloromethane (2 mL) at 0 °C was added dropwise piperidine (0.2 mL). The mixture was stirred for 1 h, and then concentrated. The residue was purified by chromatography (PE/EA, 3/1 then CH_2Cl_2/MeOH, 50/1) to give compound **31** (11 mg, 67%) as a solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.16 (s, 3H), −0.06 (s, 3H), 0.89 (s, 9H), 1.10 (d, 3H, J=6.6 Hz), 1.62–1.70 (m, 1H), 1.72 (s, 3H), 1.76 (s, 3H), 2.30–2.42 (m, 1H), 2.58–2.69 (m, 1H), 3.02 (s, 3H), 3.88 (d, 1H, J=8.7 Hz), 4.12–4.20 (m, 2H), 4.33 (d, 1H, J=10.5 Hz), 5.13 (d, 1H, J=17.4 Hz), 5.23 (d, 1H, J=10.8 Hz), 5.64 (s, 1H), 5.76 (d, 1H, J=1.8 Hz), 6.12 (dd, 1H, J=10.8, 17.4 Hz), 7.06–7.19 (m, 2H), 7.30 (s, 1H), 7.40–7.63 (m, 5H), 8.05 (d, 2H, J=8.4 Hz) ppm. ESI-MS (*m/z*): 500.3 (M^+-131).

4.1.12. Cyclomarin C.[3] $[\alpha]_D^{20}=-72.8$ (c 0.8, $CHCl_3$); IR: ν_{max} 703, 742, 1095, 1376, 1458, 1509, 1649, 1686, 2873, 2962, 3310 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.62 (d, 3H, J=6.3 Hz), 0.65 (m, 1H), 0.73 (d, 3H, J=6.7 Hz), 0.82 (d, 3H, J=6.6 Hz), 0.88 (d, 3H, J=6.6 Hz), 0.93 (d, 3H, J=6.6 Hz), 1.05 (d, 3H, J=6.3 Hz), 1.05 (m, 1H), 1.25 (s, 3H), 1.31 (d, 3H, J=7.2 Hz),1.43 (m, 2H), 1.55 (s, 3H), 1.63 (m, 1H), 1.70 (s, 3H), 1.73 (s, 3H), 2.15–2.35 (m, 3H), 2.42 (br s, 1H), 2.72 (s, 3H), 2.82 (s, 3H), 3.10–3.30 (m, 2H), 3.35 (s, 3H), 4.07 (t, 1H, $J=9.8$ Hz), 4.30 (s, 1H), 4.40 (t, 1H, $J=8.7$ Hz), 4.56 (br s, 1H), 4.77 (d, 1H, $J=10.0$ Hz), 4.78–4.83 (m, 2H), 4.88 (t, 1H, $J=4.9$ Hz), 4.82–4.93 (m, 1H), 5.07 (d, 1H, $J=5.2$ Hz), 5.15 (d, 1H, $J=17.4$ Hz), 5.22 (d,1H, $J=10.7$ Hz), 5.31 (d, 1H, $J=3.0$ Hz), 6.07 (dd, 1H, $J=10.7$, 17.4 Hz), 6.72 (d, 1H, $J=2.9$ Hz), 7.05 (dd, 1H, $J=7.2$, 7.2 Hz), 7.12 (d, 1H, $J=5.3$ Hz), 7.15–7.21 (m, 2H), 7.22–7.30 (m, 4H), 7.33 (s, 1H), 7.48 (d, 1H, $J=8.3$ Hz), 7.55 (d, 1H, $J=7.8$ Hz), 7.95 (d, 1H, $J=7.7$ Hz), 8.05 (d, 1H, $J=9.3$ Hz), 8.17 (d, 1H, $J=10.3$ Hz) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.8, 18.5, 18.9, 19.3, 20.0, 20.8, 22.4, 23.5, 25.1, 25.7, 27.9, 27.9, 29.3, 29.5, 30.9, 33.2, 33.4, 35.6, 38.9, 50.6, 52.7, 55.3, 55.9, 57.7, 58.1, 58.6, 59.3, 59.3, 66.4, 68.5, 80.0, 111.4, 113.8, 114.4, 118.7, 119.5, 121.5, 123.1, 124.8, 126.8, 127.9, 128.3, 128.6, 128.7, 134.5, 135.1, 135.8, 143.7, 168.4, 168.8, 169.6, 170.6, 171.1, 171.6, 172.6 ppm. ESI-MS (*m/z*): 1049.4 ($M+Na^+$), 1009.5 (MH^+-H_2O).

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References and notes

1. Renner, M. K.; Shen, Y. C.; Cheng, X. C.; Jensen, P. R.; Frankmolle, W.; Kauffman, C. A.; Fenical, W.; Lobkovsky, E.; Clardy, J. J. *J. Am. Chem. Soc.* **1999**, *121*, 11273–11276.
2. (a) Wen, S.-J.; Zhang, H.-W.; Yao, Z.-J. *Tetrahedron Lett.* **2002**, *43*, 5291–5294. (b) Sugiyama, H.; Shioiri, T.; Yokokawa, F. *Tetrahedron Lett.* **2002**, *43*, 3489–3492. (c) Tarver, J. E.; Joullie, M. M., Jr. *J. Org. Chem.* **2004**, *69*, 815–820.
3. Wen, S.-J.; Yao, Z.-J. *Org. Lett.* **2004**, *6*, 2721–2724.
4. See Supporting Information for Ref. 3 at http://pubs.acs.org/.
5. Hasuoka, A.; Nishikimi, Y.; Nakayama, Y.; Kamiyama, K.; Nakao, M.; Miyagawa, K.-i.; Nishimura, O.; Fujino, M. *J. Antibiot.* **2002**, *55*, 322–336.
6. Hoeg-Jensen, T.; Holm, A.; Sorensen, H. *Synthesis* **1996**, 383–387.
7. Umemiya, H.; Kagechika, H.; Hashimoto, Y.; Shudo, K. *Nucleotides Nucleosides* **1996**, *15*, 465–467.
8. Naegele, E.; Schelhaas, M.; Kuder, N.; Waldmann, H. *J. Am. Chem. Soc.* **1998**, *120*, 6889–6902.
9. Waldmann, H.; Kunz, H. *Liebigs Ann. Chem.* **1983**, *10*, 1712–1725.
10. Kiho, T.; Nakayama, M.; Yasuda, K.; Miyakoschi, S.; Inukai, M.; Kogen, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2315–2318.
11. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
12. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.

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Synthesis, structural studies and desilylation reactions of some *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates

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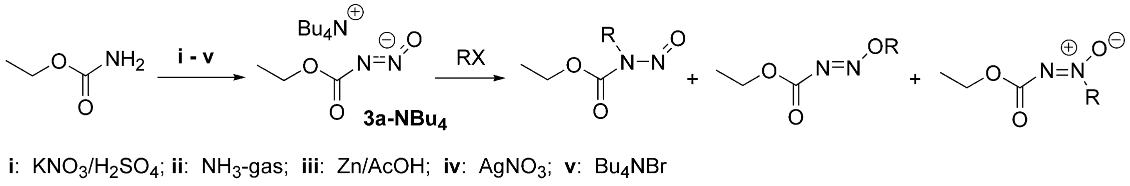
Abstract—The present report describes the preparation and characterization of several *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates, designed as precursors to thermally unstable secondary *N*-nitrosocarbamate anions via fluoride-assisted cleavage. X-ray structural studies demonstrate that the core *N*-nitrosocarbamate moiety has a nearly planar geometry, with an *s-E* orientation at the N–N bond. DFT calculations (B3LYP/6-31 + G(d)) reproduce accurately the structural features of the title compounds and detailed conformational analysis at the same level of theory addresses the long-standing issue of preferred geometries for three classes of related structures: *N*-nitrosocarbamates, *N*-nitrosoureas and *N*-nitrosoamides. Desilylation studies demonstrate that both the solvent and the fluoride concentration influence the rate of the process.

1. Introduction

The work reported in this article is a reflection of our continuing efforts to generate and study secondary *N*-nitrosocarbamates, a class of thermally unstable and chemically labile structures.[1] The only known example of a secondary *N*-nitrosocarbamate, *N*-nitrosourethane, was prepared more than a 100 years ago from urethane, in a sequence of nitration followed by partial reduction, and conversion to the silver salt.[2] Its chemistry was recently revisited, the authors using primarily the organic-soluble tetrabutylammonium salt (Scheme 1).[1]

The experimental studies and gas-phase calculations led to the conclusion that the *N*-nitrosourethane anion is a typical ambident nucleophile, yielding products of both N-alkylation and O-alkylation, and the ratio of N- to O-alkylated product depends, as expected, on the steric demand of the alkyl halide. Smaller and less branched alkyl groups lead primarily to N-alkylation, while the O-alkylated product becomes predominant with the use of more sterically hindered substrates. In addition, the theoretical analysis and kinetic measurements demonstrated that the *N*-nitrosourethane anion and its O-alkylated derivatives undergo thermal decomposition in a concerted fashion, through a four-membered cyclic TS.

Certain questions, however, were not addressed, such as the potential influence of the size of the carbamate alkyl (or aryl) group on the thermal stability of the resultant *N*-nitrosocarbamate anions and their O-alkylated derivatives. In addition, varying the size of the carbamate alkyl (aryl) group would be expected to influence the regioselectivity in their alkylation reactions. Hence, our goal has



i: KNO_3/H_2SO_4; **ii**: NH_3-gas; **iii**: Zn/AcOH; **iv**: $AgNO_3$; **v**: Bu_4NBr
R = Me, Et, *i*-Pr, benzyl

Scheme 1.

Keywords: *N*-Nitrosocarbamates; X-ray structures; 2-(Trimethylsilyl)ethyl group; Desilylation; DFT calculations.

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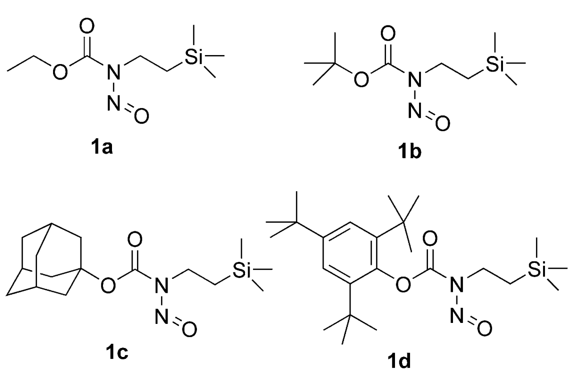


Figure 1. Four *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates with a gradually increasing size of the carbamate ester group.

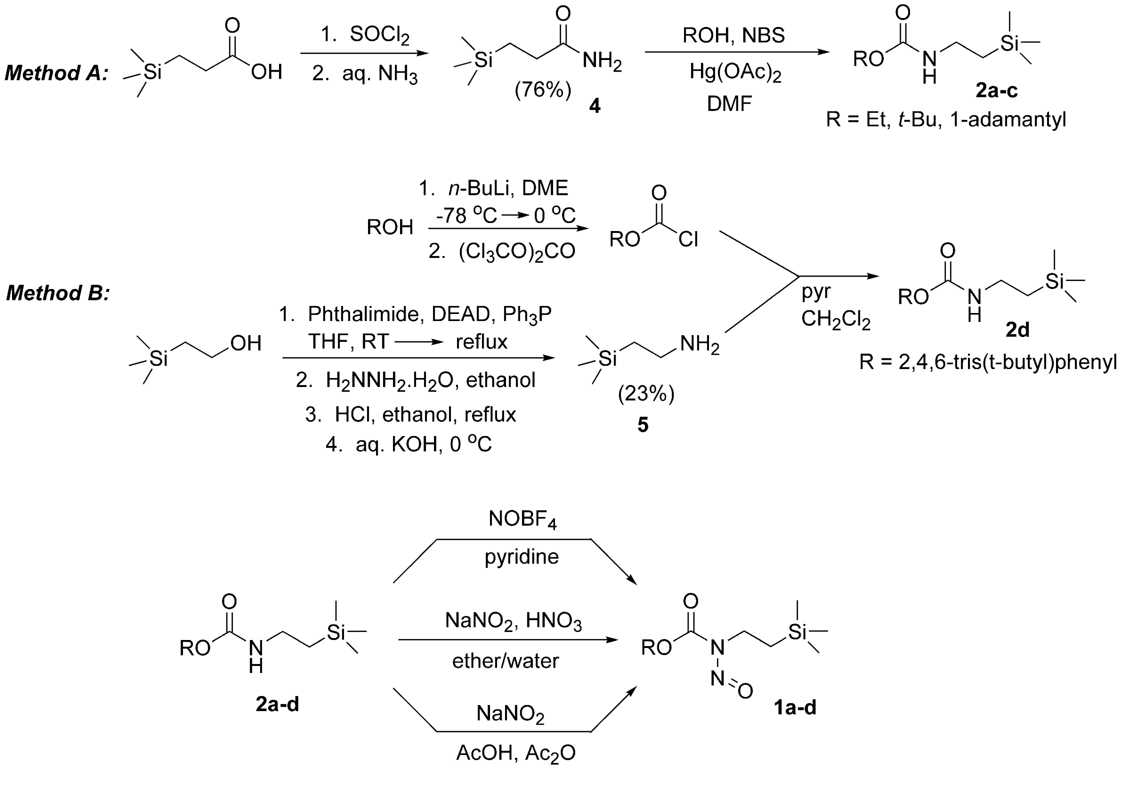
been to prepare and study other secondary *N*-nitrosocarbamates, with varying size of the carbamate alkyl (aryl) group, in an attempt to prepare exceptionally stable structures, suitable for isolation and solid-state studies. The initial efforts were focused on adaptation of the synthetic strategy used in the preparation of *N*-nitrosourethane salts. Such attempts have proved futile, prompting us to design and implement a new methodology, based on the fluoride-assisted cleavage of a 2-(trimethylsilyl)ethyl group (TMSE) incorporated into thermally and chemically stable *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates. The TMSE group has been used in the past as a protecting group for alcohols and carboxylic acids,[3] thiols[4,5] and phosphates.[6] In a recent article, Bottaro et al. described its use as a removable moiety at a nitrogen center, in the generation of the dinitroamide anion.[7]

The present report outlines the details of our synthetic work leading to the preparation of four *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates (**1a–d**, Fig. 1) and the current results from desilylation experiments. This article also reports the first crystal structures of *N*-nitrosocarbamates, compounds **1c** and **1d**, and compares those with results from gas-phase DFT calculations (B3LYP/6-31+G(d)).

2. Results and discussion

2.1. Synthesis

The preparation of carbamates **1a–d** is outlined in Scheme 2. The corresponding alcohol (for compounds **1a–c**) or 2,4,6-tris(*t*-butyl)phenol (for compound **1d**) is converted to an *N*-2-(trimethylsilyl)ethylcarbamate (**2a–d**), by means of one of two general methods (A and B). Method A utilizes a Hoffmann-type rearrangement reaction of 3-(trimethylsilyl)propanamide (**4**)[8] with the corresponding alcohol, in the presence of NBS and $Hg(OAc)_2$.[9] The reaction works very well for preparation of the ethyl carbamate (**2a**), but the yields drop significantly for the *t*-butyl (**2b**) and 1-adamantyl (**2c**) carbamates, and no reaction is observed in an attempt to generate the 2,4,6-tris(*t*-butyl)phenyl carbamate (**2d**). The latter was prepared using an alternative approach (Method B) that includes deprotonation of the starting phenol followed by reaction with triphosgene, leading to the generation of 2,4,6-tris(*t*-butyl)phenyl chloroformate.[10] Reaction of the chloroformate with 2-(trimethylsilyl)-ethanamine (**5**) affords the carbamate **2d**. Compound **5** was prepared according to a patented procedure utilizing 2-(trimethylsilyl)ethanol, which is converted, in a Mitsunobu reaction, to *N*-2-(trimethylsilyl)ethylphthalimide.[11] The phthalimide, upon reaction with hydrazine



Scheme 2.

hydrate followed by acidification, yields 2-(trimethylsilyl)ethanamine hydrochloride. In strongly basic conditions the hydrochloride is converted to the free amine **5**.

Nitrosation reactions were carried out utilizing three different approaches. Nitrosation in a two-phase (water–ether) system was carried out, with the nitrosating agent generated in the aqueous layer, upon reaction of $NaNO_2$ with HNO_3.[12] The method gives satisfactory results for carbamates **2a**–**b**, but the yield is significantly reduced in the case of **2c** and only traces of nitrosated product are observed in the reaction of **2d**. Better yields for carbamates **2a**–**c** (but not **2d**) were observed when the nitrosation was performed in anhydrous conditions, with $NOBF_4$ as a nitrosating agent and the presence of pyridine base.[13] The difficulties in nitrosation of **2d** have to be attributed to the steric effect (hindrance) of the two *ortho*-positioned *t*-butyl groups of the aromatic ring. Successful nitrosation of **2d** was eventually achieved following a modified version of the method of Overberger and Anselm, which employs $NaNO_2$ in a mixture of acetic acid and acetic anhydride.[14]

2.2. Structural studies

X-ray crystallographic analysis was conducted on compounds **1c** and **1d**, providing the first reported crystal structures of *N*-nitrosocarbamates (Figs. 2 and 3). The asymmetric unit in the unit cell of **1d** contains two unique molecules and the only major difference between them is the value of one of the dihedral angles in the TMSE group. Experimental and theoretical data on selected bond lengths, angles and torsional angles are shown in Table 1, together with structural parameters for *N*-methyl-*N*-nitroso-*p*-nitrobenzamide (**MNNB**) and *N*-methyl-*N*-nitrosourea (**MNU**).[15] Bond lengths in **1c** and **1d** are more comparable to those in the *N*-nitrosoamide **MNNB**, particularly the N=O and C=O bonds. The deviations are slightly greater between **1c**–**d** and **MNU**. The C=O bond is longer in **MNU**, while the N–N bond is ∼0.04 Å shorter.

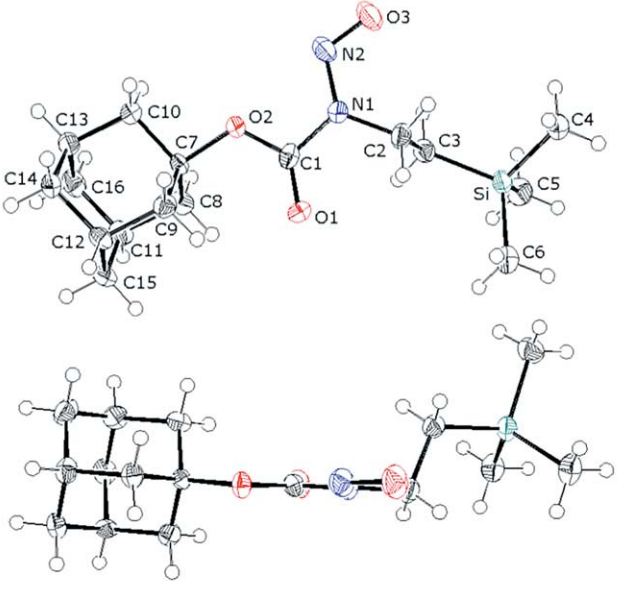


Figure 2. Two orientations of the X-ray structure of 1-adamantyl *N*-nitroso-*N*-(2-trimethylsilyl)ethylcarbamate (**1c**). The thermal ellipsoids are drawn at 50% probability. Hydrogen atoms are given arbitrary radii.

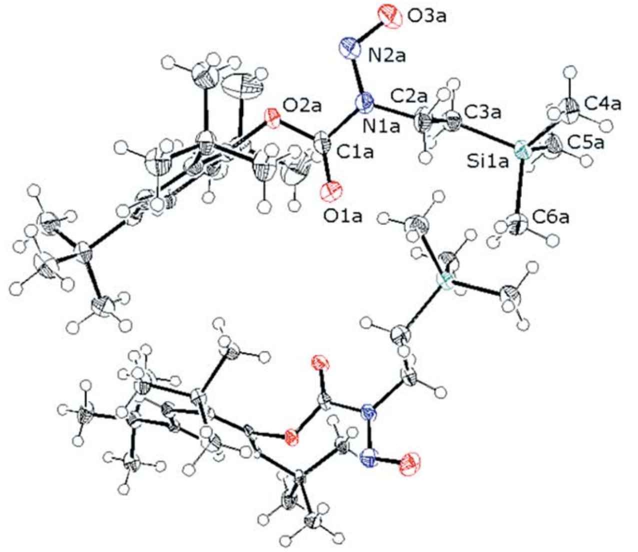


Figure 3. X-ray structure of 2,4,6-tris(*t*-butyl)phenyl *N*-nitroso-*N*-(2-trimethylsilyl)ethylcarbamate (**1d**). The asymmetric unit contains two unique molecules. The thermal ellipsoids are drawn at 50% probability. Hydrogen atoms are given arbitrary radii.

The *N*-nitrosocarbamate sub-structure in **1c** and **1d**, O–C(=O)–N–N=O, is very nearly planar, with a slightly greater twist around the C(1)–N(1) bond in the case of **1c**. The overall distortion from planarity is larger than the observed in **MNU**, but less than that of the *N*-nitrosoamide **MNNB**, particularly the N(2)–N(1)–C(1)–O(1) dihedral angle. The 2-(trimethylsilyl)ethyl group in both **1c** and **1d** adopts an *anti* conformation. The values for the dihedral angle C(4)–Si–C(3)–C(2) demonstrate that in the case of **1c** and one of the unique molecules in the unit cell of **1d** there is a nearly ideal *gauche* positioning of the $C(2)H_2$-group relative to the methyl groups at the silicon center. The second unique structure for **1d** exhibits an almost 18° deviation from the *gauche*-orientation.

As evident from Table 1, the theoretically derived structural data are in very close agreement with the experimental parameters. The reported results are from gas-phase DFT studies at the B3LYP/6-31+G(d) level of theory.[16–18]

All optimized minima structures, except for those of carbamate **1d**, were further validated by frequency calculations at the same level of theory and they had sets of only positive second derivatives. Values of Gibbs free energy differences were obtained after frequency calculations. The computed values for bond lengths are usually within ±0.01 Å of the experimental ones and the differences between theoretical and experimental values for angles and dihedral angles are usually within ±3°. In general, DFT tends to slightly exaggerate the planarity of the core structures for both the carbamates **1c**–**d** and **MNU**. The global minimum structures for the four *N*-nitrosocarbamates **1a**–**d** are shown in Figure 4.

Table 1. Selected experimental (bold characters) and theoretical bond lengths (Å) and angles (°) for *N*-nitrosocarbamates **1a**-**1d**, *N*-methyl-*N*-nitroso-*p*-nitrobezamide (**MNNB**) and *N*-methyl-*N*-nitrosourea (**MNU**)

Bond lengths and angles[a]	**1a**	**1b**	**1c**	**1d**[b]	**MNNB**[c]	**MNU**[c]
O(1)–C(1)	—	—	**1.200(4)**	**1.203(5)**, **1.197(5)**	**1.204(5)**	**1.215(2)**
	1.216	1.217	1.217	1.210	1.219	1.220
N(1)–N(2)	—	—	**1.360(4)**	**1.374(5)**, **1.359(5)**	**1.352(5)**	**1.326(2)**
	1.369	1.366	1.370	1.369	1.369	1.353
N(1)–C(1)–	—	—	**1.417(4)**	**1.410(4)**, **1.405(6)**	**1.405(6)**	**1.431(2)**
	1.412	1.418	1.419	1.413	1.416	1.431
O(3)–N(2)–	—	—	**1.220(3)**	**1.231(4)**, **1.222(6)**	**1.218(4)**	**1.231(2)**
	1.218	1.220	1.220	1.218	1.216	1.220
O(2)–C(1)	—	—	**1.323(4)**	**1.331(5)**, **1.342(4)**	—	—
	1.333	1.328	1.328	1.345	—	—
Si–C(3)	—	—	**1.880(3)**	**1.872(5)**, **1.886(4)**	—	—
	1.912	1.911	1.912	—	—	—
N(2)–N(1)–C(1)	—	—	**118.3(2)**	**117.1(3)**, **117.4(3)**	**116.9(4)**	**116.6(2)**
	118.0	118.3	118.3	118.2	117.8	117.1
O(3)–N(2)–N(1)	—	—	**113.2(2)**	**112.6(4)**, **113.0(4)**	**113.8(4)**	**114.4(2)**
	114.0	114.1	114.1	114.0	113.8	116.3
C(1)–N(1)–N(2)–O(3)	—	—	**−177.2(3)**	**−176.6(3)**, **−176.8(5)**	**−176.8**	**−177.7**
	−178.0	−178.0	−177.4	−178.4	−173.3	180.0
N(2)–N(1)–C(1)–O(1)	—	—	**173.4(3)**	**177.3(3)**, **176.7(5)**	**165.4**	**178.5**
	178.4	178.1	175.8	179.2	162.5	180.0
C(4)–Si–C(3)–C(2)	—	—	**−56.7(3)**	**−76.7(3)**, **−56.3(4)**	—	—
	−59.0	−60.2	−62.9	−59.5	—	—

Theoretical data from global minimum structures optimized at the B3LYP/6-31+G(d) level.

[a] Atom labels in accordance with the crystallographic designation for compounds **1c**–**d**.

[b] X-ray data given for each of the two unique molecules in the asymmetric unit of **1d**.

[c] X-ray data from Ref. 15.

2.3. Conformational analysis

Calculations demonstrate that each of the compounds **1a–d** has a set of rotamers with respect to both the C(1)–N(1) bond (*s-trans* and *s-cis*) and the N(1)–N(2) bond (*s-E* and *s-Z*). Table 2 lists the relative Gibbs free energies and the Boltzmann statistical contributions for the rotamers of structures **1a**–**c**, **MNU** and **MNNB**. In each case the corresponding (*s-E*, *s-trans*) isomer is the global minimum, and in the cases of **1c**, **1d**, **MNU** and **MNNB** these are in agreement with the X-ray crystal structures. Nakayama and Kikuchi reached similar conclusions in their recent theoretical studies of several *N*-nitroso compounds.[19] In almost all of the studied cases the (*s-E*, *s-cis*) isomer is the lowest energy local minimum. One exception is **MNU**, in whose case the lowest energy local minimum is the (*s-Z*, *s-trans*) structure. The latter is most likely due to stabilizing hydrogen bonding realized between the NH_2 and NO groups in **MNU**, which has been suggested earlier based on NMR and IR studies.[20] In the cases of **MNNB** and **MNU** calculations could not locate a minimum structure corresponding to the (*s-Z*, *s-cis*) isomer, which appears to be due to steric repulsion.

The currently available experimental and theoretical data provide valuable information towards resolving the issue of thermal stability of *N*-nitroso compounds, which in turn bears relation to the relative stability of the *s-E* and *s-Z* conformations. In the *s-Z* conformation there is spatial proximity of the nitroso group oxygen center to the carbonyl

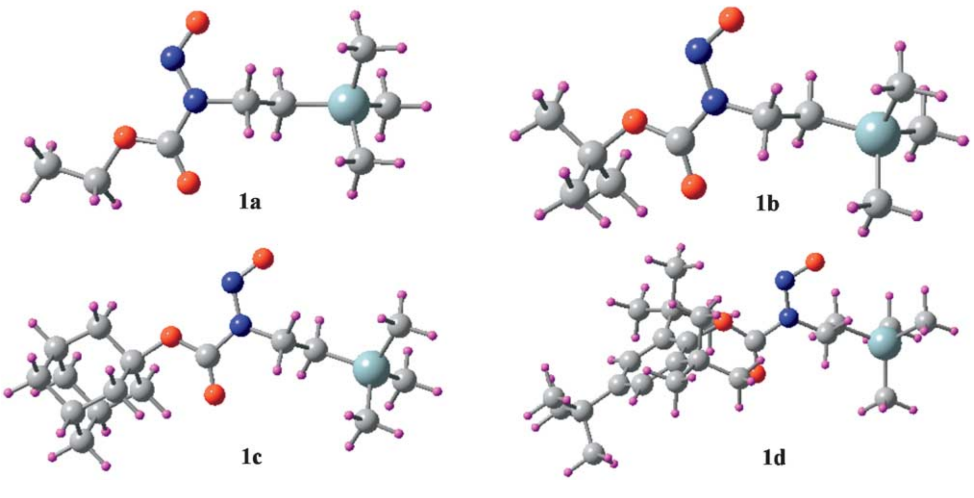


Figure 4. Optimized global minima structures of compounds **1a**–**d**. Data from B3LYP/6-31+G(d) calculations.

Table 2. Relative Gibbs free energies of conformers of *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates **1a**-**1c**, *N*-methyl-*N*-nitroso-*p*-nitrobezamide (**MNNB**) and *N*-methyl-*N*-nitrosourea (**MNU**), with respect to rotation around the N(1)–N(2) bond (*s-E* and *s-Z*) and the C(1)–N(1) bond (*s-cis* and *s-trans*)[a]

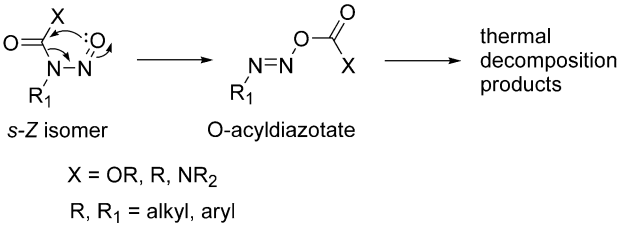
	1a	1b	1c	MNNB	MNU
s-E, *s-trans*	0.0 (89.9)	0.0 (99.5)	0.0 (97.2)	0.0 (100)	0.0 (99.9)
s-E, *s-cis*	+1.3 (10.1)	+3.2 (0.5)	+2.1 (2.8)	+6.0 (∼0)	+9.2 (∼0)
s-Z, *s-trans*	+5.5 (∼0)	+6.2 (∼0)	+5.8 (∼0)	+6.3 (∼0)	+3.9 (0.1)
s-Z, *s-cis*	+6.7 (∼ 0)	+7.7 (∼ 0)	+6.0 (∼ 0)	—[b]	—[b]

Data from B3LYP/6-31+G(d) geometry optimizations and frequency calculations. Boltzmann contributions (%) at 298 K given in parentheses.

[a] Atom labels in accordance with the crystallographic designation for compound **1c**.

[b] Minimum structure was not located.

carbon, and an intramolecular reaction becomes possible, leading to decomposition (Scheme 3).[21,22] No such reaction is feasible starting with the *s-E* isomer. Thus, it seems that greater relative stability of the *s-E* isomer is a pre-requisite for thermal stability of the corresponding *N*-nitroso compound.



Scheme 3.

Our results and other recent studies are in agreement that the *s-E* isomer is indeed the predominant form in the solid state and in the gas phase.[15,19,23] However, it should be noted that the NMR and IR studies of Snyder and Stock were interpreted in favor of the *s-Z* isomers of several *N*-alkyl- and *N*,*N*′-dialkyl-*N*-nitrosoureas being exclusively present in solution.[20]

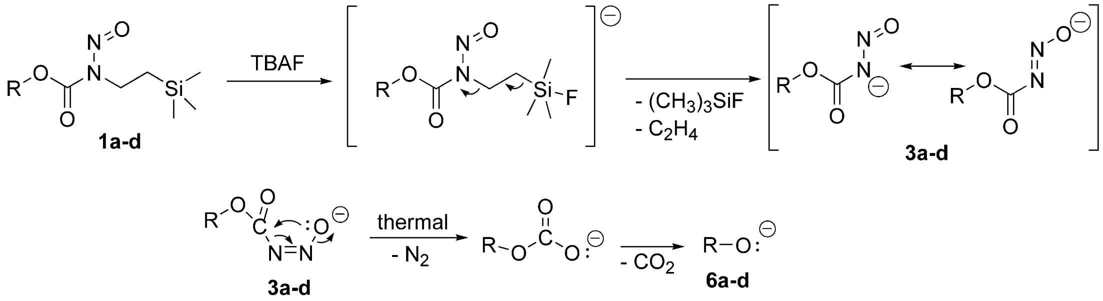
2.4. Desilylation studies

The use of the TMSE group as a temporary protecting group at a nitrogen center was reported recently in the successful preparation of the dinitroamide anion from *N*-2-(trimethylsilyl)ethyl-*N*,*N*-dinitroamine.[7] The dinitroamide anion is a resonance-stabilized species with a delocalized negative charge and reduced basicity. Thus, not surprisingly, relatively mild deprotection conditions were employed for the cleavage reaction. Secondary *N*-nitrosocarbamate anions are also characterized by resonance stabilization and charge delocalization.[1] It has been estimated, for example, that *N*-nitrosourethane is more acidic than acetic acid.[24] Hence, cleavage of the TMSE group, according to Scheme 4, would be expected to occur readily. One significant obstacle arises from the fact that structures **3a**–**d** have been demonstrated (in the case of **3a**)[1] or are anticipated to be thermally unstable, thus making it a challenge to find the appropriate conditions for desilylation that would be sufficiently mild not to trigger further decomposition of **3a**–**d** to the corresponding alkoxide (phenoxide) anion **6a**–**d**.

Our current results are based on studies conducted in DMF or acetonitrile as solvents, with $Bu_4NF\cdot3H_2O$ as a fluoride source. Experiments were performed using compounds **1a**, with available independent literature data for comparison,[1] and **1d**, whose decomposition was particularly easy to follow by NMR, giving distinct peaks in the aromatic region. All experiments were performed on an NMR scale, in deuterated solvents, so that the progress of reaction and product composition could be monitored directly. Results from the desilylation experiments are shown in Table 3.

(a) *Role of the solvent and temperature*. The studied reactions showed clear dependence on the nature of the solvent, with the rates being consistently higher in DMF. Thus, in the case of **1a** desilylation is complete in 0.5 h even at −15 °C, while in acetonitrile complete desilylation of **1a** requires 16 h even at +10 °C. The desilylation reaction is slower in the case of **1d**, and the solvent has an even more pronounced effect. Compound **1d** is practically stable in acetonitrile at +10 °C and only traces of decomposition products were detected after 24 h and 5-fold excess of Bu_4NF.

(b) *Amount of* $Bu_4NF\cdot3H_2O$. As Table 3 indicates, the



Scheme 4.

Table 3. Experimental conditions and product composition data for desilylation reactions of carbamates **1a** and **1d**

Carbamate	Solvent	Temperature (°C)	$Bu_4NF \cdot 3H_2O$ (equiv)	Time	Anion **3** (%)	Anion **6** (%)
1a	DMF	−15	3	30 min	29	71[a]
	DMF	−15	5	30 min	31	69
	DMF	+5	1	24 h	29[b]	57
	DMF	+10	5	10 min	19	81
	CH_3CN	+5	5	22 h	35	65
	CH_3CN	+10	5	16 h	41	59
1d	DMF	+10	3	48 h	72[c]	14[d]
	DMF	+5	5	23 h	98[e]	0
	CH_3CN	+10	5	24 h	0[f]	<1

[a] Structure validated by comparison with authentic sample of **6a**–NMe_4 in DMF-d_7.

[b] Reaction not complete after 24 h, with 14% residual carbamate **1a** present in the mixture.

[c] Reaction not complete after 48 h, with 13% residual carbamate **1d** present in the mixture.

[d] Structure validated by comparison with a sample of **6d–Li** in DMF-d_7.

[e] 2% Residual carbamate **1d**.

[f] 98% Residual carbamate **1d**.

process required some excess of the tetrabutylammonium salt in order to be conducted to completion. In the case of **1a** and 1 equiv of $Bu_4NF \cdot 3H_2O$, the reaction mixture after 24 h at 5 °C showed 14% of unreacted carbamate and the desilylation of **1d** with 3 equiv $Bu_4NF \cdot 3H_2O$ at +10 °C was not complete even after 48 h. Typical runs were conducted with 3 to 5-fold excess of $Bu_4NF \cdot 3H_2O$. We anticipate that the relative amounts of fluoride and rates of desilylation are influenced by the fact that the used fluoride source is a trihydrate. It has been shown, both spectroscopically and theoretically, that the fluoride anion forms hydrate clusters with varying number of water molecules.[25–27] This is certain to affect the anion's reactivity and decelerate the desilylation process.

(c) *Product composition*. The immediate product of desilylation in each case is the corresponding *N*-nitrosocarbamate anion **3**. However, in most runs, particularly of carbamate **1a**, anion **6** was observed as well, in varying quantities. In the case of **1a** the product structures, **3a** and **6a**, were validated by comparison with the available in literature spectral data in acetonitrile solvent.[1] In DMF, the NMR pattern of the product mixtures is similar, and we have assigned the quartet at δ 4.14 and the triplet at δ 1.23 to the anion **3a**, while the quartet at δ 3.50 and triplet at δ 1.06 were assigned to **6a**, after comparison with the spectrum of an independently prepared sample of **6a–NMe$_4$** in DMF-d_7. The maximum amount of **3a** in the studied cases, determined by integration of NMR signals, was about 40%, but in most runs it was lower and not significantly affected by temperature or the nature of the solvent. The desilylation of **1d** on the other hand occurs with predominant and in some cases exclusive formation of the desired product **3d**, whose signal for the aromatic H-atoms appears at δ 7.34 ppm in deuterated DMF (Fig. 5a–d).

As Figure 5e demonstrates, subjection of **3d** to elevated temperature leads to its decomposition. The product of decomposition has a signal for the aromatic protons at δ 6.86 ppm, and was identified as the aryloxide anion **6d**. For comparison, we prepared the lithium salt of 2,4,6-tris(*t*-butyl)phenol by deprotonation with *n*-BuLi. The resultant **6d–Li** has a signal in the aromatic region at δ 6.90 ppm, in DMF-d_7. Anion **6d**, in the presence of water in the reaction mixture, is eventually protonated and after several days at ambient temperature the sole product is 2,4,6-tris(*t*-butyl)phenol, whose signal in the aromatic region is at δ 7.20 ppm (Fig. 5f).

The high percentage of ethoxide **6a** in the product mixtures from desilylation of **1a** cannot be entirely accounted for by a process of secondary decomposition of the anion **3a**, particularly at temperatures below 0 °C, since it would correspond to a much lower thermal stability of **3a** than established in earlier studies. An alternative explanation can be offered based on the hypothesis that the major portion of ethoxide is formed via parallel reaction of decomposition of **1a**. Previous studies have shown that tetrabutylammonium

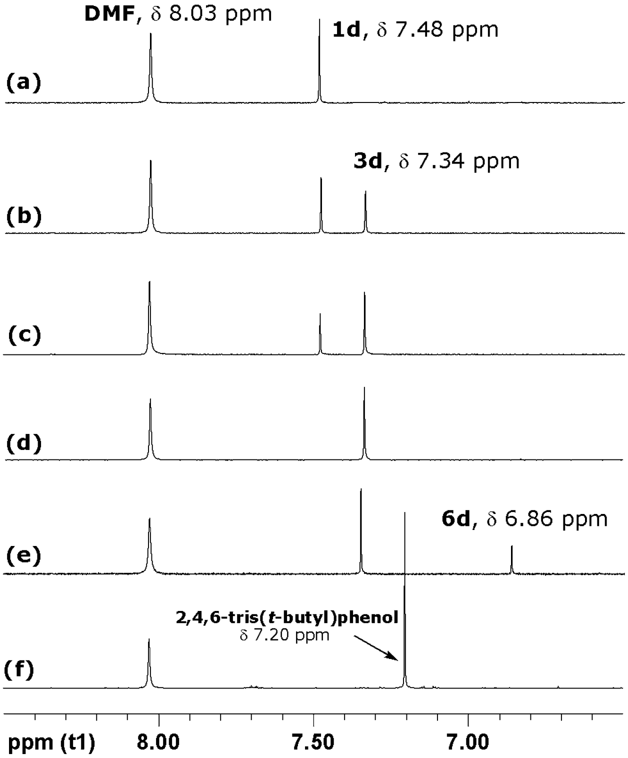
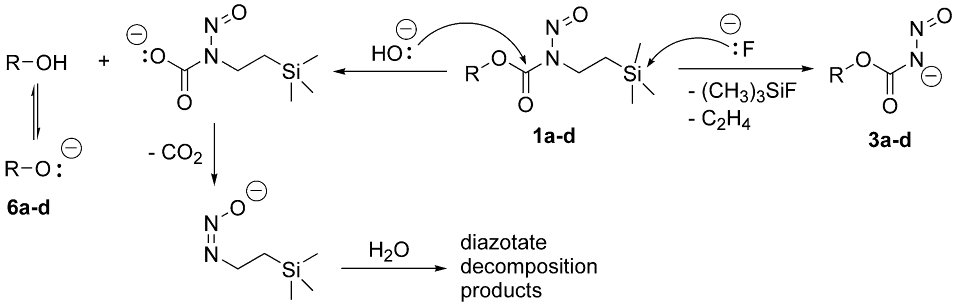


Figure 5. (a) Carbamate **1d** in DMF-d_7; (b)–(d) carbamate **1d** with 5 equiv of $Bu_4NF \cdot 3H_2O$ in DMF-d_7: (b) 1 h at +5 °C; (c) 3 h at +5 °C; (d) 23 h at +5 °C; (e) anion **3d** after 3 h at +45 °C; (f) anion **3d** after 5 days at ambient temperature. All spectra referenced to the solvent (DMF-d_7, δ 8.03 ppm).



Scheme 5.

fluoride solutions are strongly basic and the presence of water leads to the generation of hydroxide anions.[28–30] At the same time *N*-nitrosocarbamates are known to be hydrolytically labile in such conditions.[31–33] To verify the hypothesis, we conducted a trial experiment with ethyl *N*-butyl-*N*-nitrosocarbamate,[33–37] which is a close structural analog of **1a** but lacks a silicon center. An NMR sample of it in DMF-d_7 was added to 5 equiv of $Bu_4NF \cdot 3H_2O$ at 5 °C. The spectrum after 30 min indicated complete decomposition of the starting material with the almost exclusive formation of **6a**, which in this case could occur only via basic hydrolysis of the starting *N*-nitrosocarbamate. Hence we are proposing a two-pathway decomposition for carbamates **1a–d** in the presence of $Bu_4NF \cdot 3H_2O$, as reflected in Scheme 5. The significant differences in product composition between **1a** and **1d** are due to the presence of the bulky and branched *t*-butyl groups in **1d** (Figs. 3 and 4), which render the carbonyl group virtually inaccessible, thus slowing down considerably the rate of basic hydrolysis.

3. Conclusions

Our efforts to date have led to the formulation and implementation of a new synthetic strategy aimed at the preparation and isolation of secondary *N*-nitrosocarbamates via desilylation of chemically and thermally stable tertiary *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamates.

The current article presents the first solid-state structural studies of *N*-nitrosocarbamates. X-ray data have demonstrated that both of the studied target structures exist as *s*-*E* conformers in the solid state, with a nearly planar *N*-nitrosocarbamate moiety. Further, DFT calculations demonstrate that the *s*-*E* orientation at the N–N bond is the preferred arrangement in the gas phase as well, for *N*-nitrosocarbamates, *N*-nitrosoureas and *N*-nitrosoamides.

Desilylation studies demonstrate that the process depends on several factors: temperature, solvent and concentration of fluoride anion. The current methodology, employing $Bu_4NF \cdot 3H_2O$, seems to be most effective in cases of structures with large and branched carbamate groups, whose steric hindrance effectively suppresses the competing process of basic hydrolysis of the *N*-nitrosocarbamate, the latter leading directly to the anions **6** rather than **3**. Use of anhydrous fluoride ion sources, such as tetrabutylammonium triphenyldifluorosilicate (TBAT)[28,30] or CsF/CsOH mixtures[38] may prove advantageous in optimizing the yields of the *N*-nitrosocarbamate anions **3**.

4. Experimental

^1H and ^{13}C spectra of intermediate and target compounds were recorded at 300 and 75 MHz, respectively, and referenced to the solvent ($CDCl_3$: 7.27 ppm and 77.0 ppm; D_2O: 4.76 ppm; acetonitrile-d_3: 1.93 ppm; DMF-d_7: 8.03 ppm). X-ray structures were obtained using an Oxford Diffraction Xcalibur3 diffractometer with graphite monochromatic Cu K_α radiation. Structure solution and refinement was preformed using the SHELXTL 6.10 software package.[39] All calculations were performed utilizing the Gaussian03-Linda/GaussView software package[40] on a Linux-operated cluster (QuantumCube QS4-2400C by Parallel Quantum Solutions, Fayetteville, AR). Constant temperature desilylation reactions were conducted using the Isotemp 1016S circulating bath. Elemental analysis was provided by Atlantic Microlab, Norcross, GA. HRMS data was provided by the Mass Spectrometry and Proteomics facility at the Ohio State University. Nitrosonium tetrafluoroborate was purchased from the Lancaster chemical company. 3-(Trimethylsilyl)propanamide (**4**)[8] and 2,4,6-tris(*t*-butyl)phenyl chloroformate[10] were synthesized, following previously reported procedures. 2-(Trimethylsiyl)ethanamine (**5**) was prepared according to a published patent work.[11] The ^1H NMR data for compounds **4** and **5**, and precursors to them, are reported in this article, as they were previously unavailable.

4.1. X-ray crystallography of 1-adamantyl *N*-2-trimethylsilylethyl-*N*-nitrosocarbamate (1c)

A crystal (yellow plate) of **1c** ($C_{16}H_{28}N_2O_3Si$) having approximate dimensions 0.324×0.206×0.083 mm was mounted on a glass fiber. The preliminary set of cell constants was calculated from reflections harvested from 5 sets of 15 frames, merged to 139 peaks that were used to derive the initial orientation matrix. Data acquisition was conducted at 150 K using the phi and omega scans technique. Final cell constants were determined based on the full data set, leading to a triclinic cell (*P*-1) with these dimensions: $a=6.6116\ (13)$ Å, $b=9.8970\ (18)$ Å, $c=13.958\ (2)$ Å, $\alpha=75.660\ (14)°$, $\beta=84.856\ (14)°$, $\gamma=89.407\ (14)°$, $V=881.3\ (3)$ Å^3. For $Z=2$ and FW=324.49, the calculated density is 1.223 g/cm^3. Non-hydrogen atoms were refined anisotropically; hydrogen atoms were assigned based on geometry. The final structure has values for the unweighted agreement factor $R1=0.0459$ based on 1300 strong reflections ($I>4\sigma$) and $R1=0.0489$ based on all data.

4.2. X-ray crystallography of 2,4,6-tris(*t*-butyl)phenyl *N*-2-trimethylsilylethyl-*N*-nitrosocarbamate (1d)

A crystal (yellow needle) of **1d** ($C_{24}H_{42}N_2O_3Si$) having approximate dimensions 0.358×0.079×0.072 mm was mounted on a glass fiber. The preliminary set of cell constants was calculated from reflections harvested from 5 sets of 15 frames, merged to 155 peaks that were used to derive the initial orientation matrix. Data acquisition was conducted at 100 K using the phi and omega scans technique. Final cell constants were determined based on the full data set, leading to an orthorhombic cell (*Pbca*) with these dimensions: $a=11.3845$ (11) Å, $b=20.5755$ (18) Å, $c=45.454$ (4) Å, $\alpha=90.00°$, $\beta=90.00°$, $\gamma=90.00°$, $V=10647.2$ (17) $Å^3$. For $Z=16$ and FW=434.67, the calculated density is 1.075 g/cm^3. Non-hydrogen atoms were refined anisotropically; hydrogen atoms were assigned based on geometry. The final structure has values for the unweighted agreement factor $R1=0.0917$ based on 7208 strong reflections ($I>4\sigma$) and $R1=0.1193$ based on all data.

4.3. Fluoride-assisted desilylation of ethyl *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamate (1a) and 2,4,6-tris(*t*-butyl)phenyl *N*-2-(trimethylsilyl)ethyl-*N*-nitrosocarbamate (1d). General procedure

In a typical run 0.020 mol of carbamate **1**, dissolved in 0.7 mL of the appropriate deuterated solvent (acetonitrile-d_3 or DMF-d_7), was added to $Bu_4NF\cdot 3H_2O$ (0.020–0.10 mol, 1–5 equiv) at −15 °C (ice–acetone bath). The resultant solution was transferred into an NMR tube and kept at the appropriate temperature until disappearance of the signals of the starting material. NMR measurements were conducted at regular time intervals. The change in quantity of reactant/product was monitored via integration of the NMR signals relative to the multiplet of Bu_4N^+ centered at δ 1.76 ppm.

4.3.1. Ethyl *N*-nitrosocarbamate, tetrabutylammonium salt (3a–Bu$_4$N). ^{1}H NMR (DMF-d_7, anion only) δ 4.14 (q, $J=7.1$ Hz, 2H), 1.23 (t, $J=7.1$ Hz, 3H); ^{1}H NMR (acetonitrile-d_3, anion only) δ 4.09 (q, $J=7.1$ Hz, 2H), 1.21 (t, $J=7.1$ Hz, 3H).[1]

4.3.2. 2,4,6-Tris(*t*-butyl)phenyl *N*-nitrosocarbamate, tetrabutylammonium salt (3d–Bu$_4$N). ^{1}H NMR (DMF-d_7, anion only) δ 7.34 (s, 2H), 1.43 (s, 18H), 1.31 (s, 9H).

4.3.3. 3-(Trimethylsilyl)propanamide (4).[8] *(A) 3-(Trimethylsilyl)propanoyl chloride.* ^{1}H NMR ($CDCl_3$) δ 2.80–2.87 (m, 2H), 0.91–0.97 (m, 2H), 0.03 (s, 9H).

(B) 3-(Trimethylsilyl)propanamide. ^{1}H NMR ($CDCl_3$) δ 5.61 (broad s, 2H), 2.17–2.24 (m, 2H), 0.80–0.87 (m, 2H), 0.02 (s, 9H).

4.3.4. 2-(Trimethylsilyl)ethanamine (6).[8,11] *(A) N-2-(trimethylsilyl)ethylphthalimide.* ^{1}H NMR ($CDCl_3$) δ 7.82–7.85 (m, 2H), 7.68–7.71 (m, 2H), 3.69–3.75 (m, 2H), 0.99–1.05 (m, 2H), 0.07 (s, 9H).

(B) 2-(Trimethylsilyl)ethanamine hydrochloride. ^{1}H NMR (D_2O) δ 2.94–3.01 (m, 2H), 0.84–0.91 (m, 2H), −0.03 (s, 9H).

(C) 2-(Trimethylsilyl)ethanamine. ^{1}H NMR ($CDCl_3$) δ 2.71–2.78 (m, 2H), 2.10 (broad s, 2H), 0.73–0.80 (m, 2H),-0.01 (s, 9H).

4.4. Preparation of *N*-2-(trimethylsilyl)ethylcarbamates. General procedure (Method A)

To a stirred solution of 3-(trimethylsilyl)propanamide (3.40 mmol) and mercuric acetate (4.60 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL), was added a solution of the corresponding alcohol (102.50 mmol) in anhydrous *N*,*N*-dimethylformamide (3 mL), at ambient temperature and under nitrogen. This was followed by addition of a solution of NBS (4.50 mol) in *N*,*N*-dimethylformamide (2 mL). The reaction was stirred for 12 h at room temperature. The solvents were removed at reduced pressure and methylene chloride (50 mL) was added to the residual solid. The organic extract was washed with water (5×25 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resultant residue was passed through a short silica gel filter (hexane–ethyl acetate=3:1) and the solvents were removed under reduced pressure to give the desired carbamate. Further purification via column chromatography (hexane: ethyl acetate=3:1) led to isolated products that were >95% pure by NMR.

4.4.1. Ethyl *N*-2-trimethylsilylethylcarbamate (2a) (Method A). Yield: 85%. Colorless liquid: ^{1}H NMR ($CDCl_3$) δ 4.69 (bs, 1H), 4.10 (q, $J=7.0$ Hz, 2H), 3.18–3.24 (m, 2H), 1.24 (t, $J=7.0$ Hz, 3H), 0.77–0.83 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 156.5, 60.6, 37.4, 18.2, 14.6, −1.7; HRMS (FAB$^+$) *m/z* Calcd for $C_8H_{19}NO_2Si$ $[M+Na]^+$ 212.1083, found 212.1076.

4.4.2. *t*-Butyl *N*-2-trimethylsilylethylcarbamate (2b) (Method A). Yield: 36%. Colorless liquid: ^{1}H NMR ($CDCl_3$) δ 5.80 (bs, 1H), 3.10–3.19 (m, 2H), 1.41 (s, 9H), 0.81–0.87 (m, 2H), 0.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 155.7, 79.0, 37.0, 28.4, 18.1, −1.7; HRMS (FAB$^+$) *m/z* Calcd for $C_{10}H_{24}NO_2Si$ $[M+Na]^+$ 240.1396, found 240.1396. Anal. Calcd for $C_{10}H_{23}NO_2Si$: C, 55.25; H, 10.66; N, 6.44. Found: C, 55.50; H, 10.54; N, 6.14.

4.4.3. 1-Adamantyl *N*-2-trimethylsilylethylcarbamate (2c) (Method A). Yield: 50%. White solid: mp 92–94 °C. ^{1}H NMR ($CDCl_3$) δ 4.42 (bs, 1H), 3.12–3.20 (m, 2H), 2.17 (m, 3H), 2.10 (m, 6H), 1.65 (m, 6H), 0.75–0.82 (m, 2H), 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 155.4, 41.7, 37.0, 36.2, 30.8, 18.2, 11.2, −1.7; HRMS (FAB$^+$) *m/z* Calcd for $C_{16}H_{30}NO_2Si$ $[M+Na]^+$ 318.1865, found 318.1876; Anal. Calcd for $C_{16}H_{29}NO_2Si$: C, 65.03; H, 9.89; N, 4.74. Found: C, 65.24; H, 9.93; N, 4.58.

4.4.4. 2,4,6-Tris(*t*-butyl)phenyl *N*-2-trimethylsilylethylcarbamate (2d) (Method B). A solution of 2-(trimethylsilyl)ethanamine (0.22 g, 1.85 mmol) and pyridine (0.15 g, 1.85 mmol, 0.15 mL) in dichloromethane (5 mL) was added dropwise over 15 min period to a solution of 2,4,6-tris(*t*-butyl)phenyl chloroformate (0.60 g, 1.85 mmol) in dichloromethane (5 mL) at 0 °C (ice–water), followed by stirring at ambient temperature for 12 h. The mixture was washed with water, the organic layer was separated, dried ($MgSO_4$) and the solvent removed. The resultant residue was passed through a short silica gel filter and the product eluted with methylene chloride. The solvent was removed to yield the product as a white solid (0.34 g, 45%). Further purification via recrystallization from hexane, to yield white needles: mp 157–159 °C. ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 2H), 4.99 (bs, 1H, NH), 3.28–3.36 (m, 2H), 1.37 (s, 18H), 1.32 (s, 9H), 0.88–0.94 (m, 2H), 0.06 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 155.2, 146.7, 145.7, 141.9, 123.1, 37.8, 35.6, 34.8, 31.52, 31.47, 18.4, −1.6; Anal. Calcd for $C_{24}H_{43}NO_2Si$: C, 71.05; H, 10.68; N, 3.45. Found: C, 71.23; H, 10.90; N, 3.45.

4.5. Preparation of *N*-2-trimethylsilylethyl-*N*-nitrosocarbamates. General procedure (aqueous method)

To a solution of the corresponding alkyl *N*-2-(trimethylsilyl)ethylcarbamate **2a–c** (1.25 mmol) in ether (2 mL) was added a solution of sodium nitrite (11.21 mmol) in water (2 mL). Without stirring or cooling, nitric acid (1.5 mL, 35%) was added directly to the lower layer over 1 h. The organic layer was separated, washed with water, then washed four times with saturated sodium bicarbonate, dried ($MgSO_4$) and the solvent removed to afford the corresponding alkyl *N*-nitroso-*N*-2-(trimethylsilyl)ethylcarbamate **1a–c**. Further purification by column chromatography (silica gel, hexane: $CH_2Cl_2 = 2:1$) led to isolated products that were >95% pure by NMR.

4.6. Preparation of *N*-2-trimethylsilylethyl-*N*-nitrosocarbamates. General procedure (anhydrous method)

To a stirred solution of the corresponding alkyl *N*-2-(trimethylsilyl)ethylcarbamate **2a–c** (5.00 mmol) and anhydrous pyridine (10.00 mmol) in anhydrous acetonitrile (2.5 mL) at −20 °C, was added in one portion solid $NOBF_4$ (10.00 mmol). The solution was stirred at 0 °C under nitrogen for a period of 1–2 h and the progress of the reaction was followed by thin layer chromatography (hexane–$CH_2Cl_2 = 2:1$), the product exhibiting a rapidly moving yellow spot. The solvent was removed and the resultant mixture was passed through a short silica gel filter (hexane–$CH_2Cl_2 = 2:1$). Removal of the solvents at reduced pressure gave the product **1a–c** that were >95% pure by NMR.

4.6.1. Ethyl *N*-2-trimethylsilylethyl-*N*-nitrosocarbamate (1a). Yield: 72% by the aqueous method, 78% by the anhydrous method. Yellow liquid: ^{1}H NMR ($CDCl_3$) δ 4.53 (q, $J = 7.3$ Hz, 2H), 3.70–3.77 (m, 2H), 1.45 (t, $J = 7.3$ Hz, 3H), 0.65–0.72 (m, 2H), 0.03 (s, 9H); ^{1}H NMR (acetonitrile-d_3) δ 4.43 (q, $J = 7.0$ Hz, 2H), 3.67–3.73 (m, 2H), 1.38 (t, $J = 7.0$ Hz, 3H), 0.63–0.68 (m, 2H),-0.01 (s, 9H); ^{1}H NMR (DMF-d_7) δ 4.54 (q, $J = 7.2$ Hz, 2H), 3.75–3.81 (m, 2H), 1.42 (t, $J = 7.2$ Hz, 3H), 0.67–0.73 (m, 2H), 0.05 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 153.7, 64.2, 37.6, 15.4, 14.2, −2.0; HRMS (FAB$^+$) *m/z* Calcd for $C_8H_{19}N_2O_3Si$ $[M+Na]^+$ 241.0984, found 241.0981. Anal. Calcd for $C_8H_{18}N_2O_3Si$: C, 44.01; H, 8.31; N, 12.83. Found: C, 44.34; H, 8.38; N, 12.63.

4.6.2. *t*-Butyl *N*-2-trimethylsilylethyl-*N*-nitrosocarbamate (1b). Yield: 69% by the aqueous method. Yellow liquid: ^{1}H NMR ($CDCl_3$) δ 3.68–3.74 (m, 2H), 1.65 (s, 9H), 0.66–0.72 (m, 2H), 0.04 (s, 9H); ^{1}H NMR (acetonitrile-d_3) δ 3.65–3.72 (m, 2H), 1.60 (s, 9H), 0.63–0.69 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 152.0, 85.0, 37.4, 28.0, 15.5, −2.0; HRMS (FAB$^+$) *m/z* Calcd for $C_{10}H_{23}N_2O_3Si$ $[M+Na]^+$ 269.1297, found 269.1301.

4.6.3. 1-Adamantyl *N*-2-trimethylsilylethyl-*N*-nitrosocarbamate (1c). Yield: 30% by the aqueous method, 81% by the anhydrous method. Yellow solid, recrystallized from petroleum ether at −25 °C, to yield yellow plates: mp 98–99 °C. ^{1}H NMR ($CDCl_3$) δ 3.67–3.73 (m, 2H), 2.29 (m, 9H), 1.72 (m, 6H), 0.66–0.72 (m, 2H), 0.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 151.4, 85.0, 41.3, 37.4, 36.0, 31.0, 15.5, −1.9. Anal. Calcd for $C_{16}H_{28}N_2O_3Si$: C, 59.22; H, 8.70; N, 8.63. Found: C, 59.03; H, 8.73; N, 8.45.

4.6.4. 2,4,6-Tris(*t*-butyl)phenyl *N*-2-trimethylsilylethyl-*N*-nitrosocarbamate (1d). Carbamate **2d** (0.23 g, 0.57 mmol) was dissolved in a mixture of acetic acid (3.0 mL) and acetic anhydride (4.5 mL). The resultant solution was cooled to 5 °C (ice–water) and $NaNO_2$ (0.83 g, 12.06 mmol) was added in small portions, over 2-h period, while keeping the temperature below 10 °C. Upon complete addition, the resultant mixture was stirred for additional 12 h at ambient temperature. The solvents were removed at reduced pressure and the residue was portioned between water and ether. The ether extract was stirred for 10 min with saturated aq $NaHCO_3$, the organic layer was separated, dried ($MgSO_4$) and the solvent removed at reduced pressure. The resultant yellow oil was flash chromatographed on a short silica gel column (hexane: methylene chloride = 5:1). The yellow colored, highly mobile fraction was collected and the solvent removed, leaving 0.21 g (84%) of the product as a yellow oil, which slowly solidified. Recrystallized from methanol at −30 °C to yield yellow needles: mp 62–64 °C. ^{1}H NMR ($CDCl_3$) δ 7.41 (s, 2H), 3.84–3.89 (m, 2H), 1.39 (s, 18H), 1.37 (s, 9H), 0.74–0.78 (m, 2H), 0.07 (s, 9H); ^{1}H NMR (acetonitrile-d_3) δ 7.43 (s, 2H), 3.79–3.85 (m, 2H), 0.68–0.74 (m, 2H), 0.02 (s, 9H); ^{1}H NMR (DMF-d_7) δ 7.48 (s, 2H), 3.89–3.95 (m, 2H), 0.72–0.78 (m, 2H), 0.07 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 153.5, 148.0, 145.4, 141.4, 123.5, 37.9, 35.6, 34.9, 31.51, 31.48, 15.4, −2.0. Anal. Calcd for $C_{24}H_{42}N_2O_3Si$: C, 66.31; H, 9.74; N, 6.44. Found: C, 66.09; H, 9.77; N, 6.46.

5. Supporting information available

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 258576 and 258577. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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Supplementary data

Energies and thermodynamic parameters of optimized minimum structures of compounds **1a–d**, **MNU** and **MNNB** are summarized in Table S1. Images of ^{1}H NMR spectra of compounds **1b** and **2a** are shown in Figure S1.

Supplementary data associated with this article can be found at 10.1016/j.tet.2005.03.044

References and notes

1. Benin, V.; Kaszynski, P.; Radziszewski, G. *J. Am. Chem. Soc.* **2002**, *124*, 14115–14126.
2. Thiele, J.; Lachman, A. *Justus Liebigs Ann. Chem.* **1895**, *288*, 304–311.
3. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley: New York, 1999; pp 64, 399–400.
4. Yang, W.; Koreeda, M. *J. Am. Chem. Soc.* **1994**, *116*, 10793–10794.
5. Hamm, M. L.; Cholera, R.; Hoey, C. L.; Gill, T. J. *Org. Lett.* **2004**, *6*, 3817–3820.
6. Wada, T.; Sekine, M. *Tetrahedron Lett.* **1994**, *35*, 757–760.
7. Bottaro, J. C.; Penwell, P. E.; Schmitt, R. J. *J. Am. Chem. Soc.* **1997**, *119*, 9405–9410.
8. Sommer, L. H.; Rockett, J. *J. Am. Chem. Soc.* **1951**, *73*, 5130–5134.
9. Jew, S.; Park, H. G.; Park, H.; Park, M.; Cho, Y. *Tetrahedron Lett.* **1990**, *31*, 1559–1562.
10. Nagel, M.; Hansen, H.-J. *Helv. Chim. Acta* **2000**, *83*, 1022–1045.
11. Schirlin, D.; Collard, J.- N.; Danzin, C. (Merrell Dow Pharmaceuticals Inc.) USA. Eur. Pat. Appl. EP0291787, 1988, p 32.
12. Waddell, S. T.; Santorelli, G. M. *Tetrahedron Lett.* **1996**, *37*, 1971–1974.
13. Romea, P.; Urpi, F.; Vilarassa, J. *J. Org. Chem.* **1989**, *54*, 3209–3211.
14. Overberger, C. G.; Anselme, J.-P. *J. Org. Chem.* **1963**, *28*, 592–593.
15. Prout, K.; Fail, J.; Hernandez-Cassou, S.; Miao, F. M. *Acta Crystallogr.*. **1982**, *B38*, 2176–2181.
16. Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100.
17. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.
18. Frisch, M. J.; Pople, J. A.; Binkley, J. S. *J. Chem. Phys.* **1984**, *80*, 3265–3269.
19. Nakayama, N.; Kikuchi, O. *J. Mol. Struct. (Theochem)* **2001**, *536*, 213–218.
20. Snyder, J. K.; Stock, L. M. *J. Org. Chem.* **1980**, *45*, 886–891.
21. White, E. H.; Aufedermarsh, C. A. J. *J. Am. Chem. Soc.* **1961**, *83*, 1174–1178.
22. Darbeau, R. W.; Perez, E. V.; Sobieski, J. I.; Rose, W. A.; Yates, M. C.; Boese, B. J.; Darbeau, N. R. *J. Org. Chem.* **2001**, *66*, 5679–5686.
23. Yamaguchi, K.; Matsumura, G. *Acta Crystallogr.* **1992**, *C48*, 1051–1054.
24. Hantzsch, A.; Schumann, M.; Egler, A. *Chem. Ber.* **1899**, *32*, 1703–1716.
25. Xantheas, S. S.; Dunning, T. H., Jr *J. Phys. Chem.* **1994**, *98*, 13489–13497.
26. Xantheas, S. S. *J. Phys. Chem.* **1996**, *100*, 9703–9713.
27. Cabarcos, O. M.; Weinheimer, C. J.; Lisy, J. M.; Xantheas, S. S. *J. Chem. Phys.* **1999**, *110*, 5–8.
28. Pilcher, A. S.; Ammon, H. L.; DeShong, P. *J. Am. Chem. Soc.* **1995**, *117*, 5166–5167.
29. Cox, D. P.; Terpinski, J.; Lawrynowicz, W. *J. Org. Chem.* **1984**, *49*, 3216–3219.
30. Pilcher, A. S.; DeShong, P. *J. Org. Chem.* **1996**, *61*, 6901–6905.
31. Gold, B.; Linder, W. B. *J. Am. Chem. Soc.* **1979**, *101*, 6772–6773.
32. Moss, R. A. *J. Org. Chem.* **1966**, *31*, 1082–1087.
33. Wilds, A. L.; Meader, A. L. J. *J. Org. Chem.* **1948**, *13*, 763–779.
34. Hartman, W. W.; Phillips, R. In *Organic Syntheses, Collect. Vol. II*; Wiley: New York, 1943; p 464.
35. Hartman, W. W.; Brethen, M. R. In *Organic Syntheses, Collect. Vol. II*; Wiley: New York, 1943; p 278.
36. Moss, R. A. *Tetrahedron Lett.* **1966**, *7*, 711–718.
37. Yellow liquid: ^{1}H NMR (CDCl$_3$) δ 4.55 (q, *J*.H., 2H), 3.75 (t, $J=7.1$ Hz, 2H), 1.47 (t, $J=7.2$ Hz, 3H), 1.33–1.43 (m, 2H), 1.18–1.30 (m, 2H), 0.89 (t, $J=7.2$ Hz, 3H); ^{13}C NMR (CDCl$_3$) δ 154.0, 64.3, 40.7, 29.0, 20.0, 14.3, 13.5.
38. Busch-Petersen, J.; Bo, Y.; Corey, E. J. *Tetrahedron Lett.* **1999**, *40*, 2065–2068.
39. *SHELXTL, version 6.10*; Bruker AXS, Inc.: Madison, WI, 2000.
40. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, R.C. Gaussian, Inc.: Wallingford, CT, 2004.

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A new dimeric bidentated NHC–Pd(II) complex from *trans*-cyclohexane-1,2-diamine for Suzuki reaction and Heck reaction

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Abstract—A new dimeric bidentated NHC–Pd(II) complex was synthesized from *trans*-cyclohexane-1,2-diamine and its structure was characterized by MALDI Mass spectrum. This NHC–Pd(II) complex was fairly effective in Suzuki and Heck reactions to give the products in good to excellent yields in most cases.

1. Introduction

With its unique ligating properties, *N*-heterocyclic carbenes (NHC) have long been the subject of both synthetic and catalytic studies. Owing to the high stability of the metal complexes of NHC's toward heat, moisture and oxygen, these complexes have been successfully used in a wide range of catalytic reactions.[1] Among these versatile catalysts, bis(carbene)palladium(II)dihalide complexes are the efficient catalysts for various C–C and C–N coupling reactions.[2]

The palladium-catalyzed biaryl fomation of aryl halides with arylboronic acids (the Suzuki reaction)[3] and arylation and alkenylation of olefins (the Heck reaction)[4] have turned out to be two of the most powerful methods for the formation of carbon–carbon bond in organic synthesis. A number of palladium/*N*-heterocyclic carbene systems have been employed in these processes with great success.[5] Most of them used in situ formed palladium-NHC species from imidazolium salts and palladium sources in the presence of base.[5c,e,f,k,l] There are only a few examples using prepared bis(carbene)palladium(II)dihalide complexes as catalysts in above two coupling reactions.[2,6] These carbene–Pd(II) complexes have fixed ligand/metal ratio and are easy to handle. In this paper, we wish to report the synthesis of a novel dimeric bidentated NHC–Pd(II) complex derived from *trans*-cyclohexane-1,2-diamine and its application in Suzuki–Miyaura reaction and Heck reaction.

2. Results and discussion

The synthesis of dimeric bidentated NHC–Pd(II) complex **5** is shown in Scheme 1. Using *trans*-cyclohexane-1,2-diamine (1.0 equiv) as starting material to react with 2-fluoronitrobenzene (2.0 equiv) in anhydrous ethanol, *N*,*N*′-bis(2-nitrophenyl)cyclohexane-1,2-diamine **1** was obtained in 77% yield under reflux in the presence of $NaHCO_3$ for 10 h. The reduction of **1** with Pd–C in dichloromethane under H_2 atmosphere (1.0 atm) at room temperature for 2 days afforded *N*,*N*′-bis(2-nitrophenyl)-cyclohexane-1,2-diamine **2** in 92% yield. The treatment of **2** by triethyl orthoformate containing a catalytic amount of TsOH gave compound **3** in 77% yield at room temperature for 2 days. The benzimidazolium iodide **4** was obtained by stirring compound **3** with CH_3I in CH_2Cl_2 at room temperature as a yellow precipitate in 79% yield. Reaction of NHC precursor **4** with $Pd(OAc)_2$ in DMSO at 85 °C for 6 h afforded the desired NHC–Pd(II) complex **5** in 80% yield. This pale green powder is stable even at more than 350 °C under ambient atmosphere. We can not obtain its single crystals for X-ray diffraction because it could only be poorly dissolved in DMA or DMF and could hardly be recrystallized from organic solvents. Therefore, its structure was determined by ^1H NMR, MALDI Mass spectrum

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Keywords: *trans*-Cyclohexane-1,2-diamine; Suzuki–Miyaura cross-coupling reaction; Heck reaction; Dimeric bidentated NHC–Pd(II) complex; Benzimidazolium iodide.

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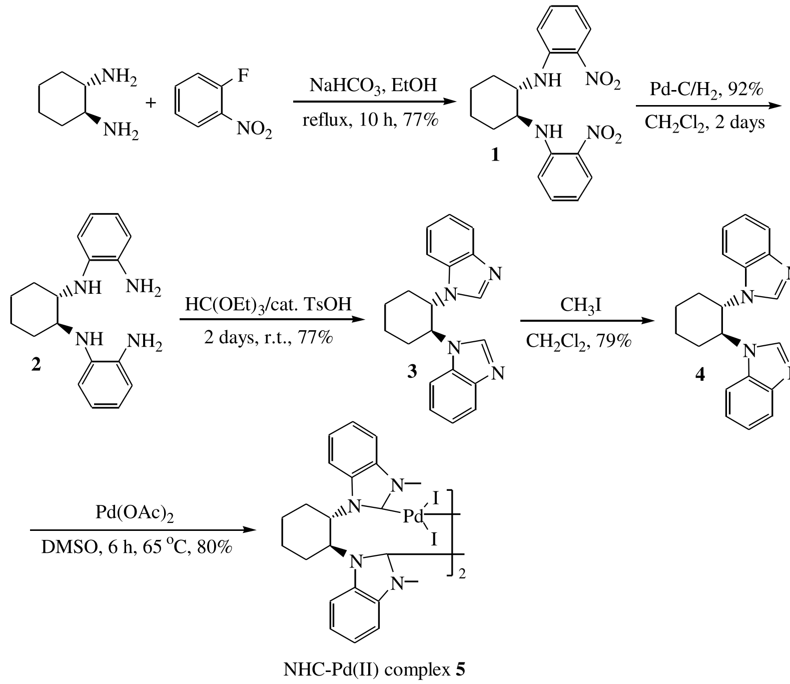


Scheme 1. Synthesis of the benzimidazolium salt and Pd(II)–NHC complex **5**.

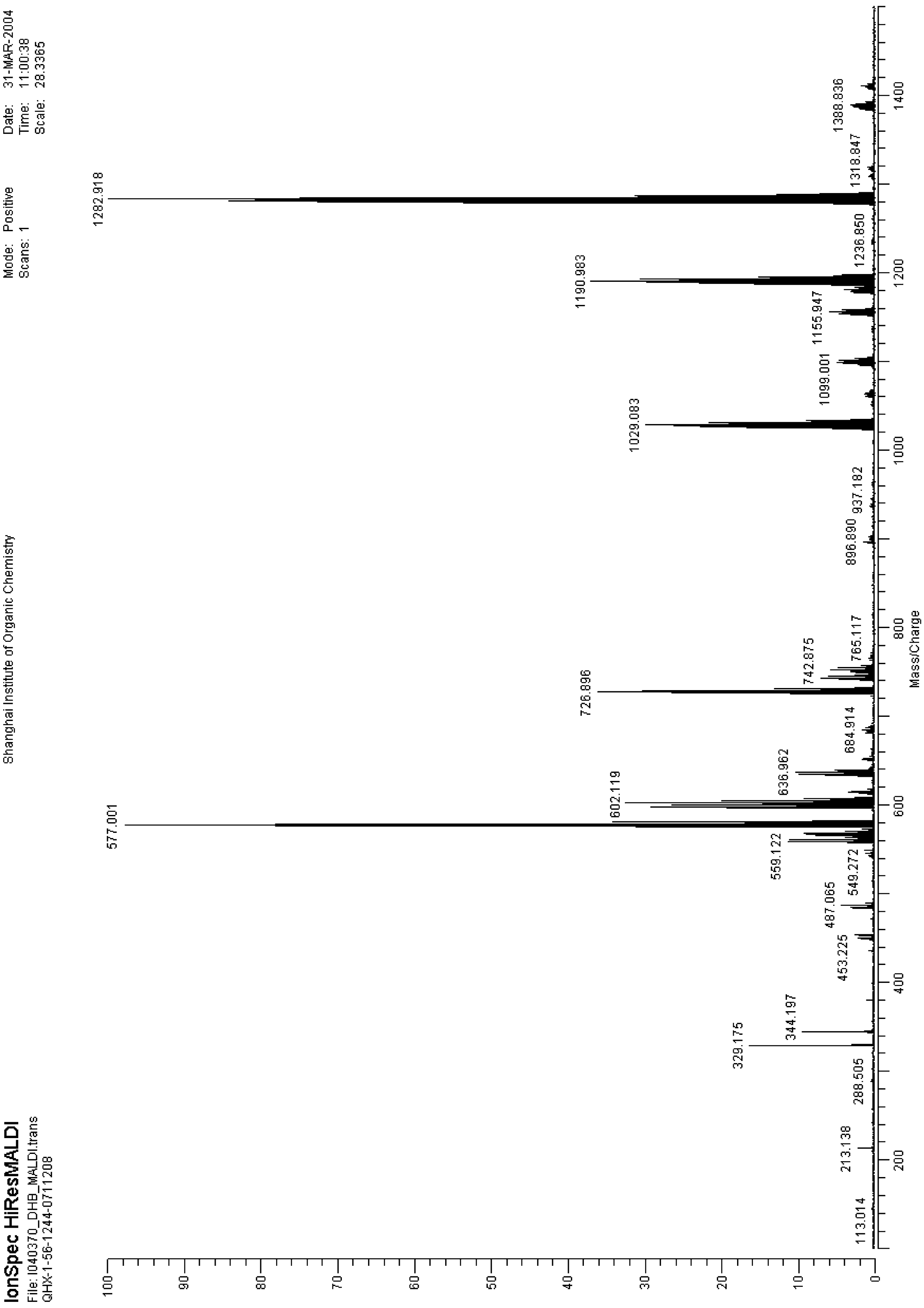
(Matrix-assisted laser desorption/ionization)[7] and elemental analysis. On the basis of MALDI Mass spectrum (Fig. 1), a molecular ion peak was identified at 1283 ($M^+ - I$) along with a ion peak at 577 ($1/2M^+ - H–I$) by this soft and sensitive ionization technique. Thus, this NHC–Pd(II) complex **5** should be a dimeric bidentated NHC–Pd(II) complex.

The application of Pd(II)–NHC complex **5** as a catalyst for Suzuki–Miyaura cross-coupling reaction was first examined where base, reaction temperature and additive effects were carefully examined in the reaction of phenylboronic acid with bromobenzene under ambient atmosphere in *N*,*N*-dimethylacetamide (DMA). This is because Pd(II)–NHC complex **5** could only be poorly dissolved in DMA. The results are summarized in Tables 1 and 2, respectively. The use of K_2CO_3 or Cs_2CO_3 (1.5 equiv) as the base in DMA (2.0 mL) at 120 °C gave the coupled product biphenyl **6a** in 94 and 92% yield after 2 h, respectively in the presence of additive $C_{16}H_{33}Me_3NBr$ (1.0 mmol%)[8] (Table 1, entries 1 and 2). Under the identical conditions, **6a** was obtained in 84% yield using KOAc or $K_3PO_4 \cdot 3H_2O$ as a base (Table 1, entries 3 and 5). The other bases such as $KF \cdot 2H_2O$, $NaHCO_3$ and KOH are not as effective as K_2CO_3 and Cs_2CO_3. In the presence of organic bases such as DMAP and DBU, no reaction occurred (Table 1, entries 6 and 7). Thus, the relatively cheap K_2CO_3 was the preferred base for this reaction. We also examined the effect of the reaction temperature and the additive $C_{16}H_{33}Me_3NBr$ on this reaction. The results are summarized in Table 2. We found that under identical conditions, the coupled product **6a** was obtained in higher yield at higher temperature after 2 h and no reaction occurred at temperature 15 °C (Table 1, entries 10–13). Without addition of additive $C_{16}H_{33}Me_3NBr$, **6a** was obtained in similar yield (95%) at 120 °C after 12 h (Table 1, entries 13 and 14).

Using these optimized reaction conditions, the Suzuki–Miyaura reaction of a variety of arylhalides, including arylbromides and phenyliodide, with phenylboronic acid were studied. The results are summarized in Table 3. As can be seen, arylbromides with different substituents on the benzene ring afforded coupling products **6** in 54–99% yield under ambient atmosphere within 2–10 h (Table 2, entries 1–6).

On the other hand, we also examined the Suzuki–Miyaura cross-coupling reaction of arylchlorides with phenylboronic acid using this novel dimeric bidentated NHC–Pd(II) complex **5** in DMA. The results are elucidated in Table 3. We found that under the similar conditions as those shown in Table 2 with $KOBu^t$ as a base, **6a** was obtained in only 15% (Table 3, entry 1). Previously, Nolan et al. reported that using alcohols as solvent, the Suzuki–Miyaura cross-coupling reaction of arylchlorides with phenylboronic acid could be achieved at room temperature in the presence of NaO^tBu.[9] Therefore, we examined the additive effects of *iso*-propanol and *tert*-amyl alcohol (1.5 mL) on this type of Suzuki–Miyaura cross-coupling reaction. We found that with the addition of *tert*-amyl alcohol (1.5 mL), **6a** can be obtained in 55% yield under identical conditions. For two other arylchlorides such as 4-methylphenylchloride and 4-acetylphenylchloride, the corresponding coupled products **6d** and **6e** were obtained in 45 and 62% yields, respectively (Table 3, entries 4 and 5).

Heck reaction was also examined in DMA by the reaction of bromobenzene with butyl acrylate in the presence of various bases and additives (Table 4, entries 1–6). We found that K_2CO_3 afforded the best results for this reaction and allowed the coupling product **7a** to be obtained in 80% under ambient atmosphere at 160 °C in the presence of $C_{16}H_{33}Me_3NBr$ (1.0 mol%) (Table 4, entry 6).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1029.083
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1190.983
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1282.918
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1388.836
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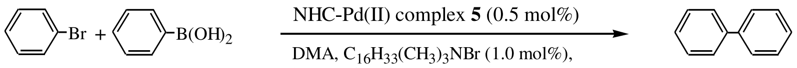
400
600
800
1000
1200
1400
Mass/Charge

Figure 1.

Table 1. Screening for base, additive and reaction temperature in the NHC–Pd(II) complex **5** catalyzed Suzuki-cross coupling reaction of bromobenzene (1.0 equiv) with phenylboronic acid (1.2 equiv) in DMA (2.0 mL)



6a

Entry	Temp. (°C)	Base	Time (h)	**6a** Yield (%)[a]
1	120	K_2CO_3	2	94
2	120	Cs_2CO_3	2	92
3	120	KOAc	2	84
4	120	$KF\cdot 2H_2O$	2	58
5	120	$K_3PO_4\cdot 3H_2O$	2	84
6	120	DMAP	2	N.R[b]
7	120	DBU	2	N.R[b]
8	120	$NaHCO_3$	2	65
9	120	KOH	2	32
10	90	K_2CO_3	12	84
11	50	K_2CO_3	12	32
12	15	K_2CO_3	12	N.R.
13	120	K_2CO_3	12	94
14[c]	120	K_2CO_3	12	95

[a] Isolated yields.

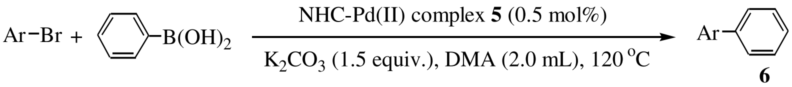
[b] No reaction occurred.

[c] Without addition of additive $C_{16}H_{33}Me_3NBr$.

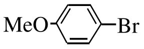
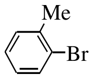
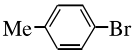
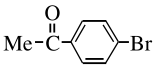
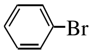
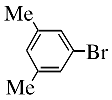
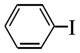
Using these reaction conditions, we next examined the Heck coupling of a variety of arylhalides with butyl and methyl acrylate. The results are summarized in Table 5. We found that the Heck reaction products **7** were obtained in good to high yields in most cases under ambient atmosphere (Table 5, entries 1–3 and 5–10). For methyl acrylate, the coupled product **7f** was obtained in moderate yield (Table 5, entry 7). Product structures were determined by ^1H and ^{13}C NMR spectroscopy (see Supporting information).

In conclusion, we disclosed a novel dimeric bidentated NHC–Pd(II) complex **5** as an effective catalyst for Suzuki–Miyaura reaction and Heck cross-coupling reaction. The corresponding coupled products were obtained in good to high yields in most cases by this Pd(II)-catalyst under normal conditions. Efforts are underway to elucidate the mechanistic details of this C–C bond forming reaction catalyzed by Pd(II)–NHC complex and the use of **5** to catalyze other C–C bond forming transformations.

Table 2. NHC–Pd(II) complex **5** catalyzed Suzuki-cross coupling reaction of arylbromide (1.0 equiv) with phenylboronic acid (1.2 equiv)

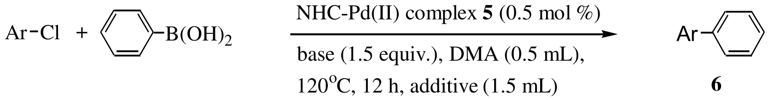


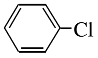
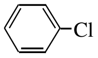
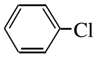
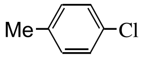
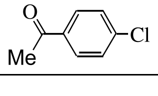
6

Entry	Ar	Time (h)	**6** Yield (%)[a]
1		10	**6b**, 80
2		3	**6c**, 92
3		3	**6d**, 99
4		10	**6e**, 73
5		2	**6a**, 94
6		10	**6f**, 54
7		3	**6a**, 85

[a] Isolated yields.

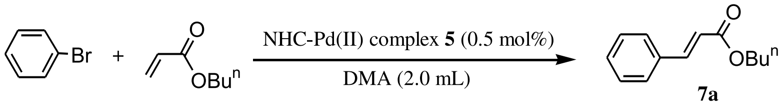
Table 3. NHC–Pd(II) complex **5** catalyzed Suzuki-cross coupling reaction of arylchloride (1.0 equiv) with phenylboronic acid (1.2 equiv)



Entry	Ar	Base	Additive	**6a** Yield (%)[a]
1		$KOC(CH_3)_3$	None	**6a**, 15
2		$KOC(CH_3)_3$	*iso*-Propanol	**6a**, 42
3		$KOC(CH_3)_3$	*tert*-Amyl alcohol	**6a**, 55
4		$KOC(CH_3)_3$	*tert*-Amyl alcohol	**6d**, 45
5		$KOC(CH_3)_3$	*tert*-Amyl alcohol	**6e**, 62

[a] Isolated yields.

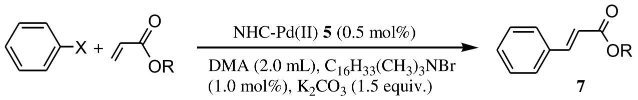
Table 4. NHC–Pd(II) complex **5** (0.5 mol%) catalyzed Heck-cross coupling reaction of phenylbromide (1.0 equiv) with butyl acrylate (1.5 equiv)

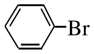
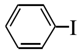
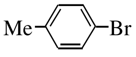
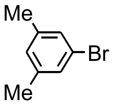
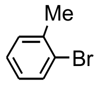
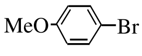
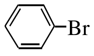


Entry	Additive	Base[a]	Temp (°C)	Time (h)	**7a** Yield (%)[b]
1	None	K_2CO_3	160	12	15
2	None	Cs_2CO_3	160	12	Trace
3	4A MS	$K_3PO_4 \cdot 3H_2O$	160	12	<10
4	No	KOAc	160	12	<10
5	No	Et_3N	160	12	N.R.
6	$C_{16}H_{33}(CH_3)_3NBr$	K_2CO_3	160	12	80

[a] 1.5 equiv of base.
[b] Isolated yields.

Table 5. NHC–Pd(II) **5** (0.5 mol%) catalyzed Heck-cross coupling reaction of arylhalide (1.0 equiv) with butyl acrylate (1.5 equiv)



Entry	Ar–X	R	Temp (°C)	Time (h)	**7** Yield (%)[a]
1		Bu^n	160	6	**7a**, 80
2		Bu^n	160	6	**7a**, 99
3		Bu^n	160	5	**7b**, 90
4		Bu^n	160	5	**7c**, 88
5		Bu^n	160	3	**7d**, 88
6		Bu^n	160	10	**7e**, 80
7		Me	160	6	**7f**, 45

[a] Isolated yield.

3. Experimental

3.1. General remarks

All reactions were conducted in oven (135 °C) and flame-dried glassware under ambient atmosphere of air and moisture; dichloromethane was distilled from calcium hydride; diethyl ether, tetrahydrofuran were distilled from sodium metal/benzophenone ketyl. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. Mass spectra were recorded by the EI method. All of the solid compounds reported in this paper gave satisfactory CHN microanalyses. Commercially obtained reagents were used without further purification. All reactions were monitored by TLC with silica gel coated plates.

3.1.1. Synthesis of *N*,*N*′-bis(2-nitrophenyl)cyclohexane-1,2-diamine 1. *trans*-Cyclohexanediamine (a racemic compound, 228 mg, 2 mmol), 2-fluoronitrobenzene (564 mg, 4 mmol), and $NaHCO_3$ (370 mg, 4.4 mmol) were stirred in refluxing anhydrous ethanol for 10 h. Then the reaction mixture was poured into 15 mL of ice water when hot and was further stirred for 20 min. The organic layers were extracted with CH_2Cl_2 (2×20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by a silica gel flash column chromatography (eluent: hexane/CH_2Cl_2=1/1) to give **1** as an orange solid (547 mg, yield: 77%). This is a known compound.[10]

^{1}H NMR ($CDCl_3$, TMS, 300 MHz): δ 1.30–1.70 (4H, m, CH_2), 1.72–2.00 (2H, m, CH_2), 2.18–2.25 (2H, m, CH_2), 3.65–3.73 (2H, m, CH), 6.52 (2H, t, J=7.5 Hz, ArH), 6.94 (2H, d, J=8.4 Hz, ArH), 7.28 (2H, t, J=7.5 Hz, ArH), 7.95 (2H, d, J=8.4 Hz, ArH), 8.15 (2H, d, J=5.7 Hz, NH).

3.1.2. Synthesis of *N*,*N*′-bis(2-aminophenyl)cyclohexane-1,2-diamine 2. Under 1.0 atm of hydrogen atmosphere, a mixture of **1** (2.86 g, 8.03 mmol) and 10 wt% Pd/C (450 mg, 0.43 mmol) were stirred in anhydrous CH_2Cl_2 at room temperature for 2 days. After filtration, the solvent was removed under reduced pressure and the residue was purified by a silica gel flash column chromatography (eluent: CH_2Cl_2) to give **2** as a white solid (2.18 g, yield: 92%). mp 120–122 °C; IR (CH_2Cl_2): ν 3296, 2933, 1597, 1508, 1267, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS, 300 MHz): δ 1.10–1.26 (2H, m, CH_2), 1.36–1.43 (2H, m, CH_2), 1.56 (2H, s, NH), 1.71–1.78 (2H, m, CH_2), 2.33–2.41 (2H, m, CH_2), 3.10–3.30 (2H, m, CH), 3.37 (4H, s, NH_2), 6.70–6.82 (8H, m, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.8, 32.2, 57.4, 113.4, 116.6, 119.1, 120.1, 135.6, 136.4; MS (EI) *m/z*: 296 (M$^+$), 189 (86.80), 145 (15.66), 119 (100); Anal. Calcd for $C_{18}H_{24}N_4$ C, 72.94; H, 8.16; N, 18.90; found: C, 72.56; H, 7.81; N, 18.64%. HRMS (MALDI/DHB) Calcd for $C_{18}H_{25}N_4^{+1}$(M+H$^+$) 297.2074; found: 297.2076.

3.1.3. Synthesis of compound 3. A mixture of **2** (2.18 g 7.36 mmol), TsOH (30 mg), and $HC(OEt)_3$ (30 mL) were stirred at room temperature for 2 days. The volatiles were removed under reduced pressure and the residue was purified by a silica gel flash column chromatography (eluent: EtOAc/CH_2Cl_2/CH_3OH/Et_3N =8/2/1/0.2) to give **3** as a gray solid (1.8 g, yield: 77%). mp 252–254 °C; IR (CH_2Cl_2): ν 3443, 2941, 1655, 1492, 741 cm^{-1}; ^{1}H NMR ($CDCl_3$, TMS, 300 MHz): δ 1.65–1.80 (2H, m, CH_2), 2.12–2.19 (2H,m, CH_2), 2.20–2.40 (4H, m, CH_2), 4.60–4.75 (2H, m, CH), 7.00–7.15 (6H, m, ArH), 7.50–7.65 (2H, m, ArH), 7.73 (2H, s, N=CH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.0, 32.2, 59.1, 109.1, 120.2, 122.0, 122.7, 132.5, 140.5, 143.3; MS (EI) *m/z*: 317 (MH$^+$, 25.32), 316 (M$^+$, 100), 118 (M$^+$−198, 46.32), 171 (M$^+$−145, 40.75); HRMS (MALDI/DHB) Calcd for $C_{20}H_{21}N_4^{+1}$(M+H$^+$) 317.1761; found: 317.1752.

3.1.4. Synthesis of compound 4. A mixture of **3** (200 mg, 0.63 mmol) and iodomethane CH_3I (0.2 mL, 3.2 mmol) were stirred in anhydrous CH_2Cl_2 at room temperature for 2 days. A white precipitation was formed. The solid compound was obtained after filtration and washed with diethyl ether (2×20 mL). The compound **4** was obtained as a white powder (300 mg, yield: 79%). mp 305–307 °C; IR (KBr): ν 3432, 3022, 1565, 769 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz): δ 1.68–1.72 (2H, m, CH_2), 2.06–2.09 (4H, m, CH_2), 2.41–2.42 (2H, m, CH_2), 3.96 (6H, s, CH_3), 5.33–5.39 (2H, m, CH), 7.42–7.48 (4H, m, ArH), 7.63–7.71 (4H, m, ArH), 10.00–10.03 (2H, s, CH); ^{13}C NMR (DMSO, 75 MHz): δ 24.7, 31.2, 34.4, 61.3, 112.4, 114.1, 127.4, 127.6, 131.5, 142.9; MS (MALDI): 473 (M$^+$−I), 345 (M$^+$−H-2I). Anal. Calcd for $C_{22}H_{26}I_2N_4$ C, 44.02; H, 4.37; N, 9.33; found: C, 44.15; H,4.21; N, 9.36%.

3.1.5. Synthesis of NHC–Pd(II) complex 5. The compound **4** (70 mg, 0.12 mmol) was dissolved in 8 mL of DMSO and was stirred at 65 °C, then $Pd(OAc)_2$ (30 mg, 0.13 mmol) was added. The oil bath was elevated to 85 °C during a period of 1 h. A pale green precipitation was formed. The solid was filtered and washed with CH_2Cl_2 (2×10 mL) and dried in a dry box under reduced pressure to give NHC–Pd(II) complex **5** (70 mg, yield: 80%). mp 360–362 °C (dec.); IR (KBr): ν 3483, 2940, 2854, 1477, 1448, 1342, 749 cm^{-1}; ^{1}H NMR (DMF-d^7, 300 MHz): δ 2.03–2.05 (3H, m, CH_2), 2.20–2.25 (1H, m, CH_2), 2.57–2.59 (3H, m, CH_2), 2.90–2.95 (1H, m, CH_2), 4.13 (3H, s, CH_3), 4.29 (3H, s, CH_3), 5.27–5.40 (2H, m, CH), 7.31–7.36 (5H, m, ArH), 7.64–7.79 (3H, m, ArH); MS (MALDI): 1283 (M$^+$−I), 577 (1/2 M$^+$−H–I); Anal. Calcd for $C_{44}H_{48}I_4N_8Pd_2$ C, 37.50; H, 3.43; N, 7.95; found: C, 37.56; H, 3.37; N, 7.80%.

3.2. General procedure for the Suzuki reaction of aryl halides with boronic acids

A typical procedure is given below on the reaction expressed in entry 1 of Table 1. NHC–Pd(II) complex **5** (7.0 mg, 0.005 mmol), potassium carbonate (200 mg, 1.5 mmol), benzenebromide (110 μL, 1.0 mmol), phenylboronic acid (148 mg, 1.2 mmol), and DMA (2.0 mL) were introduced to a Schlenk tube under ambient atmosphere. The mixture was stirred at 120 °C for 2 h. The reaction mixture was diluted with H_2O (15 mL) and Et_2O (15 mL), followed by extraction twice with Et_2O. The organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give crude product. The pure product was isolated by column chromatography (eluent: hexane) on silica gel to give 147 mg (95%) of biphenyl as a colorless solid and was analyzed by ^{1}H NMR and IR spectroscopy.

3.3. Typical reaction procedure for Heck reaction

In a Schlenk tube fitted with a septum and reflux condenser were placed aryl halide (1.0 mmol), butyl acrylate (1.5 mmol), potassium carbonate (200 mg, 1.5 mmol), cetyltrimethylammonium bromide (4 mg, 0.01 mmol), *N*,*N*-dimethylacetamide (DMA, 2.0 mL) and then NHC–Pd(II) complex **5** (7.0 mg, 0.005 mmol) was added. The reaction mixture was stirred at 160 °C for 6 h. The reaction mixture was diluted with H_2O (15 mL) and Et_2O (15 mL), followed by extraction twice with Et_2O. The organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give crude product. A pure product was isolated by column chromatography (eluent: 15/1 hexanes/ethyl acetate) on silica gel. The purified product was analyzed by ^1H NMR and IR spectroscopy.

Acknowledgements

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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.2005.03.043

References and notes

1. (a) Herrmann, W. A. *Angew. Chem., Int. Ed.* **2002**, *40*, 1290–1309. (b) Bourissou, D.; Guerret, O.; GabbaÏ, F. P.; Bertrand, G. *Chem. Rev.* **2000**, *100*, 39–91. (c) Herrmann, W. A.; Böhm, V. P. W.; Gstöttmayr, C. W. K.; Grosche, M. C.; Reisinger, C.; Weskamp, T. *J. Organomet. Chem.* **2001**, *617–618*, 616–628. (d) Enders, D.; Gielen, H. *J. Organomet. Chem.* **2001**, *617–618*, 70–80.
2. (a) Herrmann, W. A.; Elison, M.; Fisher, J.; Kocher, C.; Artus, G. R. J. *Angew. Chem., Int. Ed.* **1995**, *34*, 2371–2374. (b) Albert, K.; Gisdakis, P.; Rosch, N. *Organometallics* **1998**, *17*, 1608–1616. (c) Zhang, C.; Trudell, M. L. *Tetrahedron Lett.* **2000**, *41*, 595–598. (d) Perry, M. C.; Cui, X.-H.; Burgess, K. *Tetrahedron: Asymmetry* **2002**, *13*, 1969–1972. (e) Lee, H.-M.; Lu, C.-Y.; Chen, C.-Y.; Chen, W.-L.; Lin, H.-C.; Chiu, P.-L.; Cheng, P.-Y. *Tetrahedron* **2004**, *60*, 5807–5825. (f) Marshall, C.; Ward, M.-F.; Harrison, W. T. A. *Tetrahedron Lett.* **2004**, *45*, 5703–5706. (g) Clyne, D. S.; Jin, J.; Genest, E.; Gallucci, J. C.; RajarBabu, T. V. *Org. Lett.* **2000**, *2*, 1125–1128.
3. Suzuki, A. In *Metal-Catalyzed Cross-Coupling Reactions*; Diederich, F., Stang, P. J., Eds.; Wiley-VCH: Weinheim, 1998; pp 49–97. For recent reviews, see: (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483. (b) Stanforth, S. P. *Tetrahedron* **1998**, *54*, 263–303. (c) Suzuki, A. *J. Organomet. Chem.* **1999**, *576*, 147–168. (d) Littke, A. F.; Fu, G. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 4176–4211. (e) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633–9695. (f) Miura, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 2201–2203.
4. (a) Amatore, C.; Jutand, A. *Acc. Chem. Res.* **2000**, *33*, 314–321. (b) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009–3066. (c) Guillou, C.; Beunard, J.-L.; Gras, E.; Thal, C. *Angew. Chem., Int. Ed.* **2001**, *40*, 4745–4746. (d) Oestreich, M.; Dennison, P. R.; Kodanko, J. J.; Overman, L. E. *Angew. Chem., Int. Ed.* **2001**, *40*, 1439–1442. (e) Ashimori, A.; Bachand, B.; Calter, M. A.; Govek, S. P.; Overman, L. E.; Poon, D. J. *J. Am. Chem. Soc.* **1998**, *120*, 6488–6499. (f) Shibasaki, M.; Boden, C. J. D.; Kojima, A. *Tetrahedron* **1997**, *53*, 7371–7395. (g) de Meijere, A.; Meyer, F. E. *Angew. Chem., Int. Ed.* **1994**, *33*, 2379–2411.
5. (a) Böhm, V. P. W.; Gstöttmayr, C. W. K.; Weskamp, T.; Herrmann, W. A. *J. Organomet. Chem.* **2000**, *595*, 186–190. (b) Zhang, C.; Huang, J.; Trudell, M. L.; Nolan, S. P. *J. Org. Chem.* **1999**, *64*, 3804–3805. (c) Gstöttmayr, C. W. K.; Böhm, V. P. W.; Herdtweck, E.; Grosche, M.; Herrmann, W. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1363–1365. (d) Fürstner, A.; Leitner, A. *Synlett* **2001**, 290–292. (e) Hillier, A. C.; Grasa, G. A.; Viciu, M. S.; Lee, H. M.; Yang, C.; Nolan, S. P. *J. Organomet. Chem.* **2002**, *653*, 69–82. (f) Magill, A. M.; McGuinness, D. S.; Cavell, K. J.; Britovsek, G. J. P.; Gibson, V. C.; White, A. J. P.; Williams, D. J.; White, A. H.; Skelton, B. W. *J. Organomet. Chem.* **2001**, *617*, 546–560. (g) Yang, C.; Lee, H. M.; Nolan, S. P. *Org. Lett.* **2001**, *3*, 1511–1514. (h) Weskamp, T.; Böhm, V. P. W.; Herrmann, W. A. *J. Organomet. Chem.* **1999**, *585*, 348–352. (i) Grasa, G. A.; Viciu, M. S.; Huang, J.; Zhang, C.; Trudell, M. L.; Nolan, S. P. *Organometallics* **2002**, *21*, 2866–2873. (j) Altenhoff, G.; Goddard, R.; Lehmann, C. W.; Glorius, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 3690–3693. (k) Wang, A.-E.; Zhong, J.; Xie, J.-H.; Zhou, Q.-L. *Adv. Synth. Catal.* **2004**, *346*, 595–598.
6. Gardiner, M. G.; Herrmann, W. A.; Reisinger, C.-P.; Schwarz, J.; Spiegler, M. *J. Organomet. Chem.* **1999**, *572*, 239–247.
7. Matrix-assisted laser desorption/ionization (MALDI) is a more soft and sensitive ionization technique than others. Among the chemical and physical ionization pathways suggested for MALDI are gas-phase photoionization, ion-molecule reactions, etc. The most widely accepted ion formation mechanism involves gas-phase proton transfer or alkali metal transfer in the expanding plume with photoionized matrix molecules. The matrix will serve to minimize sample damage from the laser pulse and enhance the appearance of molecular ionsde Hoffmann, E.; Stroobant, V. *Mass Spectrometry: Principles and Applications*, 2nd ed.; Wiley: Chichester, 2001; pp 28–32.
8. Grasa, G. A.; Viciu, M. S.; Huang, J.; Nolan, S. P. *J. Org. Chem.* **2001**, *66*, 7729–7737.
9. Navarro, O.; Kelly, R.-A.; Nolan, S.-P. *J. Am. Chem. Soc.* **2003**, *125*, 16194–16195.
10. Wardell, S. M. S. V.; Wardell, J. L.; Ward, M. F.; Low, J. N.; Glidewell, C. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **2000**, *56*, 865–867.

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Substituted coumarin amidines: useful building blocks for the preparation of [1]benzopyrano[4,3-*b*]pyridin-5-one and [1]benzopyrano[4,3-*d*]pyrimidin-5-one derivatives

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Abstract—The synthesis of [1]benzopyrano[4,3-*b*]pyridin-5-ones **4a–f** and **4g–j** starting from 3-formylcoumarin and 3-cyanocoumarin *N*-functionalized amidines **3a–f** and **3g–j**, respectively, was reported. The ring-closure reaction mechanism, under basic or acidic media, was proposed. Furthermore, the reaction of 3-formylamidines **3a**,**c–f** with ammonium acetate gave good yields of 2-substituted [1]benzopyrano[4,3-*d*]pyrimidin-5-ones **7**.

1. Introduction

In recent years nitrogen polyheterocycles containing a coumarin nucleus have received increasing attention due to their potential biological properties, and considerable efforts have been undertaken to exploit synthetic routes to these compounds. In particular, the coumarin nucleus is present in compounds endowed with analgesic[1] and antiplatelet[2] properties, and in promising drugs useful as phosphodiesterase VII inhibitors[3] for the treatment of immunity-associated diseases.

The introduction of substituents in the C-3 position of the coumarin moiety is of great importance as this generates intermediates in the synthesis of heterocycles condensed with the benzopyran-2-one nucleus.

In the last few years we focused our attention on tertiary amidines bearing a 3-substituted coumarin group on N-1 as synthons to give nitrogen fused triheterocycles, through intramolecular cyclization reactions.

From acetamidines bearing a 3-nitrocoumarin group on N-1, we recently developed an easy method for the preparation of coumarin fused to imidazoles.[4]

In this context, we reported a preliminary communication[5] on acetamidines bearing the 3-formylcoumarin group on N-1 as a starting material to synthesize benzopyranopyridin-5-ones.

As a continuation of this study, we describe here, in full detail, the synthesis of substituted 2-amino- and 2,4-diaminobenzopyranopyridin-5-ones through acetamidines bearing a 3-formyl or 3-cyano coumarin group on N-1.

With the purpose to extend this strategy to different nitrogen polyheterocycle systems we report, for the first time, a new synthetic approach to 2-substituted benzopyranopyrimidin-5-ones. The key intermediates were again the acetamidines substituted at N-1 with the 3-formyl coumarin group.

2. Results and discussion

Amidines **3a–j** were prepared from azides **1a–b** and enamines **2a–f**. The new azide **1b** was achieved by reaction of sodium azide with the corresponding 4-chloro-2-oxo-2*H*-[1]benzopyran-3-carbonitrile.[6] We performed the reactions using equimolar amounts of 4-azido-3-formylcoumarin **1a**[7] or of 4-azido-3-cyanocoumarin **1b** and the appropriate enamine **2a–f**,[8–11] previously synthesized, in chilled dichloromethane solution. These reaction conditions allowed the isolation in good yields of the amidines **3a–f** and **3g–j**, respectively (Scheme 1).

Keywords: 3-Formylcoumarin amidines; 3-Cyanocoumarin amidines; Enediamino tautomers; Aldols; Intramolecular cyclization.

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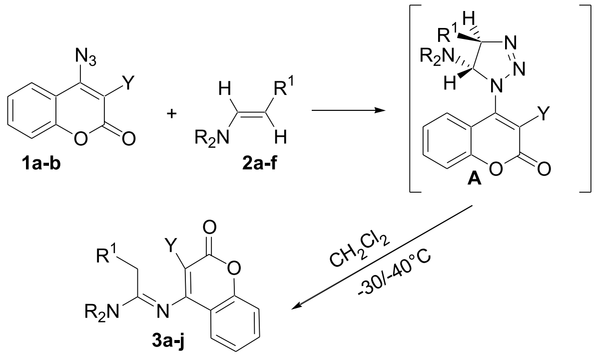


1a Y = CHO
1b Y = CN

2a R^1 = C_6H_5, NR_2 = morpholine
2b R^1 = C_6H_5, NR_2 = diethylamine
2c R^1= 4-BrC_6H_4, NR_2 = morpholine
2d R^1 = 4-$CH_3OC_6H_4$, NR_2 = morpholine
2e R^1 = *i*Pr, NR_2 = morpholine
2f R^1 = $CH_2C_6H_5$, NR_2 = morpholine

3a R^1 = C_6H_5, NR_2 = morpholine, Y = CHO
3b R^1 = C_6H_5, NR_2 = diethylamine, Y = CHO
3c R^1= 4-BrC_6H_4, NR_2 = morpholine, Y = CHO
3d R^1 = 4-$CH_3OC_6H_4$, NR_2 = morpholine, Y = CHO
3e R^1 = *i*Pr, NR_2 = morpholine, Y = CHO
3f R^1 = $CH_2C_6H_5$, NR_2 = morpholine, Y = CHO
3g R^1 = C_6H_5, NR_2 = morpholine, Y = CN
3h R^1 = C_6H_5, NR_2 = diethylamine, Y = CN
3i R^1 = *i*Pr, NR_2 = morpholine, Y = CN
3j R^1 = $CH_2C_6H_5$, NR_2 = morpholine, Y = CN

Scheme 1.

Despite the low reaction temperature, the 4,5-dihydrotriazole **A** could never be isolated, owing to its thermal instability as a result of the electron-withdrawing effect of the N-1 substituent[12] which facilitates the cleavage of the N1–N2 bond and promotes amidine rearrangement.

The proposed structures of amidines **3a**–**f** and **3g**–**j** were validated by spectral data, which were consistent with the available literature data for similar substitution patterns in coumarin derivatives.[13]

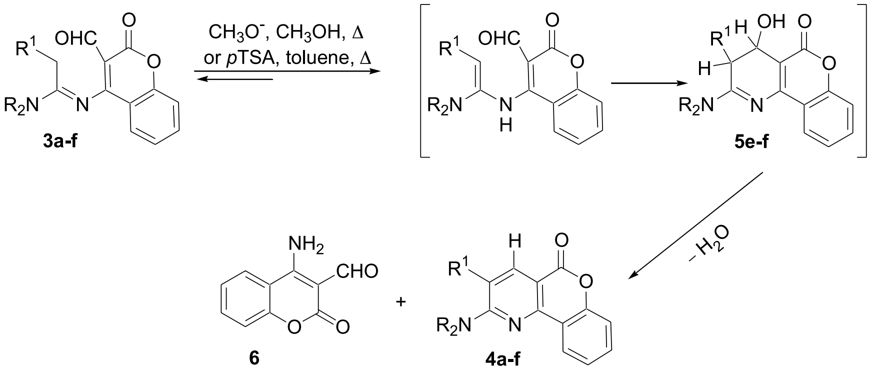
It is well known that the activation of the α-methylene group of amidines, with respect to condensation reaction, is attributed to the existence of amidine–enediamine tautomerism.[14]

Taking into account the structural features of amidines **3a**–**f** and **3g**–**j** we proposed, at first, to take advantage of the nucleophilic character of the α-methylene group of amidines,[14b,c] as well as the presence of formyl or cyano groups linked to C-3 of the coumarin ring, to plan an intramolecular condensation reaction.

The amidines **3a**–**d** were heated in methanol at reflux under basic conditions, and easily transformed into the expected 2-amino-benzopyranopyridinones **4a**–**d**. In the case of substrates **3e**–**f**, in addition to the desired products **4e**–**f**, the aldol intermediates **5e**–**f** and the side product **6**, arising from the hydrolytic reaction of amidine were also isolated (Scheme 2).

In aldol intermediate **5**, the subsequent water loss should be assisted by the substituent group bound to C-3.

To determine the best conditions to favour this process, and to enhance the yield of derivatives **4a**–**b**,**e**–**f**, we decided



3a, 4a, 5a R^1 = C_6H_5, NR_2 = morpholine
3b, 4b, 5b R^1 = C_6H_5, NR_2 = diethylamine
3c, 4c, 5c R^1= 4-BrC_6H_4, NR_2 = morpholine
3d, 4d, 5d R^1 = 4-$CH_3OC_6H_4$, NR_2 = morpholine
3e, 4e, 5e R^1 = *i*Pr, NR_2 = morpholine
3f, 4f, 5f R^1 = $CH_2C_6H_5$, NR_2 = morpholine

Scheme 2.

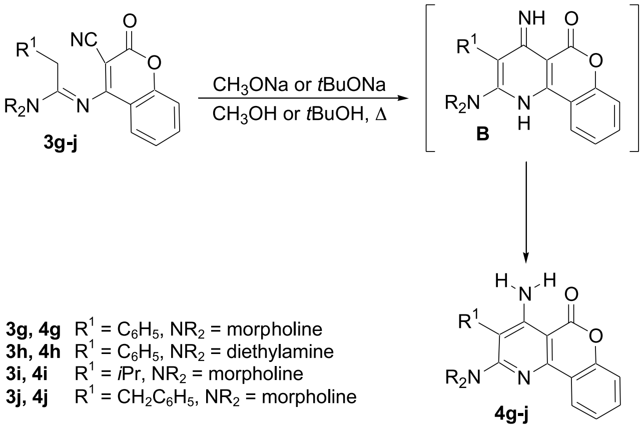
Table 1. Effect of experimental conditions on **4**,**5**,**6** ratio

Amidines **3**	Compd. **4** yield (%)		Compd. **5**[a] yield (%)	Compd. **6**[a] yield (%)
	Method A[b]	Method B[c]		
a	46	70	0	
b	58	88	0	
c	94	—	0	
d	85	—	0	
e	36[d]/18[e]	60	28[e]	35[e]
f	38[d]/22[e]	66	27[e]	33[e]

[a] Compound formed only with method A.
[b] CH_3ONa/CH_3OH: see Section 4.
[c] *p*-TSA/toluene: see Section 4.
[d] Total yield: see Section 4.
[e] Yields of **4**, **5**, **6** from chromatographic column: see Section 4.

to perform the reaction of related amidines in acidic medium.

The reaction of the amidines **3a**–**b**,**e**–**f** in toluene at reflux, containing a catalytic amount of *p*-toluensulfonic acid, afforded the expected benzopyranopyridin-5-ones **4a**–**b**,**e**–**f**. Table 1 shows the results obtained under basic and acidic media. Significant improvements in yields were achieved by acidic reaction conditions (Table 1, method B), thus confirming the hypothesis that the ring-closure reaction occurs through isomerization of the amidine **3** into its enediamino tautomer,[14] followed by an intramolecular condensation of the α-carbon with the formyl group. It is plausible to postulate, in basic medium, a similar mechanistic hypothesis.



3g, 4g $R^1 = C_6H_5$, NR_2 = morpholine
3h, 4h $R^1 = C_6H_5$, NR_2 = diethylamine
3i, 4i R^1 = *i*Pr, NR_2 = morpholine
3j, 4j $R^1 = CH_2C_6H_5$, NR_2 = morpholine

Scheme 3.

We furthermore investigated the possibility to react 3-cyano substituted acetamidines **3g**–**j** with the aim to achieve 2,4-diaminobenzopyranopyridin-5-ones. By heating acetamidines **3g**–**j** in basic medium (methoxide for **3g**–**h** and *t*butoxide for **3i**–**j**, respectively), the expected tricyclic derivatives **4g**–**j** bearing an NH_2 group on C-4 were produced.

^{13}C NMR spectra of **4g**–**j** showed a quaternary carbon at about 150.6–151.7 ppm, as a diagnostic signal for C-4 linked to the amino group of the pyridine ring.[15] In the IR spectrum we also observed an absorption band in the range 1691–1698 cm^{-1}, assigned to the stretching of the coumarin carbonyl group, whose frequency is lowered[16] owing to the intramolecular hydrogen bonding, with the neighbouring amino function. The production of fused heterocycles **4g**–**j** can be rationalized as follows (Scheme 3).

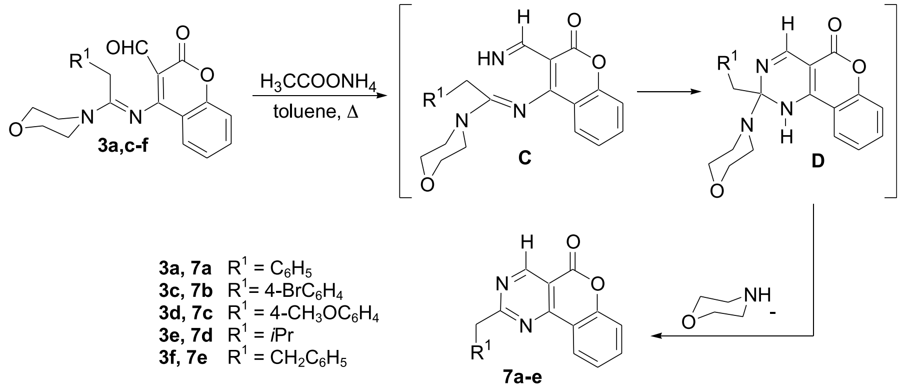
In a basic medium, the iminic intermediate **B** arising from the nucleophilic addition of the C-α amidinic carbon to the cyano group, through rapid isomerization gave the desired 4-amino group.

The described success to synthesize 2-aminobenzopyranopyridinones **4a**–**f** from the corresponding 3-formyl acetamidines **3a**–**f** prompted us to react the same compounds to obtain 2-substituted benzopyranopyrimidin-5-ones **7**. As a synthetic route, we considered a mixture of 3-formyl acetamidines **3a**,**c**–**f** with an excess of ammonium acetate in toluene at reflux. In a short reaction time, expected tricyclic derivatives **7a**–**e** were achieved in very good yield (Scheme 4).

The structural assignments of 2-alkyl-benzopyranopyrimidin-5-ones **7a**–**e** established by ^1H and ^{13}C NMR spectroscopy relating to the coumarin[13a] and pyrimidine[17] nuclei, were in agreement with the literature data.

In each case, we observed a typical pattern for the coumarin[13a] moiety. ^{13}C NMR spectra showed a first signal at about 160 ppm, corresponding to a singlet[17b] at about 9.5 δ related to CH-4 in HETCOR experiment, and a second signal at 174.3–176.2 ppm associated to the lactone carbonyl group, the latter validated also by IR (nujol) stretching at about 1720 cm^{-1}.

In the ^1H NMR spectra we noted a deshielding effect on the



3a, 7a $R^1 = C_6H_5$
3c, 7b $R^1 = 4\text{-}BrC_6H_4$
3d, 7c $R^1 = 4\text{-}CH_3OC_6H_4$
3e, 7d R^1 = *i*Pr
3f, 7e $R^1 = CH_2C_6H_5$

Scheme 4.

methylene group linked to C-2 of the pyrimidine nucleus for the compounds **7a–e**, relative to the starting amidines **3a**,**c–f**, as a consequence of two nitrogen atoms in its *ortho* position.

The achievement of fused heterocycles **7** can be rationalized by the reaction path depicted in Scheme 4.

The reaction with ammonium acetate gave the imino intermediate **C**, which rapidly underwent ring-closure through addition to the C═N group of the amidine to give an unstable 2,2-disubstituted-benzopyranopyrimidone **D**. Morpholine elimination gave aromatic pyrimidine derivative **7**.

3. Conclusions

We have described new methods for the preparation of both substituted 5*H*[1]benzopyrano[4,3-*b*]pyridin-5-ones and 5*H*[1]benzopyrano[4,3-*d*]pyrimidin-5-ones in good yields. At the same time, these synthetic routes represent an expeditious access to polyheterocycles containing a coumarin moiety, adding further proof of the utility of substituted acetamidines as building blocks in organic synthesis. Although the synthesis of a limited number of derivatives is described in this paper, the flexibility of this methodology should provide access to many variously [1]benzopyrano[4,3-*b*]pyridin-5-ones and [1]benzopyrano-[4,3-*d*]pyrimidin-5-ones.

4. Experimental

4.1. General

Melting points were determined using a Buchi 510 (capillary) or an Electrothermal 9100 apparatus and are uncorrected. IR spectra were measured using Perkin–Elmer FT-IR 16 P.C. (Nujol), and Perkin–Elmer FT-IR Spectrum One (KBr) spectrometers. ^{1}H and ^{13}C NMR spectra were recorded on EM Varian Gemini 200 and on Bruker Avance 300 spectrometers in $CDCl_3$ solution unless otherwise stated. Chemical shifts are expressed in ppm from internal standard tetramethylsilane (δ), coupling constants (J) are given in Hz. Column chromatography was performed on Kieselgel 60 (Merck) 0.063–0.200 mm with eluents and ratios indicated in Section 4.

Materials. Azide **1a**,[7] enamines **2a**,**c**,**d**,[8] **2b**,[9] **2e**,[10] **2f** 11 and compounds **3a**,[5] **4a**,[5] **5e**[5] have already been described. 4-Chloro-2-oxo-2*H*-[1]benzopyran-3-carbonitrile[6] and 4-amino-3-formylcoumarin[7] **6** are known compounds.

4.1.1. 4-Azido-2-oxo-2*H*-[1]benzopyran-3-carbonitrile 1b. NaN_3 (1.092 g, 0.0168 mmol), protected from light, was suspended in dry DMF (30 mL) and chilled in an ice bath. A solution of 4-chloro-2-oxo-2*H*-[1]benzopyran-3-carbonitrile (3.3 g, 0.016 mmol) in dry DMF (50 mL) was added over 20 min. The mixture was then stirred a further 3 h at 0 °C before being poured onto ice. The red–orange precipitate was filtered and recrystallized from ethanol to give the azide **1b** (2.9 g, 85%) as orange crystals, mp 171 °C (decomp.); IR ν_{max} (KBr) 2228 (CN), 2148 and 2130 (N_3), 1722 (C═O) cm^{-1}; ^{1}H NMR δ 7.36–7.44 (2H, m, H-6, H-7); 7.71–7.79 (1H, m, H-5), 7.91 (1H, dd, J=8.4, 1.4 Hz, H-8); ^{13}C NMR ($DMSO_{d6}$): 90.5 (C-3), 112.8 (C-4a), 114.7 (CN), 118.1, 125.3, 126.3, 137.0 (CH), 153.5 (C-4), 157.7 (C-8a), 158.5 (C═O). Anal. Calcd for $C_{10}H_4N_4O_2$: C, 56.61; H, 1.90; N, 26.41; found: C, 56.49; H, 1.82; N, 26.59.

4.2. General procedure for the preparation of amidines 3a–f

The suitable enamine **2a–f** (10 mmol) was dissolved in CH_2Cl_2 (20 mL). With careful monitoring of the internal temperature (−40 °C, acetone–CO_2 bath), an equimolar amount of azide **1a**, dissolved in CH_2Cl_2 (30 mL), was slowly added dropwise with stirring. The mixture was kept at −30 °C until disappearance (TLC: cyclohexane/EtOAc, 3:2) of the starting azide **1a**. The stirring was continued until room temperature was reached. The solvent was then evaporated and the crude product purified by crystallization from *i*Pr_2O to give pure **3a–f**.

4.2.1. *N*,*N*-Diethyl-*N*′-(3-formyl-2-oxo-2*H*-[1]benzopyran-4yl)-2-phenyl-acetamidine 3b. Reaction time: 1.5 h. Yield 65%; pale yellow crystals, mp 127 °C; IR ν_{max} (Nujol) 1703 (C═O), 1668 (CHO) cm^{-1}; ^{1}H NMR: 1.24 (3H, t, J=7.3 Hz, CH_3), 1.29 (3H, t, J=7.3 Hz, CH_3), 3.30–4.11 (6H, m, CH_2NCH_2, CH_2), 7.10–7.30 (5 H+2 H, m, ArH, H-6, H-8), 7.53 (1H, td, J=7.3, 1.8 Hz, H-7), 8.05 (1H, dd, J=8.0, 1.8 Hz, H-5), 10.13 (1H, s, CHO); ^{13}C NMR: 11.8 (CH_3), 13.8 (CH_3), 38.0 (CH_2), 44.1 (CH_2N), 44.2 (CH_2N), 100.2 (C-3), 117.2, 123.9, 126.9, 133.3 (CH), 120.4 (C-4a), 127.3, 128.5, 129.2 (ArCH) 134.8 (ArC), 154.3 (C-4), 157.2 (C-8a), 165.4 (N═C–N), 165.6 (C═O), 189.1 (CHO). Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73; found: C, 72.68; H, 5.94; N, 7.89.

4.2.2. 4-{[2-(4-Bromophenyl)-1-morpholin-4-ylethylidene]amino}-2-oxo-2*H*-[1]benzopyran-3-carbaldehyde 3c. Reaction time: 1 h. Yield 78%; pale yellow crystals, mp 153 °C; IR ν_{max} (Nujol) 1700 (C═O), 1660 (CHO) cm^{-1}; ^{1}H NMR: 3.59–4.03 (10H, m, morph., CH_2), 6.98 and 7.32 (4H, J=8.4 Hz, ArH), 7.21–7.35 (2 H, m, H-6, H-8), 7.57 (1H, td, J=8.4, 1.8 Hz, H-7), 7.97 (1H, dd, J=8.4, 1.8 Hz, H-5), 10.14 (1H, s, CHO); ^{13}C NMR: 37.3 (CH_2), 46.6 (CH_2NCH_2), 66.2 (CH_2OCH_2), 100.5 (C-3), 117.2, 124.1, 126.7, 133.2 (CH), 119.6 (C-4a), 121.5 (Ar*C*Br), 129.8, 132.3 (ArCH), 136.4 (ArC), 154.3 (C-4), 157.9 (C-8a), 164.6 (N═C–N), 165.0 (C═O), 189.2 (CHO). Anal. Calcd for $C_{22}H_{19}BrN_2O_4$: C, 58.04; H, 4.21; N, 6.15; found: C, 57.91; H, 4.02; N, 6.37.

4.2.3. 4-{[2-(4-Methoxyphenyl)-1-morpholin-4-ylethylidene]amino}-2-oxo-2*H*-[1]benzopyran-3-carbaldehyde 3d. Reaction time: 3 h. Yield 63%; yellow crystals, mp 138 °C; IR ν_{max} (Nujol) 1700 (C═O), 1658 (CHO) cm^{-1}; ^{1}H NMR: 3.54–4.01 (10H, m, morph., CH_2), 3.76 (3H, s, OCH_3), 6.76 and 7.00 (4H, J=8.4 Hz, ArH), 7.20–7.29 (2H, m, H-6, H-8), 7.55 (1H, td, J=7.3, 1.8 Hz, H-7), 7.98 (1H, dd, J=8.4, 1.8 Hz, H-5), 10.12 (1H, s, CHO); ^{13}C NMR: 37.2 (CH_2), 46.7 (CH_2NCH_2), 55.3 (OCH_3), 66.1(CH_2OCH_2), 100.7 (C-3), 117.1, 124.0, 127.0, 133.6 (CH), 120.1 (C-4a), 114.4, 130.0 (ArCH), 129.6 (ArC), 154.2 (C-4), 157.5 (C-8a), 158.6 (Ar*C*OCH$_3$) 164.7 (N=C–N), 165.4 (C=O), 189.3 (CHO). Anal. Calcd for $C_{23}H_{22}N_2O_5$: C, 67.97; H, 5.46; N, 6.89; found: C, 67.87; H, 5.74; N, 7.01.

4.2.4. 4-[(3-Methyl-1-morpholin-4-ylbutylidene)amino]-2-oxo-2*H*-[1]benzopyran-3-carbaldehyde 3e. Reaction time: 2 h. Yield 70%; yellow powder, mp 134 °C; IR ν_{max} (Nujol) 1699 (C=O), 1661 (CHO) cm^{-1}; ^{1}H NMR: 0.79 (3H, d, J=6.6 Hz, CH$_3$), 0.94 (3H, d, J=6.6 Hz, CH$_3$), 1.77–1.89 (1H, m, (CH$_3$)$_2$C*H*), 2.22–2.47 (2H, m, CH$_2$), 3.60–4.08, (8H, m, morph.), 7.18–7.31 (2H, m, H-6, H-8), 7.55 (1H, td, J=7.3, 1.8 Hz, H-7), 7.92 (1H, dd, J=8.0, 1.4 Hz, H-5), 10.07 (1H, s, CHO); ^{13}C NMR: 22.3 (CH$_3$), 23.3 (CH$_3$), 27.3 (*C*H), 39.6 (CH$_2$), 54.5 (CH$_2$NCH$_2$), 66.4 (CH$_2$OCH$_2$), 99.8 (C-3), 117.2, 123.9, 127.0, 133.5 (CH), 119.9 (C-4a), 154.3 (C-4), 157.7 (C-8a), 165.4 (N=C–N), 167.8 (C=O), 188.8 (CHO). Anal. Calcd for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48; N, 8.18; found: C, 66.78; H, 6.57; N, 7.97.

4.2.5. 4-[(1-Morpholin-4-yl-3-phenyl-propylidene)-amino]-2-oxo-2*H*-[1]benzopyran-3-carbaldehyde 3f. Reaction time: 2 h. Yield 69%; yellow powder, mp 108 °C; IR ν_{max} (Nujol) 1700 (C=O), 1653 (CHO) cm^{-1}; ^{1}H NMR: 2.60–2.99 (4H, m, CH$_2$–CH$_2$), 3.60–3.93 (8 H, m, morph.), 7.02–7.27 (5 H+2 H, m, ArH, H-6, H-8), 7.54 (1H, td, J=8.0, 1.8 Hz, H-7), 7.69 (1H, dd, J=8.0, 1.8 Hz, H-5), 10.08 (1H, s, CHO); ^{13}C NMR: 32.3 (CH$_2$), 33.3 (CH$_2$), 46.5 (CH$_2$NCH$_2$), 66.1 (CH$_2$OCH$_2$), 99.7 (C-3), 117.1, 124.0, 126.8, 133.5 (CH), 119.6 (C-4a), 127.1, 128.4, 128.9 (ArCH), 139.1 (ArC), 154.2 (C-4), 157.6 (C-8a), 165.2 (N=C–N), 167.3 (C=O), 188.9 (CHO). Anal. Calcd for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17; found: C, 70.94; H, 5.54; N, 6.99.

4.3. General procedure for the preparation of amidines 3g–j

A solution of the appropriate enamine **2a–b**,**e–f** (10 mmol) in CH$_2$Cl$_2$ (20 mL) was added to a solution of an equimolar amount of azide **1b** dissolved in CH$_2$Cl$_2$ (30 mL), with careful monitoring of internal temperature (−30 °C, acetone–CO$_2$ bath). The resulting dark red solution was stirred at −30 °C until disappearance (TLC: cyclohexane/EtOAc, 1:1) of the starting azide **1b**. The stirring was continued until room temperature was reached. The solvent was then evaporated and the crude product purified by silica gel column, eluent EtOAc/cyclohexane (3:7). The crude oil crystallized from an appropriate solvent to give pure **3g–j**.

4.3.1. 4-[(1-Morpholin-4-yl-2-phenylethylidene)amino]-2-oxo-2*H*-[1]benzopyran-3-carbonitrile 3g. Reaction time: 2 h. Yield 60%; pale yellow crystals from AcOEt, mp 215 °C; IR ν_{max} (Nujol) 2206 (CN), 1700 (C=O) cm^{-1}; ^{1}H NMR: 3.55–4.10 (10H, m, morph., CH$_2$), 7.15–7.38 (5H+2 H, m, ArH, H-6, H-8), 7.61 (1H, td, J=8.0, 1.8 Hz, H-7), 7.89 (1H, dd, J=8.1, 1.8 Hz, H-5); ^{13}C NMR: 37.8 (CH$_2$), 46.2 (CH$_2$NCH$_2$), 66.4 (CH$_2$OCH$_2$), 81.4 (C-3), 116.9 (CN), 117.9 (C-4a), 117.5, 124.6, 126.0, 134.3 (CH), 127.8, 128.2, 129.5 (ArCH), 133.9 (ArC), 153.7 (C-4), 160.3 (C-8a), 162.1 (N=C–N), 164.1 (C=O). Anal. Calcd for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25; found: C, 70.83; H, 5.34; N, 10.95.

4.3.2. *N*′-(3-Cyano-2oxo-2*H*-[1]benzopyran-4yl)-*N*,*N*-diethyl-2-phenyl-acetamidine 3h. Reaction time: 2 h. Yield 83%; apricot powder from AcOEt, mp 140 °C; IR ν_{max} (KBr) 2214 (CN), 1715 (C=O) cm^{-1}; ^{1}H NMR: 1.15 (3H, t, J=7.3 Hz, CH$_3$), 1.35 (3H, t, J=7.3 Hz, CH$_3$), 3.30–4.22 (6H, m, CH$_2$NCH$_2$ and CH$_2$), 7.12–7.33 (5 H+2 H, m, ArH, H-6, H-8), 7.58 (1H, td, J=6.9, 1.8 Hz, H-7), 7.90 (1H, dd, J=8.0, 1.8 Hz, H-5); ^{13}C NMR: 12.4 (CH$_3$), 13.8 (CH$_3$), 38.3 (CH$_2$), 44.4 (CH$_2$N), 44.6 (CH$_2$N), 79.7 (C-3), 117.4 (CN), 118.2 (C-4a), 117.4, 124.3, 126.0, 133.9 (CH), 127.6, 128.3, 129.3 (ArCH), 134.4 (ArC), 153.7 (C-4), 160.8 (C-8a), 162.9 (N=C–N), 163.0 (C=O). Anal. Calcd for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69; found: C, 73.41; H, 5.66; N, 11.94.

4.3.3. 4-[(3-Methyl-1-morpholin-4-ylbutylidene)amino]-2-oxo-2*H*-[1]benzopyran-3-carbonitrile 3i. Reaction time: 3 h. Yield 70%; pink powder from *i*Pr$_2$O, mp 139 °C; IR ν_{max} (KBr) 2207 (CN), 1715 (C=O) cm^{-1}; ^{1}H NMR: 0.80 (3H, d, J=6.6 Hz, CH$_3$), 1.00 (3H, d, J=6.6 Hz, CH$_3$), 1.80–2.00 (1H, m, C*H*), 2.38–2.72 (2H, m, CH$_2$), 3.60–3.80 and 3.80–4.00 (8H, 2 m, morph.), 7.22–7.38 (2H, m, H-6, H-8), 7.59 (1H, td, J=8.4, 1.8 Hz, H-7), 7.79 (1H, dd, J=8.0, 1.8 Hz, H-5); ^{13}C NMR: 22.0 (CH$_3$), 23.3 (CH$_3$), 27.6 (*C*H), 39.9 (CH$_2$), 48.3 (CH$_2$NCH$_2$), 66.6 (CH$_2$OCH$_2$), 79.7 (C-3), 117.1 (CN), 117.9 (C-4a), 117.5, 124.4, 126.2, 134.2 (CH), 153.8 (C-4), 160.8 (C-8a), 163.7 (N=C–N), 164.6 (C=O). Anal. Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38; found: C, 67.43; H, 6.52; N, 12.13.

4.3.4. 4-[(1-Morpholin-4-yl-3-phenylpropylidene)-amino]-2-oxo-2*H*-[1]benzopyran-3-carbonitrile 3j. Reaction time: 3 h. Yield 60%; pink powder from AcOEt, mp 105 °C; IR ν_{max} (KBr) 2210 (CN), 1716 (C=O) cm^{-1}; ^{1}H NMR: 2.75–3.12 (4H, m, CH$_2$–CH$_2$), 3.60–3.97 (8H, m, morph.), 7.02–7.36 (5 H+2 H, m, ArH, H-6, H-8), 7.45 (1H, dd, J=8.0, 1.7 Hz, H-5), 7.58 (1H, td, J=8.5, 1.7 Hz, H-7); ^{13}C NMR: 32.4 (CH$_2$), 33.8 (*CH$_2$*C$_6$H$_5$), 53.5 (CH$_2$NCH$_2$), 66.6 (CH$_2$OCH$_2$), 80.4 (C-3), 117.0 (CN), 117.7 (C-4a), 117.5, 124.6, 126.6, 134.3 (CH), 127.4, 128.6, 129.2 (ArCH), 138.7 (ArC), 153.8 (C-4), 160.7 (C-8a), 163.8 (N=C–N), 164.3 (C=O). Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85; found: C, 71.08; H, 5.39; N, 11.04.

4.4. General procedure for the synthesis of [1]benzopyrano[4,3-*b*]pyridin-5-one derivatives 4a–f

Method A. A catalytic amount of sodium methoxide (0.5 ml, 30% solution) was added to a stirred suspension of the appropriate 3-formylamidine **3a–f** (4 mmol) in methanol (100 mL). The mixture was heated at reflux until the starting amidine disappeared. The solvent was evaporated under reduced pressure and the crude residue was crystallized from *i*Pr$_2$O to afford compounds **4a–d**. In the case of amidines **3e–f**, after disappearance of the starting compound (2 h) the crude reaction mixture was chromatographed on a silica gel column (EtOAc/cyclohexane, 1:4) affording a first fraction containing **4e–f**, a second fraction of 4-amino-3-formylcoumarin[7] **6** and finally the aldols **5e–f**, respectively. A catalytic amount of sodium methoxide (0.3 ml, 30% solution) was added to the previously obtained aldols **5e–f**. The mixture heated for an additional time (5 h), yielding a second batch of benzopyranopyridin-5-one **4e–f** then crystallized from *i*Pr_2O. The total yields of isolated and purified products **4a–f**,**5e–f** and **6** are listed in Table 1.

Method B. A suspension of the appropriate 3-formylamidine **3a–b**,**e–f** (4 mmol) in toluene (30 mL) containing a catalytic (0.02 g) amount of *p*-TSA was heated at reflux until disappearance of the starting amidine **3** (TLC: cyclohexane/EtOAc, 1:1, about 6 h), and then the solvent evaporated to dryness. The resulting solid residue was recrystallized from *i*Pr_2O to give pure **4** derivatives. Analytical data are shown above. The yields of isolated and purified compounds **4a–b**,**e–f** are listed in Table 1.

4.4.1. 2-(Diethylamino)-3-phenyl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4b. Reaction time: 1 h. Cream crystals, mp 123 °C; IR ν_{max} (Nujol) 1711 (C=O) cm^{-1}; ^1H NMR: 1.12 (6H, t, $J=7.3$ Hz, $2\times CH_3$), 3.46 (4H, q, $J=7.3$ Hz, $2\times CH_2$), 7.35–7.59 (5H +3H, m, ArH, H-7, H-8, H-9), 8.14 (1H, s, H-4), 8.48 (1H, dd, $J=8.4$, 1.4 Hz, H-10); ^{13}C NMR: 13.0 ($2\times CH_3$), 44.8 ($2\times CH_2N$), 108.0 (C-4a), 117.3, 124.4, 124.7, 131.5 (CH), 120.1 (C-10a), 125.6 (C-3), 127.8, 128.0, 129.0 (ArCH), 140.3 (ArC), 141.3 (CH-4), 150.3 (C-6a), 153.5 (C-10b), 161.0 (C-2), 162.1 (C=O). Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13; found: C, 76.81; H, 5.74; N, 7.95.

4.4.2. 3-(4-Bromophenyl)-2-morpholin-4-yl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4c. Reaction time: 0.5 h. White crystals, mp 141 °C; IR ν_{max} (KBr) 1725 (C=O) cm^{-1}; ^1H NMR: 3.44–3.52 (4H, m, CH_2NCH_2), 3.68–3.76 (4H, m, CH_2OCH_2), 7.33–7.60 (3H, m, H-7, H-8, H-9), 7.48 and 7.63 (4H, $J=8.8$ Hz, ArH), 8.26 (1H, s, H-4), 8.48 (1H, dd, $J=8.4$, 1.8 Hz, H-10); ^{13}C NMR: 48.9 (CH_2NCH_2), 66.4 (CH_2OCH_2), 110.1 (C-4a), 117.2, 124.4, 124.6, 131.8 (CH), 119.4 (C-10a), 122.3 (Ar*C*Br), 125.1 (C-3), 129.1, 132.4 (ArCH), 137.9 (ArC), 141.1 (CH-4), 150.3 (C-6a), 153.3 (C-10b), 161.3 (C-2), 162.7 (C=O). Anal. Calcd for $C_{22}H_{17}BrN_2O_3$: C, 60.43; H, 3.92; N, 6.41; found: C, 60.27; H, 3.76; N, 6.18.

4.4.3. 3-(4-Methoxyphenyl)-2-morpholin-4-yl-5*H*-[1]-benzopyrano[4,3-*b*]pyridin-5-one 4d. Reaction time: 0.5 h. Cream crystals, mp 165 °C; IR ν_{max} (Nujol) 1708 (C=O) cm^{-1}; ^1H NMR: 3.43–3.50 (4H, m, CH_2NCH_2), 3.68–3.74 (4H, m, CH_2OCH_2), 3.88 (3H, s, OCH_3), 6.99 and 7.50 (4H, $J=8.4$ Hz, ArH), 7.27–7.58 (3H, m, H-7, H-8, H-9), 8.19 (1H, s, H-4), 8.42 (1H, d, $J=8.4$ Hz, H-10); ^{13}C NMR: 48.7 (CH_2NCH_2), 55.4 (OCH_3), 66.5 (CH_2OCH_2), 110.1 (C-4a), 117.1, 124.4, 124.5, 131.5 (CH), 119.6 (C-10a), 126.4 (C-3), 114.6, 128.7 (ArCH), 131.2 (ArC), 140.7 (CH-4), 149.7 (C-6a), 153.2 (C-10b), 159.6 (Ar*C*OCH_3), 161.5 (C-2), 162.0 (C=O). Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21; found: C, 70.97; H, 5.06; N, 7.06.

4.4.4. 3-Isopropyl-2-morpholin-4-yl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4e. Reaction time: 7 h. Ivory powder, mp 175 °C; IR ν_{max} (Nujol) 1721 (C=O) cm^{-1}; ^1H NMR: 1.34 (6H, d, $J=6.9$ Hz, $2\times CH_3$), 3.20 (1H, quint, $J=6.9$ Hz, C*H*), 3.46–3.66 (4H, m, CH_2NCH_2), 3.90–3.98 (4H, m, CH_2OCH_2), 7.20–7.60 (3H, m, H-7, H-8, H-9), 8.37 (1H, s, H-4), 8.48 (1H, dd, $J=8.0$, 1.4 Hz, H-10); ^{13}C NMR: 24.3 ($2\times CH_3$), 28.4 (CH), 51.2 (CH_2NCH_2), 67.0 (CH_2OCH_2), 112.1 (C-4a), 117.2, 124.61, 124.64, 131.5 (CH), 119.9 (C-10a), 136.6 (C-3), 137.0 (CH-4), 148.8 (C-6a), 153.0 (C-10b), 161.9 (C-2), 164.7 (C=O). Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64; found: C, 70.23; H, 6.08; N, 8.79.

4.4.5. 3-Benzyl-2-morpholin-4-yl-5*H*-[1]benzopyrano-[4,3-*b*]pyridin-5-one 4f. Reaction time: 7 h. White crystals, mp 174 °C; IR ν_{max} (Nujol) 1721 (C=O) cm^{-1}; ^1H NMR: 3.51–3.58 (4H, m, CH_2NCH_2), 3.81–3.88 (4H, m, CH_2OCH_2), 4.1 (2H, s, CH_2), 7.14–7.60 (5H +3H, m, ArH, H-7, H-8, H-9), 8.14 (1H, s, H-4), 8.48 (1H, dd, $J=8.4$, 1.4 Hz, H-10); ^{13}C NMR: 38.3 (CH_2), 50.1 (CH_2NCH_2), 66.9 (CH_2OCH_2), 111.2 (C-4a), 117.3, 124.6, 124.7, 131.7 (CH), 127.2, 129.0, 129.1 (ArCH), 119.8 (C-10a), 127.1 (C-3) 138.9 (ArC), 141.4 (CH-4), 149.5 (C-6a), 153.2 (C-10b), 161.6 (C-2), 164.5 (C=O). Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52; found: C, 74.28; H, 5.45; N, 7.43.

4.4.6. 3-Benzyl-4-hydroxy-2-morpholin-4-yl-3,4-dihydro-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 5f. Reaction time: 2 h. Pale yellow oil, IR ν_{max} (Nujol) 3400 (OH), 1680 (C=O) cm^{-1}; ^1H NMR: 1.81 (1H, br s, OH), 2.40–2.52 (1H, m, CH-3), 2.65–3.00 (3H, m, C*H*NCH_2, CH_2Ph), 3.30–3.80 (4H+1H, m, CH_2OCH_2, C*H*NCH_2), 4.05–4.20 (2H, m, C$H_2$$NCH_2$), 4.92 (1H, s, CH-4), 7.10–7.60 (5H +3H, m, ArH, H-7, H-8, H-9), 8. 26 (1H, dd, $J=8.4$, 1.8 Hz, H-10); ^{13}C NMR: 37.0 (CH_2), 41.8 (CH-3), 45.0 and 46.9 (CH_2NCH_2), 65.9 and 67.1 (CH_2OCH_2), 68.7 (CH-4), 99.6 (C-4a), 117.1, 124.0, 125.8, 132.1 (CH), 120.0 (C-10a), 127.7, 129.2, 129.7 (ArCH), 137.6 (ArC), 154.6 (C-6a), 154.8 (C-10b), 164.2 (C-2), 168.2 (C=O). Anal. Calcd for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.68; N, 7.17; found: C, 71.07; H, 5.84; N, 6.95.

4.5. General procedure for the synthesis of 4-amino-[1]-benzopyrano[4,3-*b*]pyridin-5-one derivatives 4g–h

A suspension of cyano amidine **3g** or **3h** (3.0 mmol) in methanol (40 mL) was added to a well-stirred solution of Na (0.076 g, 3.3 mmol) in methanol (15 mL). The reaction mixture was heated at reflux until disappearance (TLC: cyclohexane/EtOAc 2:3) of the starting amidine: 1 h for **4g** and 2 h for **4h**. The mixture was cooled to room temperature and afforded a solid product which was recrystallized from methanol to give **4g** or **4h**, respectively.

4.5.1. 4-Amino-2-morpholin-4-yl-3-phenyl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4g. Yield 92%, white crystals, mp 194 °C; IR ν_{max} (Nujol) 3429 and 3300 (NH_2), 1691 (C=O) cm^{-1}; ^1H NMR: 3.25–3.35 (4H, m, CH_2NCH_2), 3.52–3.59 (4H, m, CH_2OCH_2), 5.35 (2H, br s, NH_2), 7.31–7.60 (5H +3H, m, ArH, H-7, H-8, H-9), 8.47 (1H, dd, $J=8.0$, 1.8 Hz, H-10); ^{13}C NMR ($DMSO_{d6}$): 48.8 (CH_2NCH_2), 66.5 (CH_2OCH_2), 96.5 (C-4a), 106.4 (C-10a), 117.1, 125.1, 125.4, 132.6 (CH), 120.1 (C-3), 128.6, 130.5, 130.8 (ArCH), 135.6 (ArC), 151.5 (C-4), 152.8 (C-6a), 155.1 (C-10b), 161.3 (C-2), 162.7 (C=O). Anal. Calcd for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25; found: C, 70.78; H, 5.24; N, 11.38.

4.5.2. 4-Amino-2-(diethylamino)-3-phenyl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4h. Yield 60%, white crystals, mp 180 °C; IR ν_{max} (KBr) 3487 and 3354 (NH_2), 1695 (C=O) cm^{-1}; ^1H NMR: 0.99 (6H, t, $J=6.9$ Hz, $2\times CH_3$), 3.46 (4H, q, $J=6.9$ Hz, $2\times CH_2$), 5.40 (2H, br s, NH_2), 7.30–7.46 (5H, m, ArH) 7.48–7.57 (3H, m, H-7, H-8, H-9), 8.46 (1H, dd, $J=8.0$, 1.4 Hz, H-10); ^{13}C NMR: 13.4 ($2\times CH_3$), 44.7 ($2\times CH_2N$), 96.5 (C-4a), 104.9 (C-10a), 116.8, 124.3, 125.2, 131.3 (CH), 120.7 (C-3), 128.1, 130.0, 130.9 (ArCH), 136.6 (ArC), 151.7 (C-4); 153.1 (C-6a), 154.9 (C-10b), 160.4 (C-2), 163.6 (C=O). Anal. Calcd for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69; found: C, 73.77; H, 5.84; N, 11.56.

4.6. General procedure for the synthesis of 4-amino-[1]-benzopyrano[4,3-*b*]pyridin-5-one derivatives 4i–j

A suspension of cyano amidine **4i** or **4j** (3.0 mmol) in *t*butanol (40 mL) was added to a well-stirred solution of *t*BuOK (0.38 g, 3.3 mmol) in *t*butanol (10 mL). The reaction mixture was heated at reflux until disappearance (TLC: cyclohexane/EtOAc 2:3) of the starting amidine (about 2 h) and evaporated to dryness. The residue was poured into water and extracted with CH_2Cl_2 (3×40 mL). The combined organic layers were washed twice with water (2×40 mL) and dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was recrystallized from methanol to give **4i** or **4j**, respectively.

4.6.1. 4-Amino-3-isopropyl-2-morpholin-4-yl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4i. Yield 56%, pale yellow crystals, mp > 300 °C; IR ν_{max} (KBr) 3465 (NH_2), 1698 (C=O) cm^{-1}; ^1H NMR: 1.44 (6H, d, $J=7.3$ Hz, $2\times CH_3$), 3.28–3.34 (4H, m, CH_2NCH_2), 3.57 (1H, quint, $J=7.3$ Hz, C*H*), 3.88–3.94 (4H, m, CH_2OCH_2), 5.90 (2H, br s, NH_2), 7.30–7.38 (2H, m, H-7, H-9), 7.52 (1H, td, $J=7.8$, 1.4 Hz, H-8), 8.47 (1H, dd, $J=7.8$, 1.4 Hz, H-10); ^{13}C NMR: 19.9 ($2\times CH_3$), 26.5 (CH), 51.8 (CH_2NCH_2), 67.4 (CH_2OCH_2), 99.4 (C-4a), 113.9 (C-10a), 116.9, 124.6, 125.3, 131.6 (CH), 120.6 (C-3), 150.6 (C-4), 152.7 (C-6a), 156.4 (C-10b), 164.1 (C-2), 165.1 (C=O). Anal. Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38; found: C, 67.42; H, 6.25; N, 12.36.

4.6.2. 4-Amino-3-benzyl-2-morpholin-4-yl-5*H*-[1]benzopyrano[4,3-*b*]pyridin-5-one 4j. Yield 64%, pale yellow crystals, mp 135 °C; IR ν_{max} (KBr) 3435 and 3342 (NH_2), 1697 (C=O) cm^{-1}; ^1H NMR: 3.35–3.49 (4H, m, CH_2NCH_2), 3.75–3.85 (4H, m, CH_2OCH_2), 4.06 (2H, s, CH_2), 5.35 (2H, br s, NH_2), 7.20–7.40 (5H + 2H, m, ArH, H-7, H-9), 7.54 (1H, td, $J=8.0$, 1.4 Hz, H-8), 8.51 (1H, d, $J=8.0$ Hz, H-10); ^{13}C NMR: 33.0 (CH_2), 50.7 (CH_2NCH_2), 67.2 (CH_2OCH_2), 98.2 (C-4a), 104.9 (C-10a), 116.8, 124.5, 125.2, 131.6 (CH), 120.4 (C-3), 127.3, 127.8, 129.5 (ArCH), 137.0 (ArC), 151.3 (C-4), 152.7 (C-6a), 156.4 (C-10b), 163.6 (C-2), 165.1 (C=O). Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85; found: C, 71.21; H, 5.47; N, 10.94.

4.7. General procedure for the synthesis of [1]benzopyrano[4,3-*d*]pyrimidin-5-one derivatives 7a–e

A solution of the amidine **3a**,**c–f** (20 mmol) in toluene (25 mL) was added to ammonium acetate (100 mmol). The resulting mixture was heated at reflux for the reported time (see after) until disappearance of the starting amidine **3** (TLC: cyclohexane/EtOAc, 4:1), then evaporated to dryness. The residue was poured into water and extracted with CH_2Cl_2 (3×40 mL). The combined organic layers were washed twice with water (2×40 mL) and dried over Na_2SO_4, filtered and evaporated to dryness. The resulting solid was recrystallized from *i*Pr_2O to give pure **7** derivatives.

4.7.1. 2-Benzyl-5*H*-[1]benzopyrano[4,3-*d*]pyrimidin-5-one 7a. Reaction time: 1 h. Yield 63%, cream crystals, mp 131 °C; IR ν_{max} (Nujol) 1720 (C=O) cm^{-1}; ^1H NMR: 4.49 (2H, s, CH_2), 7.27–7.50 (5H + 2H, m, ArH, H-7, H-9), 7.71 (1H, td, $J=7.3$, 1.4 Hz, H-8), 8.63 (1H, dd, $J=7.7$, 1.4 Hz, H-10), 9.53 (1H, s, H-4); ^{13}C NMR ($DMSO_{d6}$): 46.4 (CH_2), 113.7 (C-4a), 118.2 (C-10a), 118.1, 125.3, 125.8, 135.3 (CH), 127.4, 129.2, 130.0 (ArCH), 138.2 (ArC), 154.8 (C-6a), 158.3 (C-10b), 159.8 (C-2), 160.7 (CH-4), 174.4 (C=O). Anal. Calcd for $C_{18}H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72; found: C, 74.87; H, 4.24; N, 9.76.

4.7.2. 2-(4-Bromobenzyl)-5*H*-[1]benzopyrano[4,3-*d*]pyrimidin-5-one 7b. Reaction time: 0.5 h. Yield 89%, cream crystals, mp 185 °C; IR ν_{max} (KBr) 1746 (C=O) cm^{-1}; ^1H NMR: 4.44 (2H, s, CH_2), 7.34 and 7.48 (4H, $J=8.4$ Hz, ArH), 7.39–7.49 (2H, m, H-7, H-9), 7.71 (1H, td, $J=7.7$, 1.8 Hz, H-8), 8.59 (1H, dd, $J=7.7$, 1.8 Hz, H-10), 9.53 (1H, s, H-4); ^{13}C NMR: 46.0 (CH_2), 112.5 (C-4a), 117.7 (C-10a), 121.1 (Ar*C*Br), 117.6, 125.2, 125.4, 134.6 (CH), 131.2, 131.8 (ArCH), 136.1 (ArC), 154.5 (C-6a), 158.5 (C-10b), 159.5 (C-2), 160.8 (CH-4), 174.3 (C=O). Anal. Calcd for $C_{18}H_{11}BrN_2O_2$: C, 58.88; H, 3.02; N, 7.63; found: C, 58.67; H, 3.00; N, 7.55.

4.7.3. 2-(4-Methoxybenzyl)-5*H*-[1]benzopyrano[4,3-*d*]-pyrimidin-5-one 7c. Reaction time: 1 h. Yield 82%, cream crystals, mp 110 °C; IR ν_{max} (Nujol) 1723 (C=O) cm^{-1}; ^1H NMR: 3.78 (3H, s, OCH_3), 4.39 (2H, s, CH_2), 6.87 and 7.37 (4H, $J=8.8$ Hz, ArH), 7.35–7.47 (2H, m, H-7, H-9), 7.65 (1H, td, $J=7.3$, 1.8 Hz, H-8), 8.58 (1H, dd, $J=7.8$, 1.8 Hz, H-10), 9.48 (1H, s, H-4); ^{13}C NMR: 45.8 (CH_2), 55.5 (OCH_3), 112.3 (C-4a), 117.8 (C-10a), 129.3 (ArC), 117.5, 125.2, 125.4, 134.4 (CH), 114.2, 130.4 (ArCH), 154.4 (C-6a), 158.3 (C-10b), 158.7 (Ar*C*OCH_3), 159.6 (C-2), 160.7 (CH-4), 175.2 (C=O). Anal. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80; found: C, 71.69; H, 4.41; N, 8.74.

4.7.4. 2-Isobutyl-5*H*-[1]benzopyrano[4,3-*d*]pyrimidin-5-one 7d. Reaction time: 1 h. Yield 85%, white crystals, mp 117 °C; IR ν_{max} (Nujol) 1723 (C=O) cm^{-1}; ^1H NMR: 1.05 (6H, d, $J=6.6$ Hz, $2\times CH_3$), 2.45 (1H, ept, $J=6.6$ Hz, C*H*), 3.05 (2H, d, $J=7.3$ Hz, CH_2), 7.39–7.50 (2H, m, H-7, H-9), 7.69 (1H, td, $J=7.7$, 1.8 Hz, H-8), 8.64 (1H, dd, $J=7.7$, 1.4 Hz, H-10), 9.53 (1H, s, H-4), ^{13}C NMR: 22.6 ($2\times CH_3$), 28.5 (CH), 49.1 (CH_2), 112.1 (C-4a), 117.9 (C-10a), 117.5, 125.1, 125.3, 134.3 (CH), 154.4 (C-6a), 157.9 (C-10b), 159.7 (C-2), 160.1 (CH-4), 176.2 (C=O). Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02; found: C, 70.77; H, 5.48; N, 10.98.

4.7.5. 2-(2-Phenylethyl)-5*H*-[1]benzopyrano [4,3-*d*]pyrimidin-5-one 7e. Reaction time: 3 h. Yield 94%, cream crystals, mp 105 °C; IR $\nu_{\max}$ (Nujol) 1720 (C=O) cm^{-1}; ^{1}H NMR: 3.31–3.35 and 3.45–3.46 (4H, 2×m, CH_2-CH_2), 7.18–7.53 (5H+2H, m, ArH, H-7, H-9), 7.70 (1H, td, $J=$ 7.7, 1.8 Hz, H-8), 8.63 (1H, dd, $J=7.7$, 1.8 Hz, H-10), 9.53 (1H, s, H-4); ^{13}C NMR: 34.0 ($CH_2C_6H_5$), 41.6 (CH_2), 112.3 (C-4a), 117.8 (C-10a), 117.6, 125.2, 125.3, 134.5 (CH), 126.3, 128.4, 128.5, (ArCH), 140.9 (ArC), 154.4 (C-6a), 158.1 (C-10b), 159.8 (C-2), 160.3 (CH-4), 175.7 (C=O). Anal. Calcd for $C_{19}H_{14}N_2O_2$: C, 75.48; H, 4.67; N, 9.27; found: C, 75.40; H, 4.64; N, 9.25.

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References and notes

1. Heber, D.; Berghaus, T. *J. Heterocycl. Chem.* **1994**, *31*, 1353–1359.
2. Bruno, O.; Brullo, C.; Ranise, A.; Schenone, S.; Bondavalli, F.; Barocelli, E.; Ballabeni, V.; Chiavarini, M.; Tognolini, M.; Impicciatore, M. *Bioorg. Med. Chem. Lett.* **2001**, 1397–1400.
3. Eggenweiler, M.; Rochus, J.; Wolf, M.; Gassen, M.; Poeschke, O., *Merck Patent Gmbh, Germany*, PCT Int. Appl. 2001; *Chem. Abstr.*, **2001**, 134, 331619.
4. Beccalli, E. M.; Contini, A.; Trimarco, P. *Eur. J. Org. Chem.* **2003**, 3976–3984.
5. Beccalli, E. M.; Contini, A.; Trimarco, P. *Tetrahedron Lett.* **2004**, *45*, 3447–3449.
6. Checchi, S.; Pecori Vettori, L.; Vincieri, F. *Gazz. Chim. Ital.* **1968**, *98*, 1488–1502.
7. Steinführer, T.; Hantschmann, A.; Pietsch, M.; Weiβenfels, M. *Liebigs Ann. Chem.* **1992**, 23–28.
8. Broekhof, N. L. J. M.; Jonkers, F. L.; van der Gen, A. *Tetrahedron Lett.* **1979**, 2433–2436.
9. Mannich, C.; Davidsen, H. *Chem. Ber.* **1936**, *69*, 2106–2122.
10. Knorr, R.; Loew, P.; Hassel, P. *Synthesis* **1983**, 785–786.
11. Fisher, G. B.; Lee, L.; Klettke, F. W. *Synth. Commun.* **1994**, *24*, 1541–1546.
12. Kadaba, P. K.; Stanovnik, B.; Tišler, M. *Adv. Heterocycl. Chem.* **1984**, *37*, 339 and references cited therein.
13. (a) Rabaron, A.; Didry, J. R.; Kirkiacharian, B. S.; Plat, M. M. *Org. Magn. Reson.* **1979**, *12*, 284–288. (b) Michalik, M.; Peseke, K.; Radeglia, R. *J. Prakt. Chem.* **1981**, *323*, 506–510. (c) Michalik, M.; Kreutzmann, J. *Sulfur Lett.* **1986**, *4*, 73–78. (d) Ivanov, I. C.; Karagiosov, S. K.; Simeonov, M. F. *Liebigs Ann. Chem.* **1992**, 203–207.
14. (a) Boyd, G. V. In Patai, S., Rappoport, Z., Eds.; The Chemistry of Amidines and Imidates; Interscience: London, 1991; Vol. 2, p 370; and references cited therein. (b) Pfau, M.; Chiriacescu, M.; Revial, G. *Tetrahedron Lett.* **1993**, *34*, 327–330. (c) Cado, F.; Di Martino, J. L.; Jacquault, P.; Bazureau, J. P.; Hamelin, J. *Bull. Soc. Chim. Fr.* **1996**, *133*, 587–595.
15. Rasala, D. *J. Phys. Org. Chem.* **1993**, *6*, 307–315.
16. Kettermann, V.; Světlík, J.; Kratkly, C. *Acta Cryst.* **2001**, *C57*, 590–592.
17. (a) Abbotto, A.; Alanzo, V.; Bradamante, S.; Pagani, G. A. *J. Chem. Soc. Perkin Trans. 2* **1991**, 481–488. (b) Ivanov, I. C.; Karagiosov, S. K.; Alexandrova, S. V. *C.R. Acad. Bulg. Sci.* **1998**, *51*, 61–62.

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Mannich-type reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane with arylaldehydes and aromatic amines catalyzed by perfluorinated rare earth metal salts in fluorous phase

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Abstract—In this paper, we describe a useful Mannich-type reaction in fluorous phase. By use of perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) as a fluorous solvent and perfluorinated rare earth metal salts such as $Sc(OSO_2C_8F_{17})_3$ or $Yb(OSO_2C_8F_{17})_3$ (2.0 mol%) as a catalyst, the Mannich-type reaction of arylaldehydes with aromatic amines and (1-methoxy-2-methylpropenyloxy)trimethylsilane can be performed for many times without reloading the catalyst and the fluorous solvent.

1. Introduction

Mannich reaction is one of the most important fundamental reactions in organic chemistry. It is a powerful tool in the routes for the synthesis of various β-amino ketones or esters, which are versatile synthetic building blocks for the preparation of many nitrogen-containing, biologically important compounds.[1] In the bimolecular version of the classical Mannich reaction the use of preformed or in situ generated iminium[2] or *N*-acyliminium ions[3] and carbon nucleophiles has greatly expanded the versatility of this methodology allowing the use of milder reaction conditions. Recently, some significant progresses have been made in a number of Lewis acid or transition metal catalyzed Mannich reactions of (1-methoxy-2-methylpropenyloxy) trimethylsilane with imines in organic solvents. For example, the application of zirconium [$Zr(OPr^i)_4$], palladium complexes, and rare earth metal triflates [$Ln(OTf)_n$, Ln = Yb, Sc, Y, La] in Mannich reaction has enlarged its utility in organic synthesis.[4]

On the other hand, perfluorocarbon fluids, especially perfluoro-alkanes, have some unique properties that make them attractive alternatives for conventional organic solvents.[5] They have limited miscibility with conventional organic solvents. Compounds functionalized with perfluorinated groups often dissolve preferentially in fluorous solvents. This character can be used to extract fluorous components from reaction mixtures.[6] The 'Fluorous Biphase System' (FBS) technique was first reported by Horvath and Rabai.[6a] It allows the catalysis to be performed in a two-phase reaction mixture consisting of a perfluorinated solvent and an organic solvent. Therefore, by introducing a perfluorinated catalyst in a catalytic reaction system, the catalyst is solubilized and simultaneously immobilized in the 'Fluorous Phase'. By elevating the temperature the biphasic system forms a homogenous solution and the catalytic process can take place. Cooling down the reaction mixture leads to the reformation of two separate phases. Afterwards, easy product isolation and the recovery of the perfluoro-tagged metal catalyst can be achieved by simple phase separation.[7] The isolation and recovery of perfluorinated components can be accomplished not only by a phase separation of immiscible liquid layers but also by solid–liquid extraction using a perfluorinated non-polar stationary phase.[6a]

Based on this concept, we attempted the application of fluorous phase separation techniques to the one-pot 'three-component' Mannich reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane with arylaldehydes and

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Keywords: Mannich-type reaction; Perfluorinated rare earth metal salt; Perfluorinated alkali metal salt; (1-Methoxy-2-methylpropenyloxy)trimethylsilane; Perfluorodecalin ($C_{10}F_{18}$ *cis*- and *trans*-mixture); Fluorous biphase system.

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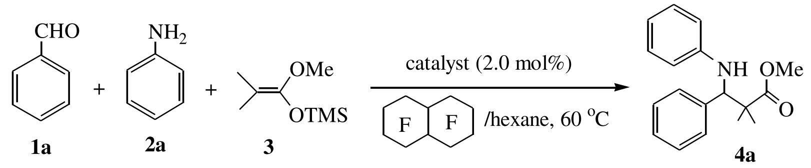


Scheme 1.

Table 1. Mannich-type reaction of **1a**, **2a**, and **3** in hexane and fluorous phase per fluorodecalin for 2 h

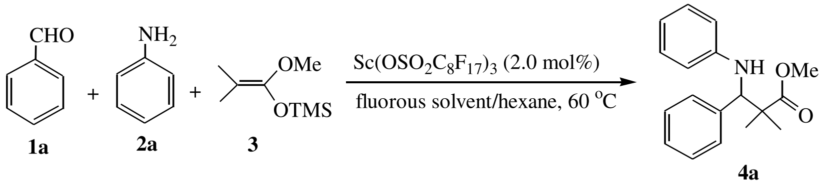
Entry	Catalyst	Yield (%)[a] **4a**
1	$HOSO_2CF_3$	50
2	$HOSOC_8F_{17}$	63
3	$Yb(OSO_2CF_3)_3$	47
4	$Yb(OSO_2C_8F_{17})_3$	65
5	$Sc(OSO_2CF_3)_3$	55
6	$Sc(OSO_2C_8F_{17})_3$	71
7	$Na(OSO_2C_8F_{17})$	24
8	$Na(OSO_2CF_3)$	14
9	$Li(OSO_2C_8F_{17})$	28

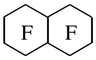
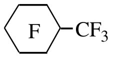
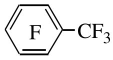
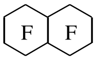
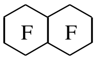
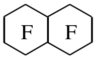
[a] Isolated yields.

aromatic amines using perfluorinated rare earth metal salts or other metal salts as catalysts. By this technology, the efficiency of the synthetic process of this Mannich-type reaction can be increased because the recovery of perfluorinated rare earth metal salt or other metal salt catalysts from the reaction solution can be avoided.

In order to perform this Mannich-type reaction in fluorous phase, we prepared several perfluorinated rare earth metal salts [$Ln(OSO_2CF_3)_3$ and $Ln(OSO_2C_8F_{17})_3$, Ln = Yb, Sc][8] and perfluorinated alkali metal salts [$M(OSO_2CF_3)$ and $M(OSO_2C_8F_{17})$, M = Li, Na][9] and used them as catalysts in the one-pot 'three-component' Mannich-type reaction of (1-methoxy-2-methyl propenyloxy)trimethylsilane **3** with benzaldehyde **1a** and aniline **2a** in hexane[10] and perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) fluorous solvent (Scheme 1). At 60 °C (upon heating), the organic phase is miscible with fluorous phase to give a homogeneous phase. At 20 °C (room temperature), it becomes a biphasic system. The results are summarized in Table 1 and as can be seen, $Ln(OSO_2C_8F_{17})_3$ Lewis acid catalysts are more effective than $Ln(OSO_2CF_3)_3$ (Table 1, entries 3–6) while perfluorinated alkali metal salts are ineffective in this FBS catalytic reaction system (Table 1, entries 7–9). It should be emphasized here that no reaction occurred in the absence of catalyst. In addition, the Bronsted acids $HOSO_2CF_3$ and $HOSO_2C_8F_{17}$ themselves can catalyze this reaction as well, with $HOSO_2C_8F_{17}$ being more effective than $HOSO_2CF_3$ under identical conditions (Table 1, entries 1 and 2). These results suggest that a long perfluorinated alkyl chain, so called 'pony tail', could indeed allow more efficient transfer of the catalyst into the fluorous phase. Overall, $Sc(OSO_2C_8F_{17})_3$ is the most active catalyst in this reaction. Although $HOSO_2C_8F_{17}$ itself can effectively catalyze this reaction as well, its salt $Sc(OSO_2C_8F_{17})_3$ is more stable under ambient atmosphere and can be easily handled (Table 1, entries 1 and 2). It should be noted that in consideration of cost of

Table 2. Mannich-type reaction of **1a**, **2a**, and **3** in different fluorous solvents for 2 h



Entry	Fluorous phase	Yield (%)[a] **4a**
1		71
2		54
3		40
4	$CF_3(CF_2)_4CF_3$	46
5[b]		72
6[b]		71
7[b]		65

[a] Isolated yields.

[b] The catalyst in fluorous phase was reused.

catalyst, $Yb(OSO_2C_8F_{17})_3$ is also an active and cheap catalyst in this reaction.

Next, we screened several other fluorous solvents, such as perfluoroheptane (C_7F_8), perfluoro (methylcyclohexane) (C_7F_{14}) and perfluorohexane (C_6F_{14}) in this FBS catalytic system. The results are summarized in Table 2. We found that during the Mannich-type reaction process, the loss of fluorous solvent is very serious at 60 °C when using perfluorohexane (C_7F_8), perfluoro(methylcyclohexane) (C_7F_{14}) and perfluorohexane (C_6F_{14}) as solvent because they are volatile (bp 58–76 °C). Perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) is the best fluorous solvent for this reaction (Table 2, entries 1–4). This new fluorous phase is not volatile because it has higher boiling point (bp 142 °C). Based on the ^{19}F NMR spectroscopic data and GC–MS, no loss of catalyst or perfluorodecalin to the organic and water phase during workup can be detected.

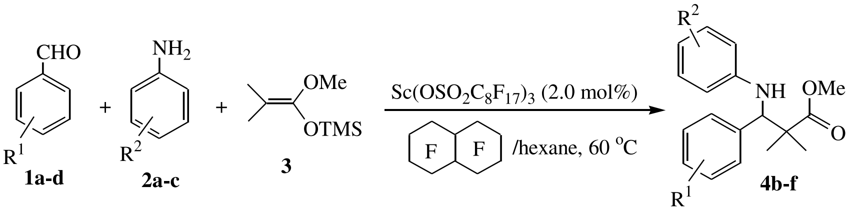
The perfluorodecalin fluorous phase containing $Sc(OSO_2C_8F_{17})_3$ catalyst can be easily isolated by simple separation of the fluorous phase. This catalytic phase can be reused for three times to give similar results without reloading fluorous solvent and the catalyst (Table 2, entries 5–7).

Using $Sc(OSO_2C_8F_{17})_3$ as a catalyst and perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) as the fluorous solvent in a biphasic way, Mannich-type reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane **3** with other arylaldehydes **1** and aromatic amines **2** were examined as well. The results are shown in Table 3. These Mannich-type reactions proceeded smoothly at 60 °C to give the corresponding adducts **4b-f** in good yields (Table 3, entries 1–5). Thus, a novel FAB catalytic process of Mannich reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane with arylaldehydes and aromatic amines has been explored. This is the first example of an Mannich reaction of arylaldehydes with aromatic amines and (1-methoxy-2-methylpropenyloxy)trimethylsilane in a fluorous phase. It should be also emphasized here that no reaction occurred when using aliphatic aldehydes as substrates with aromatic amines in this reaction under the same conditions. This is because the corresponding imines formed in situ are not stable in the presence of Lewis acids. In these cases, the reactions gave no other by-products. The starting materials could be recovered during the purification of the adducts by column chromatography.

In addition, we utilized relatively cheaper $Yb(OSO_2C_8F_{17})_3$ as a Lewis acid to catalyze the reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane **3** with arylaldehydes **1** and 2-aminophenol **5** in a biphasic way of hexane and perfluorodecalin. The results are summarized in Table 4. The initial examination of this reaction was performed at 60 °C using arylaldehyde **1a** as the substrate under the same conditions as those described above. Unfortunately, the corresponding Mannich-type adduct **6a** was formed in lower yield (40%) because of the oxidization of 2-aminophenol **5** at this high temperature (60 °C) (Table 4, entry 1). We found that with the addition of tetrahydrofuran (THF) (0.5 mL) into this hexane (1.0 mL) and perfluorodecalin (1.0 mL) system to dissolve the solid powders of 2-aminophenol **5**, the reactions proceeded smoothly at 20 °C to give **6a** in higher yield (Table 4, entry 2), although this catalytic reaction system is not a homogenous phase at 20 °C. For other arylaldehydes, these reactions proceeded smoothly under the same conditions to give the corresponding Mannich-type adducts **6** in good yields (Table 4, entries 3–6). The fluorous phase containing the employed Lewis acid $Yb(OSO_2C_8F_{17})_3$ can also be easily recovered and reused for three times to give the adduct **6a** in similar yields without reloading the catalyst and fluorous solvent (Table 4, entries 7–9).

In summary, we reported in this paper a new process to carry out the Mannich-type reaction of (1-methoxy-2-methylpropenyloxy)trimethylsilane, arylaldehydes and aromatic amines in fluorous phase. Using perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) as a fluorous solvent and $Sc(OSO_2C_8F_{17})_3$ or $Yb(OSO_2C_8F_{17})_3$ as a catalyst, this Mannich reactions can be repeated several times without reloading fluorous solvent and the catalyst. By the conventional Lewis acid catalyzed reaction, the water-stable Lewis acids, $Yb(OPf)_3$, $Sc(OTf)_3$ or $Sc(OPf)_3$ could be recovered from the water phase after the usual workup. Obviously, the water must be evaporated and this will consume a lot of energy. By this technology, the catalytic phase can be easily recovered and can be reused for the next reaction without any treatment. Further investigations to develop other types

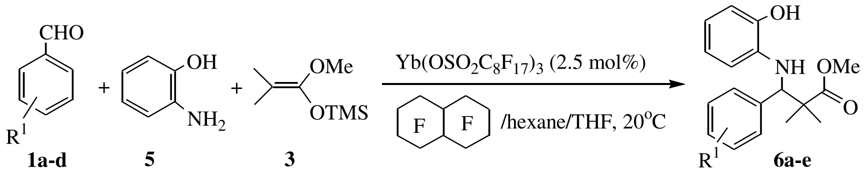
Table 3. Results of Mannich-type reaction of aryladehyde **1**, aromatic amine **2**, and **3** in fluorous phase for 2 h



Entry	R^1	R^2	Yield (%)[a] **4**
1	**1b**, $R^1 = p$-Cl	**2a**, $R^2 = H$	**4b**, 65
2	**1c**, $R^1 = p$-NO_2	**2a**, $R^2 = H$	**4c**, 65
3	**1d**, $R^1 = p$-Ome	**2a**, $R^2 = H$	**4d**, 70
4	**1a**, $R^1 = H$	**2b**, $R^2 = p$-F	**4e**, 66
5	**1a**, $R^1 = H$	**2c**, $R^2 = o$-CF_3	**4f**, 58

[a] Isolated yields.

Table 4. Results of Mannich-type reaction of arylaldehydes **1**, 2-aminophenol **5** and **3** in fluorous phase for 8 h



Entry	R^1	Yield (%)[a]
		6
1[b]	**1a**, R^1 = H	**6a**, 40
2	**1a**, R^1 = H	**6a**, 93
3	**1b**, R^1 = *p*-Me	**6b**, 73
4	**1c**, R^1 = *p*-OMe	**6c**, 63
5	**1d**, R^1 = *p*-Cl	**6d**, 79
6	**1e**, R^1 = *p*-Br	**6e**, 66
7[c]	**1a**, R^1 = H	**6a**, 60
8[c]	**1a**, R^1 = H	**6a**, 64
9[c]	**1a**, R^1 = H	**6a**, 68

[a] Isolated yields.

[b] The reaction was performed at 60 °C.

[c] The recovered catalyst in fluorous phase was used.

of reactions in fluorous phase with perfluorinated metal salt are now in progress.

2. Experimental

2.1. General remarks

MPs were obtained with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer for solution in $CDCl_3$ with tetramethylsilane (TMS) as internal standard; *J*-values are in Hz. All of the solid compounds reported in this paper gave satisfactory CHN microannalyses with a Carlo–Erba 1106 analyzer. Mass spectra were recorded with a HP-5989 instrument and HRMS was measured by a Finnigan MA$^+$ mass spectrometer. Organic solvents were dried by standard methods when necessary. Commercially obtained reagents were used without further purification. All reactions were monitored by TLC with Huanghai GF254 silica gel coated plates. The orientation of nitration was determined by NMR analysis. Flash column chromatography was carried out using 300–400 mesh silica gel.

2.2. Preparation of perfluorinated rare earth metal catalysts [$Ln(OSO_2C_8F_{17})_3$, Ln = Yb, Sc]

The reaction procedure: an excess amount of a lanthanide(III) oxide (99.9% purity) was added to an aqueous solution of $C_8F_{17}SO_3H$ (50% v/v) and heating at boiling for 30 min to 1 h. The mixture was filtered to remove the unreacted oxide. The water was then removed from the filtrate under reduced pressure. The resulting hydrate was dried by heating under vacuum at 180–200 °C for 48 h.

2.3. Preparation of perfluorinated alkali metal salt catalysts [$M(OSO_2C_8F_{17})$, M = Li, Na, K]

The reaction procedure: trifluoromethanesulfonic acid was added dropwise to a solution of the carbonate salt in methanol at 0 °C. The reaction mixture was stirred at room temperature for 30 min, then at reflux for 2 h. The clear solution concentrated under reduced pressure. The resulting white cake was dried at 125 °C for 2 h. The production was recrystallized from methanol or ethanol/diethyl ether.

2.4. Typical reaction procedure for the Mannich-type reaction in fluorous phase

To a solution of $Sc(OPf)_3$ [$(C_8F_{17}SO_3)_3Sc$] (10 mg, 0.006 mmol) in perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) (solvent, 1.0 mL) was added a solution of aniline (27 μL, 0.3 mmol) and benzaldehyde (30 μL, 0.3 mmol) in toluene (solvent, 1.0 mL). Then, (1-methoxy-2-methylpropenyloxy)trimethylsilane (90 μL, 0.45 mmol) was added in the mixture under stirring. The mixture was stirred at 60 °C for 20 h. The fluorous layer was separated for the next reaction. The reaction mixture (organic layer) was washed by water (5 mL) and extracted with dichloromethane (2×15 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc–hexane = 1:10) to give the product as a colorless solid 60 mg, yield 71%.

2.4.1. 2,2-Dimethyl-3-phenyl-3-phenylaminopropionic acid methyl ester 4a. A white solid, 60 mg, yield 71%; This is a known compound. Its ^{1}H NMR spectroscopic data are in consistent with those reported in literature (Ref. Loh, T.-P.; Liung, S. B. K. W.; Tan, K.-L.; Wei, L.-L. *Tetrahedron* **2000**, *56*, 3227–3238). ^{1}H NMR ($CDCl_3$, TMS, 300 MHz): δ 1.16 (3H, s, CH_3), 1.27 (3H, s, CH_3), 3.65 (3H, s, OCH_3), 4.49 (1H, s, CH), 4.79 (1H, s, NH), 6.49 (2H, dd, J = 1.2, 8.7 Hz, ArH), 6.59 (1H, t, J = 7.5 Hz, ArH), 7.04 (2H, dd, J = 7.5, 8.7 Hz, ArH), 7.17–7.29 (5H, m, ArH).

2.4.2. 3-(4-Chlorophenyl)-2,2-dimethyl-3-phenylaminopropionic acid methyl ester 4b. A white solid, 62 mg, yield 65%. Mp 108–110 °C. IR ($CHCl_3$): ν 1732, 1613, 1510, 1533, 1442, 1149, 873 cm^{-1}. ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.15 (3H, s, CH$_3$), 1.27 (3H, s, CH$_3$), 3.65 (3H, s, OCH$_3$), 4.45 (1H, s, CH), 4.79 (1H, s, NH), 6.46 (2H, dd, $J=1.0$, 8.8 Hz, ArH), 6.62 (1H, t, $J=7.4$ Hz, ArH), 7.05 (2H, dd, $J=7.4$, 8.8 Hz, ArH), 7.20–7.28 (4H, m, ArH). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 20.8, 24.4, 46.9, 52.1, 65.8, 113.3, 117.5, 128.2, 129.0, 129.6, 133.2, 137.9, 146.6, 176.7. MS (EI) *m/z*: 317 (M$^+$, 4.63), 216 (M$^+-101$, 100), 180 (M$^+-137$, 4.27), 104 (M$^+-213$, 13.22), 77 (M$^+-$240, 19.37). Anal. Calcd for $C_{18}H_{20}ClNO_2$ requires C, 68.03; H, 6.34; N, 4.41. Found: C, 68.26; H, 6.33; N, 4.40%.

2.4.3. 2,2-Dimethyl-3-(4-nitrophenyl)-3-phenylaminopropionic acid methyl ester 4c. A yellow solid, 64 mg, yield 65%. Mp 103–105 °C. IR (CHCl$_3$): ν 1728, 1603, 1507, 1523, 1438, 1266, 1139, 853 cm^{-1}. ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.18 (3H, s, CH$_3$), 1.32 (3H, s, CH$_3$), 3.67 (3H, s, OCH$_3$), 4.57 (1H, d, $J=7.1$ Hz, CH), 4.90 (1H, d, $J=7.1$ Hz, NH), 6.45 (2H, d, $J=8.6$ Hz, ArH), 6.64 (1H, t, $J=7.5$ Hz, ArH), 7.06 (2H, dd, $J=7.5$, 8.6 Hz, ArH), 7.49 (2H, d, $J=8.9$ Hz, ArH), 8.16 (2H, d, $J=8.9$ Hz, ArH). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 21.1, 24.5, 46.8, 52.3, 64.2, 113.3, 118.0, 123.3, 129.1, 129.2, 146.1, 147.4, 147.5, 176.2. MS (EI) *m/z*: 328 (M$^+$, 5.74), 227 (M$^+-101$, 100), 181 (M$^+-147$, 37.21), 168 (M$^+-160$, 8.99), 77 (M$^+-$251, 19.42). Anal. Calcd for $C_{18}H_{20}N_2O_4$ requires C, 65.84; H, 6.14. N, 8.53. Found: C, 65.99; H, 6.14; N, 8.44%.

2.4.4. 3-(4-Methoxyphenyl)-2,2-dimethyl-3-phenylaminopropionic acid methyl ester 4d. A yellowish oil, 66 mg, yield 70%. IR (CHCl$_3$): ν 1727, 1603, 1510, 1265, 1178, 1034 cm^{-1}. ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.15 (3H, s, CH$_3$), 1.25 (3H, s, CH$_3$), 3.65 (3H, s, OCH$_3$), 3.76 (3H, s, OCH$_3$), 4.44 (1H, s, CH), 4.74 (1H, s, NH), 6.49 (2H, d, $J=8.4$ Hz, ArH), 6.59 (1H, t, $J=7.4$ Hz, ArH), 6.81 (2H, d, $J=8.7$ Hz, ArH), 7.04 (2H, dd, $J=7.4$, 8.4 Hz, ArH), 7.18 (2H, d, $J=8.7$ Hz, ArH). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 20.9, 24.7, 47.4, 52.3, 55.4, 64.0, 113.6, 113.7, 117.4, 129.2, 129.5, 131.4, 147.2, 159.1, 177.3; MS (EI) *m/z*: 313 (M$^+$, 3.08), 212 (M$^+-101$, 100), 168 (M$^+-145$, 6.10), 104 (M$^+-209$, 15.95), 77 (M$^+-236$, 14.87). HRMS (Maldi) calcd for $C_{19}H_{24}O_3N$ (M$^++1$) requires 314.1756. Found: 314.1751.

2.4.5. 3-(4-Fluorophenylamino)-2,2-dimethyl-3-phenylpropionic acid methyl ester 4e. A white solid, 59 mg, yield 66%. Mp 89–91 °C. IR (CHCl$_3$): ν 1727, 1591, 1493, 1265, 1150 cm^{-1}. ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.15 (3H, s, CH$_3$), 1.28 (3H, s, CH$_3$), 3.66 (3H, s, OCH$_3$), 4.41 (1H, d, $J=7.3$ Hz, CH), 5.02 (1H, d, $J=7.3$ Hz, NH), 6.15 (1H, dt, $J=2.8$, 11.8 Hz, ArH), 6.25–6.31 (2H, m, ArH), 6.96 (1H, dd, $J=8.1$, 15.0 Hz, ArH), 7.23–7.29 (5H, m, ArH). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 21.1, 24.9, 47.1, 52.3, 64.6, 100.3 (d, $J=25.5$ Hz), 104.0 (d, $J=22.1$ Hz), 109.5 (d, $J=2.4$ Hz), 127.9, 128.4 (d, $J=3.6$ Hz), 130.2, 130.3, 139.0, 149.0 (d, $J=11.0$ Hz), 164.0 (d, $J=$ 254.7 Hz), 177.1. MS (EI) *m/z*: 301 (M$^+$, 3.40), 200 (M$^+-101$, 100), 122 (M$^+-179$, 21.75), 95 (M$^+-206$, 20.93), 77 (M$^+-224$, 9.22). Anal. Calcd for $C_{18}H_{20}FNO_2$ requires C, 71.74; H, 6.69; N, 4.65. Found: C, 71.80; H, 6.40; N, 4.53%.

2.4.6. 2,2-Dimethyl-3-phenyl-3-(3-trifluoromethylphenylamino)propionic acid methyl ester 4f. A white solid, 61 mg, yield 58%. Mp 108–110 °C. IR (CHCl$_3$): ν 1714, 1616, 1519, 1495, 1437, 1266, 1124, 785 cm^{-1}. ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.17 (3H, s, CH$_3$), 1.30 (3H, s, CH$_3$), 3.66 (3H, s, OCH$_3$), 4.46 (1H, s, CH), 5.14 (1H, s, NH), 6.60 (1H, d, $J=8.1$ Hz, ArH), 6.72 (1H, s, ArH), 6.82 (1H, d, $J=8.1$ Hz, ArH), 7.11 (1H, t, $J=8.1$ Hz, ArH), 7.21–7.33 (5H, m, ArH). ^{13}C NMR (CDCl$_3$, 75 MHz): δ 21.1, 25.0, 47.1, 52.4, 64.9, 110.0 (q, $J=$ 4.1 Hz), 114.1 (q, $J=4.2$ Hz), 116.2, 117.7 (q, $J=$ 277.5 Hz), 127.9, 128.4, 128.4, 129.7, 129.9 (q, $J=$ 10.1 Hz), 138.7, 147.3, 177.1. MS (EI) *m/z*: 351 (M$^+$, 2.16), 250 (M$^+-101$, 100), 172 (M$^+-179$, 11.97), 145 (M$^+-206$, 15.63). Anal. Calcd for $C_{19}H_{20}F_3NO_2$ requires C, 64.95; H, 5.74; N, 3.99. Found: C, 65.06; H, 5.88; N, 3.95%.

2.5. Typical reaction procedure for the Mannich-type reaction in fluorous phase

A solution of Yb(OPf)$_3$ [$(C_8F_{17}SO_3)_3Yb$] (25 mg, 0.025 mmol) in perfluorodecalin ($C_{10}F_{18}$, *cis*- and *trans*-mixture) (1.0 mL) was added to a solution of 2-aminophenol (33 mg, 0.3 mmol) and benzaldehyde (31 μL, 0.3 mmol) in a mixture solvent of hexane (1.0 mL) and tetrahydrofuran (0.5 mL). Then, (1-methoxy-2-methyl-propenyloxy)trimethylsilane (90 μL, 0.45 mmol) was added to the reaction mixture under stirring. The mixture was stirred at 20 °C for 8 h. Then, the reaction mixture was washed by dichloromethane (3×2 mL). And the fluorous phase was separated for the next reaction. The solvent of organic layer was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc–hexane = 1:10) to give the product.

2.5.1. 3-(2-Hydroxyphenylamino)-2,2-dimethyl-3-phenyl-propionic acid methyl ester 6a. A white solid; 84 mg, yield 93%. This is a known compound. Its ^{1}H NMR spectroscopic data are in consistent with those reported in literature (Ref. Ishitani, H.; Ueno, M.; Kobayashi, S. *J. Am. Chem. Soc.* **2000**, *122*, 34, 8180–8186). ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.21 (3H, s, CH$_3$), 1.24 (3H, s, CH$_3$), 3.69 (3H, s, OCH$_3$), 4.55 (1H, s, CH), 4.87 (1H, s, NH), 5.21 (1H, s, OH), 6.38–6.70 (4H, m, ArH), 7.22–7.29 (4H, m, ArH).

2.5.2. 3-(4-Methylphenyl)-3-(2-hydroxyphenylamino)-2,2-dimethylpropionic acid methyl ester 6b. A white solid; 68 mg, yield 73%. This is a known compound. Its ^{1}H NMR spectroscopic data are in consistent with those reported in literature (Ref. Yamashita, Y.; Ueno, M.; Kuriyama, Y.; Kobayashi, S. *Adv. Synth. Catal.* **2002**, *344*, 929–931). ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.20 (3H, s, CH$_3$), 1.24 (3H, s, CH$_3$), 2.28 (3H, s, CH$_3$), 3.68 (3H, s, OCH$_3$), 4.55 (1H, s, CH), 4.99 (1H, s, NH), 6.31–6.70 (4H, m, ArH), 7.20–7.26 (4H, m, ArH).

2.5.3. 3-(4-Methoxyphenyl)-3-(2-hydroxyphenylamino)-2,2-dimethyl-propionic acid methyl ester 6c. A yellow solid; 62 mg, yield 63%. IR (thin film): ν 3421, 2954, 2926, 2853, 1712, 1610, 1514, 1487, 1448, 1392, 1369, 1269, 1193, 1140, 1120, 1104, 1010 cm^{-1}. ^{1}H NMR (CDCl$_3$, TMS, 300 MHz): δ 1.20 (3H, s, CH$_3$), 1.23 (3H, s, CH$_3$), 3.68 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 4.53 (1H, s, CH), 4.98 (1H, s, NH), 5.76 (1H, s, OH), 6.36–6.70 (4H, m, ArH), 7.17–7.24 (4H, m, ArH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.9, 24.3, 47.4, 52., 55.1, 63.9, 113.3, 113.7, 114.1, 117.7, 120.9, 129.3, 130.9, 135.6, 144.2, 158.7, 177.9. MS (EI) *m/z*: 65 (11.32), 73 (16.81), 80 (10.75), 115 (12.65), 120 (27.24), 197 (14.72), 276 (100), 277 (16.98), 278 (96.68), 279 (14.45), 377 (M^+, 2.11). HRMS (MALDI): calcd for $C_{18}H_{21}NO_3Br$ ($M^+ + 1$) requires 378.0704, found: 378.0699.

2.5.4. 3-(4-Chlorophenyl)-3-(2-hydroxyphenylamino)-2,2-dimethylpropionic acid methyl ester 6d. A white solid; 78 mg, yield 79%. This is a known compound. Its ^1H NMR spectroscopic data are in consistent with those reported in literature (Ref. Ishitani, H.; Ueno, M.; Kobayashi, S. *J. Am. Chem. Soc.* **2000**, *122*, 8180–8186). ^1H NMR ($CDCl_3$, TMS, 300 MHz): δ 1.19 (3H, s, CH_3), 1.24 (3H, s, CH_3), 3.68 (3H, s, OCH_3), 4.55 (1H, s, CH), 4.99 (1H, s, NH), 6.31–6.70 (4H, m, ArH), 7.20–7.26 (4H, m, ArH).

2.5.5. 3-(4-Bromophenyl)-3-(2-hydroxyphenylamino)-2,2-dimethylpropionic acid methyl ester 6e. A yellow solid; 74 mg, yield 66%. IR (thin film): ν 3415, 2955, 2925, 1713, 1610, 1512, 1451, 1248, 1178, 1139, 1107, 1036 cm^{-1}. ^1H NMR ($CDCl_3$, TMS, 300 MHz): δ 1.20 (3H, s, CH_3), 1.24 (3H, s, CH_3), 3.68 (3H, s, OCH_3), 4.54 (1H, s, CH), 4.98 (1H, s, NH), 5.75 (1H, s, OH), 6.31–6.70 (4H, m, ArH), 7.15–7.41 (4H, m, ArH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.0, 24.2, 47.1, 52.4, 63.9, 113.3, 114.2, 117.9, 121.1, 121.3, 130.0, 131.1, 135.3, 138.2, 143.9, 177.5. MS (EI) *m/z*: 65 (1.99), 73 (4), 80 (2.01), 109 (3.07), 151 (3.60), 161 (2.45), 221 (2.46), 228 (100), 229 (17.11), 329 (M^+, 2.11). HRMS (MALDI): calcd for $C_{19}H_{24}NO_4$($M^+ + 1$) requires 330.1705, found: 330.1708.

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References and notes

1. (a) Kleinman, E. F. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 2, pp 893–951. (b) Tramontini, M.; Angiolini, L. In *Mannich Bases. Chemistry and Uses*; CRC: Boca Raton, FL, 1994. (c) Pilli, R. A.; Russowsky, D. *Trends Org. Chem.* **1997**, *6*, 101. (d) Risch, N.; Arend, M.; Westermann, B. *Angew. Chem., Ind. Ed.* **1998**, *37*, 1044–1070. (e) Speckamp, W. N.; Moolenaar, M. J. *Tetrahedron* **2000**, *56*, 3817–3856.
2. Pilli, R. A.; Russowsky, D. J. *Chem. Commun.* **1987**, 1053–1054.
3. (a) Pilli, R. A.; Dias, L. C.; Maldaner, A. O. *J. Org. Chem.* **1995**, *60*, 717–722. (b) Pilli, R. A.; Russowsky, D. *J. Org. Chem.* **1996**, *61*, 3187–3190.
4. (a) Kobayashi, S.; Ishitani, H.; Nagayama, S. *Chem. Lett.* **1995**, 423–424. (b) Kobayashi, S.; Busujima, T.; Nagayama, S. *Synlett* **1999**, 545–546. (c) Ali, T.; Chauham, K. K.; Frost, C. G. *Tetrahedron Lett.* **1999**, *40*, 5621–5624. (d) Iimura, S.; Nobutou, D.; Manabe, K.; Kobayashi, S. *Chem. Commun.* **2003**, 1644–1645. (e) Yamashita, Y.; Kobayashi, S. *J. Am. Chem. Soc.* **2002**, *124*, 3292–3392. (f) Ueno, M.; Kobayashi, S. *Org. Lett.* **2002**, *4*, 3395–3397.
5. (a) Zhu, D. W. *Synthesis* **1993**, 953–954. (b) Wolf, E. D.; Koten, G. V.; Deelman, B. *Chem. Soc. Rev.* **1999**, *28*, 37–41.
6. (a) Horvath, I. T.; Rabai, J. *Science* **1994**, *266*, 72–75. (b) Horvath, I. T.; Kiss, G.; Cook, R. A.; Bond, J. E.; Stevens, P. A.; Rabai, J.; Mozeliski, E. J. *J. Am. Chem. Soc.* **1998**, *120*, 3133–3143. (c) Fawcett, J.; Hope, E. G.; Kemmitt, R. D. W.; Paige, D. R.; Russell, D. R.; Stuart, A. M.; Cole-Hamilton, D. J.; Payne, M. J. *Chem. Commun.* **1997**, 1127–1128. (d) Curran, D. P. *Angew. Chem. Int. Ed.* **1998**, *37*, 1174–1196. (e) Nakamura, H.; Linclau, B.; Curran, D. P. *J. Am. Chem. Soc.* **2001**, *123*, 10119–10120.
7. (a) Schneider, S.; Bannwarth, W. *Angew. Chem.* **2000**, *112*, 4292–4295. (b) Schneider, S.; Bannwarth, W. *Helv. Chim. Acta* **2001**, *84*, 735–742.
8. For the preparation of perfluorinated rare earth metal catalysts [$Ln(OSO_2C_8F_{17})_3$, Ln = Yb, Sc], please see: Hanamoto, Y.; Sugimoto, Y.; Jin, Y. Z.; Imanaga, J. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 1239–1244. The reaction procedure: an excess amount of a lanthanide(III) oxide (99.9% purity) was added to an aqueous solution of $C_8F_{17}SO_3H$ (50% v/v) and the mixture was heated at boiling temperature for 30 min to 1 h. The mixture was filtered to remove the unreacted oxide. The water was then removed from the filtrate under reduced pressure. The resulting hydrate was dried by heating under vacuum at 180 to 200 °C for 48 h.
9. For the preparation of perfluorinated alkali metal salt catalysts [$M(OSO_2C_8F_{17})$, M = Li, Na, K], please see: Corey, E. J.; Shimoji, K. *Tetrahedron Lett.* **1983**, *24*, 169–172. The reaction procedure: trifluoromethanesulfonic or heptadecafluorooctanesulfonic acid was added dropwise to a solution of the carbonate salt in methanol at 0 °C. The reaction mixture was stirred at room temperature for 30 min, then it was refluxed for 2 h. The clear solution was concentrated under reduced pressure. The resulting white cake was dried at 125 °C for 2 h. The obtained product was recrystallized from methanol or ethanol/diethyl ether.
10. The organic substrates in hexane is miscible with various fluorous solvents upon heating at 60 °C.

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Stereoselective synthesis of new modified conformationally constrained L-tyrosine analogue with potential applications to SH2 domain ligands

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Abstract—This paper reports the stereoselective synthesis of a modified tricyclic tyrosine analogue **3**, whose conformation is constrained by the covalent bonds and designed on the basis of X-ray and solution structures of SH2 domain and its natural peptide ligand. A Michael addition followed by an alkylation in high stereoselections, a Friedel–Crafts and a Mannich reaction-based cyclization served as the key steps in the synthesis.

1. Introduction

In recent years, developments of new ligands of the SH2 (Src homology 2) domains have drawn more and more attention in drug design[1] because of its tight relationships with a variety of human cancers. As a general principle, constrained conformation has often benefited the ligand's binding with the protein, such as alanine analogues[2] and tyrosine analogues.[1,3] The previous X-ray and solution structure of liganded SH2 domain have provided a clear skeleton of the relevant ligands,[4] which plays the key role in the design of conformationally constrained tyrosine analogues. Based on these knowledge and principles, ligands or ligand platforms **1**, **2**, **3** and **4** were designed (Fig. 1). Burke and colleagues firstly reported the design and synthetic routes of racemic amino acid **1**[5] and **2**.[6] In our recent works, we reported the enantioselective syntheses of both D-**2**[7] and L-**2**.[8] However, these studies[6] showed that more modifications on the structure **2** are required to enhance the binding affinity with SH2 domain. Therefore, addition of one more carboxyl group on **2** (amino acid **3**) provides one practical possibility for such a purpose through more hydrogen-bonding generation, because this provides a site to introduce proper amino acid side chains. In this paper, we report our recent achievement on the enantiososelective synthesis of amino acid **3**.

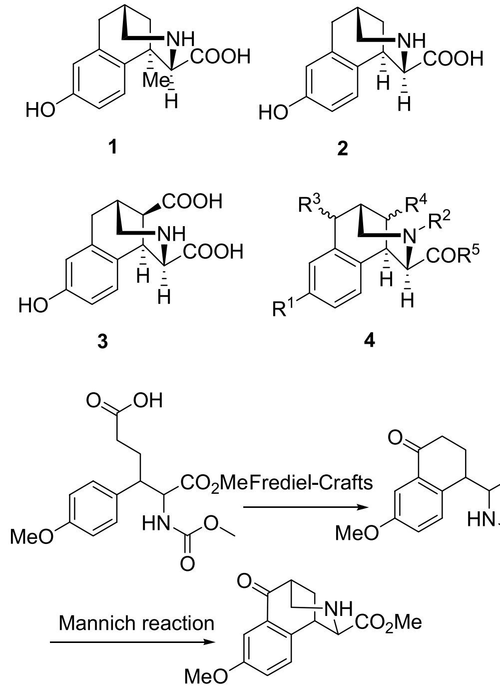

Figure 1. Structures of conformation-constrained tyrosine analogues **1**–**4**, and representation of construction of the tricylclo-skeleton.

2. Synthesis

A method for the construction of the tricyclo-skeleton of these ligands has been established during our previous

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Keywords: Src homology 2; L-Tyrosine; Mannich reaction.

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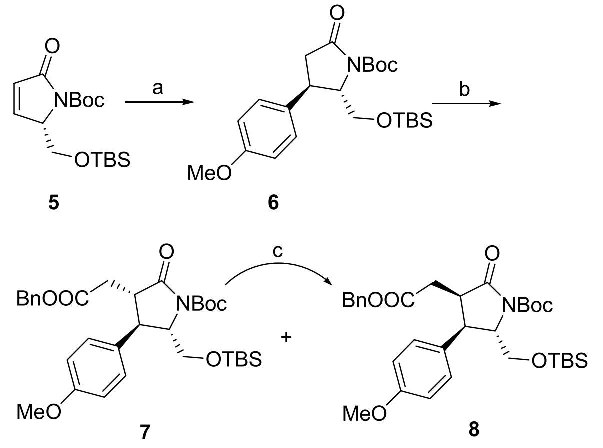
synthesis of the analogue D-**2**[7] and L-**2**,[8] which featured a Friedel–Crafts reaction followed by a Mannich reaction (Fig. 1). Accordingly, for the synthesis of L-tyrosine analogue **3**, our first task was to prepare the precursor of the Friedel–Crafts reaction, compound **10**.

Our first synthetic strategy of the precursor acid **10** is based on a Michael addition followed by an alkylation. Unsaturated lactam **5** was prepared from L-Glu over six steps in 68% overall yield.[9] The aryl group was introduced smoothly in high yield using THF as the solvent and only one isomer was obtained.[10] However, if methyl sulfide was used as the co-solvent (1:1 with tetrahydrofuran) in this step, a tandem-Michael-addition product was detected as the major product[11] (Scheme 1).



Scheme 1. Reagents and conditions: (a) CuBr·Me$_2$S, 4-MeOPhMgBr, THF, −78 to 0 °C, 90%; (b) LiHMDS, BrCH$_2$COOBn, THF, −78 to 0 °C, 98% (c) (1) LDA, THF, −78 °C, 30 min; (2) AcOH, −78 to 0 °C, 70%.

Alkylation of the lactam **6** with benzyl bromoacetate gave two isomers, **7** and **8** (ratio 1.5:1), in 98% total yield, and the 3,4-*trans*-isomer could be converted to the *cis* one completely when being treated with LHMDS (sodium bis(trimethylsilyl) amide) followed by acetic acid. Oxidation and deprotection of compound **8** afforded the precursor **10**[12] for the Friedel–Crafts reaction. Ketone **11** was obtained smoothly according to our reported method,[7,8] and the *tert*-butoxoyl carbonyl group was cleaved at the same time. The stereochemistry of **11** was confirmed by the X-ray analysis (Fig. 2).[13] Now it came to the expected Mannich reaction. No Mannich product was deducted, when ketone **11** was treated directly with formaldehyde. Reprotection and ring opening of **11** gave diester **13**, which was then treated with trifluoroacetic acid followed by formaldehyde in hope of getting the Mannich product. Unfortunately, **14** was provided as the only product. It was believed that formation of the kinetically and thermodynamically favored lactam retarded the expected Mannich reaction. Attempt to reduce the diester in **13** to corresponding diol was not successful due to the formation of a five-membered lactol (Scheme 2). So we had to prepare an alternative precursor for this Mannich reaction.

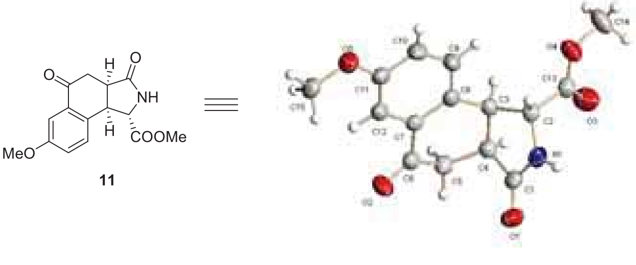
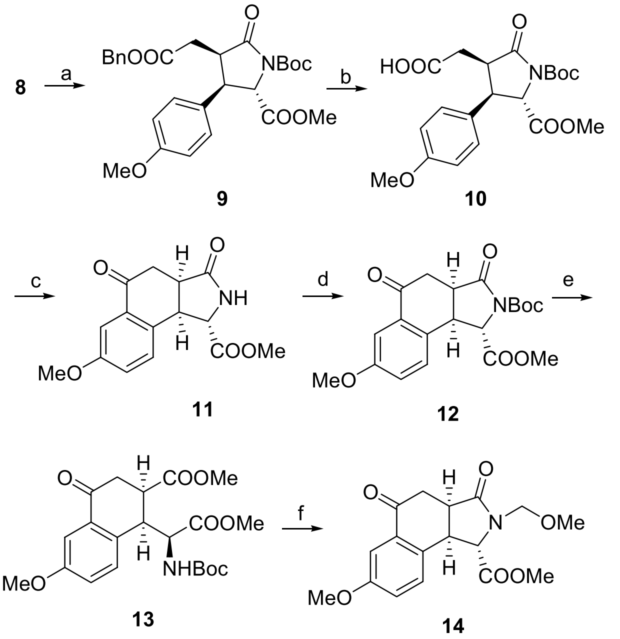


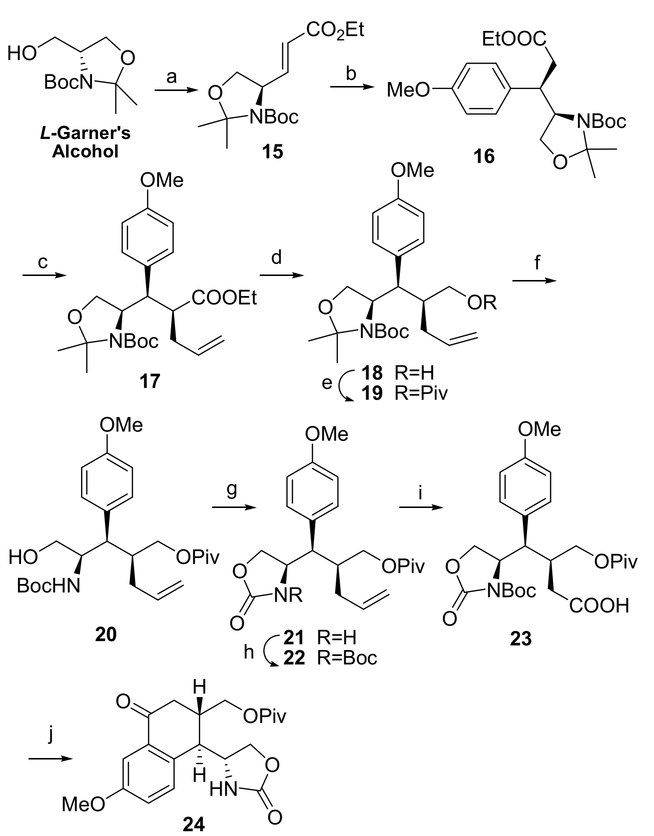
Figure 2. ORTEP drawing of ketone **11** based on the X-ray analysis.[13]



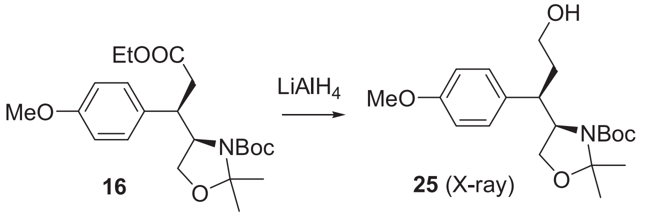
Scheme 2. Reagents and conditions: (a) (1) Jones reagent, acetone, 0 °C, 4 h; (2) CH$_2$N$_2$, Et$_2$O, rt, 10 min, 55% for two steps; (b) 1 atm H$_2$, Pd/C (10%) (cat), EtOAc, rt, 3 h, 68%; (c) (1) (COCl)$_2$, DMF (cat), CS$_2$/CH$_2$Cl$_2$, rt, 2 h; (2) AlCl$_3$, CH$_2$Cl$_2$, rt, overnight, 74%; (d) (Boc)$_2$O, DMAP (cat), CH$_3$CN, rt, 2 h, 81%; (e) K$_2$CO$_3$, MeOH, rt, 45 min, 100% (conv%: 60%); (f) (1) TFA, CH$_2$Cl$_2$, rt, 60 min; (2) HCHO, ROH, reflux, 12 h, 75%.

Synthesis of **23**, the alternative Mannich reaction substrate, commenced with acyclic unsaturated ester **15**, which was prepared form L-Garner's alcohol through Swern oxidation followed by a Wittig olefination (Scheme 3). It is noteworthy that the Wittig reagent used in this step should be free of sodium hydroxide, otherwise recemization would happen.[14] Michael addition of *p*-methoxylphenylmagnesium bromide to unsaturated ester **15** in the presence of CuI gave ester **16** as the only isomer, whose stereochemistry was confirmed by the X-ray of the corresponding alcohol **25** (Fig. 3).[13] Allylation of ester **16** also afforded one isomer **17** in nearly quantitative yield. Reduction of ester **17** with lithium aluminum hydride, protection of the resulting hydroxyl group as trimethyl acetate and cleavage of the ketal with *p*-toluenesulphonic acid gave alcohol **20**, which was converted to oxazolidone **21** after being treated with sodium hydride in DMF. Protection of the amide group with di-*tert*-butyl dicarbonate and oxidation of the terminal double bond followed by further oxidation with sodium chlorite afforded acid **23**. The subsequent Mannich reaction proceeded uneventfully and ketone **24** was afforded in 74% yield under the previously reported conditions.[8] Again, the *N*-*tert*-butoxyl carbonyl group was cleaved in this step (Scheme 3).

Protection of the amide group of ketone **24** with di-*tert*-butyl dicarbonate again and treatment with cesium carbonate in methanol afforded diol **27**, and the two hydroxyl groups were both protected with trimethylacetyl chloride. Cleavage of the *tert*-butoxoyl carbonyl followed by refluxing with formaldehyde in ethanol, and reprotection of the newly formed amino group with methyl chloroformate gave tricyclo ketone **29** in nearly quantitative yield over three steps. Attempt to reduce the carbonyl of **29** to methylene by dithioacetal/Raney-nickel was failed because the prerequisite dithioacetal cannot be formed presumably due to the steric hindrance; yet reduction with sodium borohydride smoothly gave the corresponding alcohol, which could be reduced further to compound **30** by Barton–McCombie method. Reductive cleavage of the trimethyl acetyl groups followed by oxidation gave diester **31** in total 63% yield over four steps. To get the correct stereochemistry, diester **31** was treated with sodium methoxide in methanol firstly, and **32** was separated in quantitative yield; treatment with LDA followed by acetic acid gave both **32** and **33** in about 1:1 ratio (based on ^{1}H NMR spectrum), but they cannot be separated by the



Scheme 3. Reagents and conditions: (a) (1) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C, 2 h; (2) $Ph_3P{=}CHCOOEt$, PhH, rt, overnight, 81%; (b) *p*-methoxylphenylmagnesium bromide, CuI, THF, −78 to 0 °C, 100%; (c) (1) LDA, HMPA, THF, −78 °C, 30 min; (2) allyl bromide, −78 °C–rt, 100%; (d) LAH, THF, 0 °C, 60 min, 95%; (e) PivCl, Et_3N, DMAP (cat), CH_2Cl_2, 0 °C—rt, overnight, 100%; (f) PTSA, MeOH, rt, overnight, 84%; (g) NaH, DMF, 0 °C, 60 min, 76%; (h) $(Boc)_2O$, Et_3N, DMAP (cat), CH_2Cl_2, 0 °C—rt, overnight, 93%; (i) (1) O_3, CH_2Cl_2/MeOH, −78 °C, 1 h; (2) Me_2S, −78 to 0 °C; (3) $NaClO_2$, KH_2PO_3, 2-methyl-2-butane, ^{t}BuOH/H_2O, 84%; (j) (1) $(COCl)_2$, DMF (cat), CS_2/CH_2Cl_2, rt, 2 h; (2) $AlCl_3$, CH_2Cl_2, 0 °C—rt, overnight, 74%.



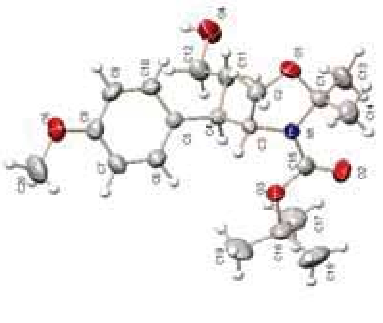
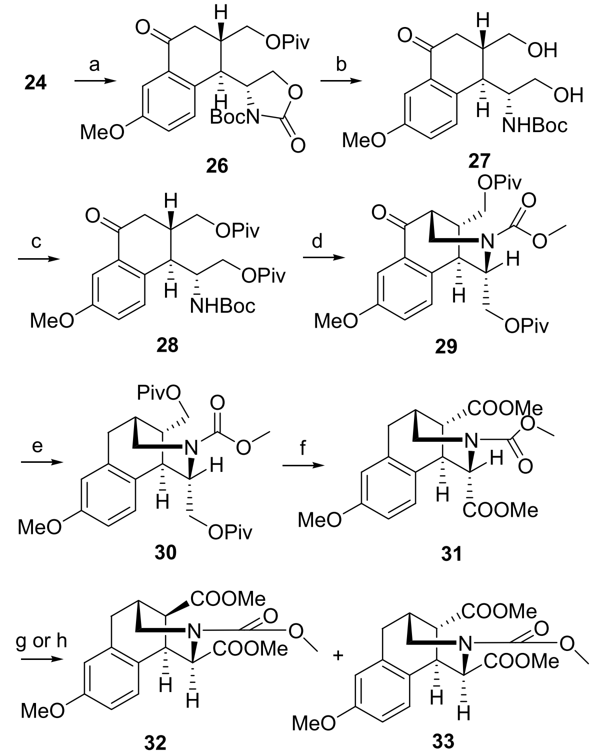


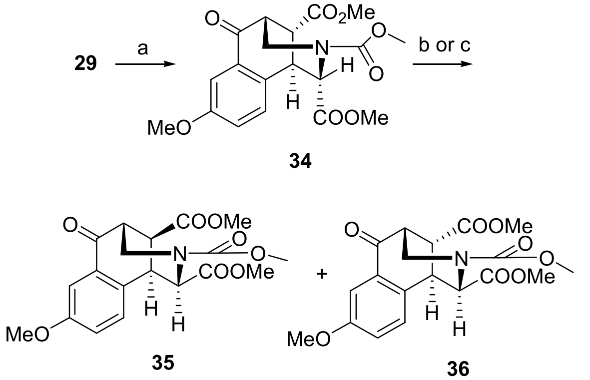
Figure 3. ORTEP drawing of alcohol **25** based on the X-ray analysis.[13]



Scheme 4. Reagents and conditions: (a) $(Boc)_2O$, Et_3N, DMAP (cat), CH_2Cl_2, 0 °C–rt, 2 h, 95%; (b) Cs_2CO_3, MeOH, rt, overnight, 86%; (c) PivCl, Et_3N, DMAP (cat), CH_2Cl_2, 0 °C—rt, overnight, 76%; (d) (1) TFA, CH_2Cl_2, rt, 2 h; (2) HCHO (36% aq), EtOH, refluxed, 2 h; (3) ClCOOMe, $NaHCO_3$, dioxane/H_2O, rt, 60 min, 100%; (e) (1) $NaBH_4$, MeOH, rt, 20 min; (2) (i) NaH (60%), THF, 0 °C, 30 min; (ii) CS_2, 0 °C—rt, 1.5 h; (iii) MeI, rt, 2 h, 86% for two steps; (3) *n*-Bu_3SnH, AIBN, toluene, refluxed, 3 h, 100%; (f) (1) DIBAL-H, CH_2Cl_2, −78 °C, 40 min; (2) DMP, CH_2Cl_2, rt, 60 min; (3) $NaClO_2$, KH_2PO_3, 2-methyl-2-butene, ^{t}BuOH/H_2O, rt; (4) CH_2N_2, Et_2O, rt, 20 min, 63% for four steps; (g) NaOMe, MeOH, 0 °C, 20 min, 100%; (h) (1) LDA, HMPA, −78 °C, 30 min; (2) AcOH, −78 °C, 10 min.

column chromatography (Scheme 4). The stereochemistry of **32** was elucidated by chemical relation (see below).

Reductive cleavage of the trimethyl acetyl group of ketone **29** with DIBAL-H (the ketone carbonyl group was reduced to the corresponding hydroxyl group) followed by oxidation and etherification gave diester **34** (the hydroxyl group was oxidized back to the carbonyl group), in 50% total yield over four steps. Similar as diester **31**, if **34** was treated with sodium methoxide in methanol, only **36** was generated; and treatment with LDA followed by acetic acid gave both **35** and **36** in about 1:1 ratio, fortunately both of them could be easily separated by column chromatography (Scheme 5). The stereochemistry of compound **34**, **35** and **36** were all confirmed by the NOESY studies (Fig. 4).



Scheme 5. Reagents and conditions: (a) (1) DIBAL-H, CH_2Cl_2, −78 °C, 40 min; (2) DMP, CH_2Cl_2, rt, 60 min; (3) $NaClO_2$, KH_2PO_3, 2-methyl-2-butene, ^{t}BuOH/H_2O, rt; 4. CH_2N_2, Et_2O, rt, 20 min, 50% for four steps; (b) NaOMe, MeOH, 0 °C, 20 min, 80% (for **36**); (c) (1) LDA, HMPA, −78 °C, 30 min; (2) AcOH, −78 °C, 10 min, 36% (for **35**) and 36% (for **36**).

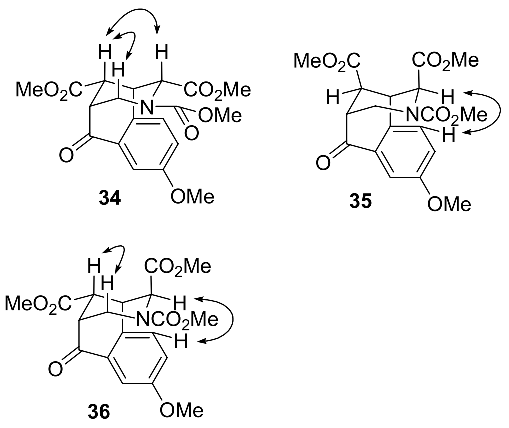
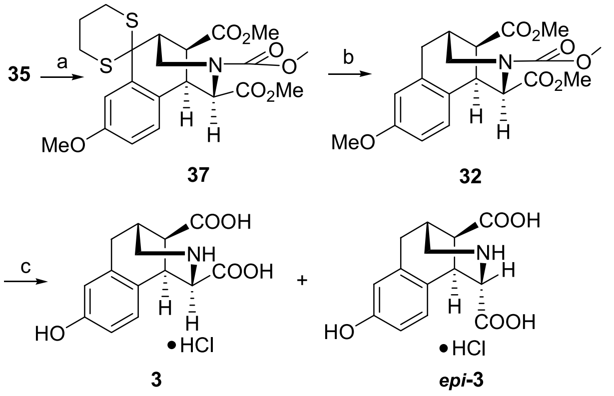


Figure 4. NOESY studies of compounds **34**, **35** and **36**.

Protection of the ketone **35** with 1,3-propandithol followed by reduction with Raney-nickel gave ester **32**. Refluxing of ester **32** with 6 N HCl for 4 days afforded amino acid **3** and its isomer *epi*-**3** (about 1:1 based on the ^{1}H NMR spectrum) (Scheme 6). Because the stereochemistry of α-carbon (in *R* configuration) of *epi*-**3** could be converted to the *S* one (**3**) automatically under basic conditions or during amide formation reactions,[5,8] both **3** and *epi*-**3** (as well as their mixture) are good for use in the further application to synthesis of new SH2 domain ligands.



Scheme 6. Reagents and conditions: (a) 1,3-propanedithiol, $BF_3 \cdot Et_2O$, CH_2Cl_2, rt, 48 h; (b) Raney-nickel, EtOH, rt, 30 min, 74% for two steps; (c) 6 N HCl, reflux, 4 days, 85%.

3. Summary

In conclusion, an enantioselective synthetic route of new tricyclic amino acid analogue **3** was developed efficiently, utilizing Michael addition followed by allyllation, intramolecular Friedel–Crafts cyclization and intramolecular Mannich cyclization as the key steps. All the stereochemistries in this route were well controlled and the key reactions were optimized and well repeated in good yields. This would give a good starting point to develop the new SH2 domain ligands based on the conformationconstrained tyrosine analogue **3** as a scaffold. The chemical diversity studies on **3** and related biochemical experiments are in progress in this laboratory.

4. Experimental

4.1. General methods

All reactions were carried out under argon or nitrogen in oven-dried glassware using standard gastight syringes, cannulas, and septa. Solvents and reagents were purified and dried by standard methods prior to use; optical rotations were measured at rt. IR spectra were recorded on an FT-IR instrument. ^{1}H NMR spectra were recorded at 300 MHz and reported in parts per million (*δ*) downfield relative to TMS as internal standard, and ^{13}C NMR spectra were recorded at 75 MHz and assigned in parts per million (*δ*). Flash column chromatography was performed on silica gel (10–40 μm) using a mixture of petroleum ether and ethyl acetate as the eluent.

4.1.1. (2*S*,3*R*)-2-(*tert*-Butyl-dimethyl-silanyloxymethyl)-3-(4-methoxy-phenyl)-5-oxo-pyrrolidine-1-carboxylic acid *tert*-butyl ester (6). To a suspension of $CuBr \cdot Me_2S$ (12.6 g, 61.3 mmol) in dry THF (40 mL) at −40 °C, was added fresh prepared *p*-anisolemagesium bromide (0.5 M in THF, 122 mL, 61.0 mmol). After 30 min the mixture was cooled to −78 °C. A mixture solution of unsaturated amide **5** (2.00 g, 6.10 mmol) and TMSCl (1.50 mL, 11.9 mmol) in dry THF (30 mL) was added to the above suspension, the reaction mixture was stirred at −78 °C for 30 min and slowly warmed to rt. Satd NH_4Cl was added and the mixture was extracted with ether, the combined ether extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography to give **6** as a pale yellow solid (2.40 g) in 90% yield. Mp: 58–59 °C. $[\alpha]_D^{20} = -29.5$ (*c* 1.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.12 (d, $J = 8.7$ Hz, 2H), 6.87 (d, $J = 8.7$ Hz, 2H), 4.07–4.02 (m, 1H), 3.99 (dd, $J = 10.2$, 3.6 Hz, 1H), 3.84–3.78 (m, 1H), 3.80 (s, 3H), 3.43 (dt, $J = 9.6$, 2.1 Hz, 1H), 3.15 (dd, $J = 17.4$, 9.6 Hz, 1H), 2.50 (dd, $J = 17.4$, 2.7 Hz, 1H), 1.55 (s, 9H), 0.94 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.3, 158.5, 149.8, 136.3, 127.3, 114.3, 82.9, 66.9, 63.5, 55.2, 40.1, 37.9, 28.0, 25.8, 18.1, −5.58 ppm. EIMS (*m*/*z*, %): 336 [$(MH-100)^+$, 4.47], 322[$(MH_2-115)^+$, 59.3]. ESIMS (*m*/*z*, %): 474.3 $(M+K)^+$, 458.3 $(M+Na)^+$, 336.2 $(MH-100)^+$. IR (KBr): 2956, 1774, 1290, 1164, 774, 538 cm^{-1}. Anal. for $C_{23}H_{37}NO_5Si$. Calcd: C, 63.41; H, 8.56; N, 3.22. Found: C, 63.67; H, 8.64; N 3.20. ee% = 96.8% (measured by HPLC).

4.1.2. (3*S*,4*R*,5*S*)-3-Benzyloxycarbonylmethyl-5-(*tert*-butyldimethyl-silanyloxymethyl)-4-(4-methoxy-phenyl)-2-oxo-pyrrolidine-1-carboxylic acid *tert*-butyl ester [7 (3*S*) and 8 (3*R*)]. To a solution of **6** (2.05 g, 4.71 mmol) in dry THF (30 mL) at −78 °C, was added fresh LHMDS (0.5 M in THF, 20 mL, 10. 0 mmol); the mixture was stirred for 30 min and $BrCH_2COOBn$ (2.20 g, 9.61 mmol) was added, then the mixture was slowly warmed to rt after another 30 min. Sat. NH_4Cl was added and the mixture was extracted with ether. The combined ether extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo, the residue was purified by flash column chromatography to give **7** (1.50 g) and **8** (1.00 g) in 98% total yield, both as pale yellow oil. Date for **7**: $[\alpha]_D^{20} = -10.5$ (*c* 2.7, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.26 (m, 3H), 7.21–7.17 (m, 2H), 7.10 (d, $J = 8.6$ Hz, 2H), 6.82 (d, $J = 8.6$ Hz, 2H), 4.94 (AB, $J_{AB} = 12.4$ Hz, 1H), 4.83 (AB, $J_{AB} = 12.4$ Hz, 1H), 4.13 (dd, $J = 10.8$, 3.1 Hz, 1H), 3.94 3.91 (m, 1H), 3.76 (s, 3H), 3.49 (dd, $J = 10.8$, 1.5 Hz, 1H), 3.17 3.11 (m, 2H), 2.83 (dd, $J = 15.9$, 5.9 Hz, 1H), 2.50 (dd, $J = 15.9$, 7.3 Hz, 1H), 1.52 (s, 9H), 0.85 (s, 9H), 0.01 (s, 3H), 0.00 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6, 171.1, 158.9, 150.1, 135.6, 132.1, 128.8, 128.4, 128.1, 114.3, 83.1, 66.4, 64.9, 60.0, 55.2, 47.3, 43.9, 34.8, 29.6, 28.1, 25.8, 18.2, −5.5 ppm. EIMS (*m*/*z*, %): 483 [$(M-100)^+$, 0.60]. ESIMS (*m*/*z*, %): 606.3 [$(M+Na)^+$, 30.0], 584.3 [$(M+H)^+$, 8.0], 484.2 [$(MH-100)^+$, 100]. IR(film): 2932, 1716, 1253, 837, 830 cm^{-1}. Anal. for $C_{32}H_{45}NO_7Si$. Calcd: C, 65.84; H, 7.77; N, 2.40. Found: C, 66.19; H, 7.77; N, 2.17.

Date for **8**: $[\alpha]_D^{20} = -31.0$ (*c* 1.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.30–7.15 (m, 5H), 6.80 (dd, $J = 8.6$ Hz, 2H), 6.65 (dd, $J = 8.6$ Hz, 2H), 5.03 (AB, $J_{AB} = 12.3$ Hz, 1H), 4.93 (AB, $J_{AB} = 12.3$ Hz, 1H), 4.13–4.10 (m, 1H), 3.95 (dd, $J = 10.6$, 4.1 Hz, 1H), 3.77 (dd, $J = 10.6$, 2.3 Hz, 1H), 3.70–3.60 (m, 4H), 3.52 (d, $J = 9.0$ Hz, 1H), 2.65 (dd, $J = 17.7$, 4.2 Hz, 1H), 1.81 (dd, $J = 17.7$, 10.3 Hz, 1H), 1.48 (s, 9H), 0.83 (s, 9H), 0.01 (s, 3H), 0.00 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.7, 171.7, 158.8, 150.0, 135.8, 132.3, 128.5, 128.3, 128.2, 114.3, 83.2, 66.3, 64.9, 63.7, 55.2, 44.1, 42.9, 31.5, 28.1, 25.8, 18.2, −5.50 ppm. EIMS (*m*/*z*, %): 483 [$(M-100)^+$, 0.77], 468 [$(M-115)^+$, 7.33]. ESIMS (*m*/*z*, %): 606.3 [$(M+Na)^+$, 11.0], 484.2 [$(MH-100)^+$, 100]. IR (film): 2932, 1741, 1254, 838, 699 cm^{-1}. Anal. for $C_{32}H_{45}NO_7Si$. Calcd: C, 65.84; H, 7.77; N, 2.40. Found: C, 66.22; H, 8.03; N, 2.30.

4.1.3. (3*R*,4*R*,5*S*)-3-Benzyloxycarbonylmethyl-5-(*tert*-butyldimethyl-silanyloxymethyl)-4-(4-methoxy-phenyl)-2-oxo-pyrrolidine-1-carboxylic acid *tert*-butyl ester (8). To a solution of **7** (0.81 mg, 1.39 mmol) in dry THF (30 mL) at −78 °C, was added fresh LHMDS (0.67 M in THF, 6.3 mL, 4.22 mmol), the mixture was stirred at −78 °C for 40 min and AcOH (0.27 mL, 4.29 mmol) was added dropwise. Satd NH_4Cl was added after additional 10 min, the mixture was warmed to rt and extracted with Et_2O. The combined Et_2O extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography to give a pale yellow oil (596 mg) in 73% yield. (The physical data has been shown in the preparation of compound **7** and **8**).

4.1.4. (2*S*,3*R*,4*R*)-4-Benzyloxycarbonylmethyl-3-(4-methoxy-phenyl)-5-oxo-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (9). To a solution of **8** (1.46 g, 2.50 mmol) in acetone (40 mL) at 0 °C, was added dropwise Jone's reagent (2.67 M, 2.40 mL, 6.41 mmol); the mixture was warmed to rt and stirred for 4 h. *iso*-propanol (15 mL) was added, and the mixture was filtered through a celite after being stirred for 30 min, the filtrate was basified with sat. $NaHCO_3$, and acetone was removed in vacuo; the residue aqueous phase was extracted with ether two times, then acidified to pH 3–4, and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed by brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was dissolved in Et_2O (20 mL) and treated with CH_2N_2 in Et_2O; after 10 min, excess CH_2N_2 was destroyed by AcOH and the mixture was concentrated in vacuo, The residue was purified by flash column chromatography to give a pale yellow oil (664 mg) in 54% yield. $[\alpha]_D^{20} = -2.2$ (*c* 1.3, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.32 (m, 3H), 7.28–7.22 (m, 2H), 6.90 (d, $J = 8.7$ Hz, 2H), 6.75 (d, $J = 8.7$ Hz, 2H), 5.10 (AB, $J_{AB} = 12.0$ Hz, 1H), 4.99 (AB, $J_{AB} = 12.0$ Hz, 1H), 4.67 (s, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 3.67 (d, $J = 9.0$ Hz, 1H), 3.55–3.45 (m, 1H), 2.81 (dd, $J = 18.0$, 3.9 Hz, 1H), 1.92 (dd, $J = 18.0$, 10.8 Hz, 1H), 1.52 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.1, 171.4, 170.6, 159.1, 149.1, 135.5, 130.0, 128.4, 128.3, 128.2, 114.4, 84.0, 66.5, 64.2, 55.2, 52.8, 43.4, 42.6, 30.9, 27.8 ppm. EIMS (*m*/*z*, %): 497 (M^+, 3.99), 397 [$(M-100)^+$, 7.78]. IR (film): 2981, 1794, 1751, 1255, 836, 531 cm^{-1}. Anal. for $C_{27}H_{31}NO_8$. Calcd: C, 65.18; H, 6.28; N, 2.82. Found: C, 65.33; H, 6.15; N, 2.80.

4.1.5. (2*S*,3*R*,4*R*)-4-Carboxymethyl-3-(4-methoxy-phenyl)-5-oxo-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (10). To a solution of **9** (250 mg, 0.50 mmol) in ethyl acetate (5 mL), was added Pd/C (10%, 25 mg), and the mixture was stirred at rt for 3 h under 1 atm hydrogen. Pd/C was filtered off and the filtrate was concentrated in vacuo, the residue was purified by flash column chromatography directly to give a pale yellow oil (140 mg) in 68% yield. $[\alpha]_D^{20} = -2.1$ (*c* 0.7, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.00 (d, $J = 8.4$ Hz, 2H), 6.86 (d, $J = 8.1$ Hz, 2H), 4.70 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.67 (d, $J = 8.7$ Hz, 1H), 3.47 (m, 1H), 2.79 (dd, $J = 12.3$, 3.9 Hz, 1H), 1.98 (dd, $J = 12.3$, 9.9 Hz, 1H), 1.53 (s, 9H) ppm. EIMS (*m/z*, %): 307 [(M−100)$^+$, 7.89]. IR (film): 3226, 2950, 1751, 1255, 838, 550 cm^{-1}. HR-MALDI for ($C_{20}H_{25}NO_8$+Na)$^+$. Calcd: 430.1478. Found 430.1472.

4.1.6. 7-Methoxy-3,5-dioxo-2,3,3*a*,4,5,9*b*-hexahydro-1*H*-benzo[*e*]isoindole-1*S*-carboxylic acid methyl ester (11). To a solution of acid **10** (100 mg, 0.245 mmol) in dry CH_2Cl_2 (4.0 mL) and CS_2 (10.0 mL) at 0 °C, was added $(COCl)^2$ (87 μL, 1.02 mmol), followed by the slow addition of dry DMF (6 μL, 0.077 mmol). The mixture was warmed to rt and stirred for 2 h, the solvent was removed to give a yellow oil. To the solution of this oil in dry CH_2Cl_2 (20 mL) at 0 °C, was added $AlCl_3$ (160 mg, 1.20 mmol) in one portion. The reaction mixture was warmed to rt and stirred overnight. Crashed ice was added and the reaction mixture was extracted with CH_2Cl_2, the CH_2Cl_2 extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography to give ketone **11** (54 mg) as a white solid in 76% yield. Mp: 178–180 °C. $[\alpha]_D^{20} = -8.7$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (d, *J*=2.9 Hz, 1H), 7.40 (d, *J*=8.7 Hz, 1H), 7.18 (dd, *J*=8.7, 2.9 Hz, 1H), 7.15 (br s, 1H), 4.25 (s, 1H), 4.02 (dd, *J*=7.8, 1.8 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 3.25–3.19 (m, 1H), 3.10 (dd, *J*=16.9, 4.1 Hz, 1H), 2.72 (dd, *J*=16.9, 7.1 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.0, 177.0, 171.5, 159.2, 133.8, 133.3, 130.0, 123.0, 108.7, 62.6, 55.6, 52.9, 40.5, 38.0, 34.7 ppm. EIMS (*m/z*, %): 289 (M$^+$, 29.73). IR (KBr): 3396, 1693, 1198, 839, 548 cm^{-1}.

4.1.7. 7-Methoxy-3,5-dioxo-1,3,3*a*,4,5,9*b*-hexahydro-benzo[*e*] isoindole-1*S*,2-dicarboxylic acid 2-*tert*-butyl ester 1-methyl ester (12). To a solution of ketone **11** (53 mg, 0.183 mmol) in dry CH_3CN (10 mL) at rt, was added Boc_2O (84 mg, 0.385 mmol) and DMAP (2 mg, 0.016 mmol) successively. After being stirred for 1.5 h, the mixture was diluted with CH_2Cl_2, washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo, the residue was purified by flash column chromatography to give ketone **12** (58 mg) as a colorless oil in 81% yield. $[\alpha]_D^{20} = +33.5$ (*c* 1.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (d, *J*=2.7 Hz, 1H), 7.45 (d, *J*=8.7 Hz, 1H), 7.23 (dd, *J*=8.7, 2.7 Hz, 1H), 4.75 (s, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.83 (d, *J*=6.9 Hz, 1H), 3.42 (dt, *J*=6.9, 3.0 Hz, 1H), 3.25 (dd, *J*=17.1, 3.0 Hz, 1H), 2.73 (dd, *J*=17.1, 6.9 Hz, 1H), 1.46 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.4, 172.3, 170.9, 160.0, 150.0, 134.2, 132.6, 130.1, 123.7, 109.2, 84.6, 65.6, 56.0, 53.4, 40.5, 37.3, 34.7, 28.2 ppm. EIMS (*m/z*, %): 389 (M$^+$, 24.0). ESIMS (*m/z*, %): 428.2 [(M+K)$^+$, 13.0], 412.2 [(M+Na)$^+$, 25.0]. IR (film): 2981, 1793, 1753, 1304, 732, 538 cm^{-1}. HR-MALDI for ($C_{20}H_{23}NO_7$+Na)$^+$. Calcd: 412.1372. Found: 412.1361.

4.1.8. (1*R*,2*R*)-1-(*tert*-Butoxycarbonylaminomethoxycarbonyl-methyl)-6-methoxy-4-oxo-1,2,3,4-tetrahydro-naphthalene-2-carboxylic acid methyl ester (13). To a solution of ketone **12** (58 mg, 0.149 mmol) in methanol (5.0 mL), was added K_2CO_3 (31 mg, 0.225 mmol), and the mixture was stirred at rt for 30 min. Water was added and the resulted mixture was extracted with ethyl acetate (50 mL×3); the organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give diester **13** as a colorless oil (32 mg) in 74% yield, and recovered material as a colorless oil (15 mg). $[\alpha]_D^{20} = +101.0$ (*c* 0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.06 (s, 2H), 5.10 (d, *J*=9.9 Hz, 1H), 4.56 (t, *J*=9.9 Hz, 1H), 3.85–3.75 (m, 7H), 3.47 (s, 3H), 3.36–3.10 (m, 2H), 2.95 (dd, *J*=18.3, 3.0 Hz, 1H), 1.43 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.5, 172.2, 159.5, 133.7, 133.1, 129.1, 121.5, 110.2, 80.6, 55.5, 52.2, 52.1, 43.3, 42.0, 35.5, 28.2, 14.2 ppm. ESIMS (*m/z*, %):460.3 [(M+K)$^+$, 3.0], 444.3 [(M+Na)$^+$, 100], 422.3 [(MH)$^+$, 3.0]. IR (film): 3387, 2850, 1730, 1212, 828, 563 cm^{-1}. HR-MALDI for ($C_{21}H_{27}NO_8$+Na)$^+$. Calcd: 444.1634. Found: 444.1633.

4.1.9. (1*S*,4*R*)-7-Methoxy-2-methoxymethyl-3,5-dioxo-2,3,3*a*,4,5,9*b*-hexahydro-1*H*-benzo[*e*]isoindole-1-carboxylic acid methyl ester (14). To a solution of ester **13** (10 mg, 0.024 mmol) in DCM (3.0 mL) was added TFA (3.0 mL), and the mixture was stirred at rt for 2 h. Solvent was removed in vacuo, and the residue was redissolved in methanol (10 mL), HCHO (30% aq, 0.30 mL, 3.00 mmol) was added to the above solution. The mixture was refluxed for 2 h and concentrated; the residue was purified by flash column chromatography directly to give a colorless oil (6.0 mg) in 75% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (d, *J*=3.0 Hz, 1H), 7.43 (d, *J*=8.7 Hz, 1H), 7.21 (dd, *J*=8.7, 3.0 Hz, 1H), 4.81 (AB, J_{AB}=11.1 Hz, 1H), 4.53 (AB, J_{AB}=11.4 Hz, 1H), 4.37 (s, 1H), 3.99 (d, *J*=7.5 Hz, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.42–3.36 (m, 1H), 3.26 (dd, *J*=17.1, 2.4 Hz, 1H), 2.80 (s, 3H), 2.75 (dd, *J*=17.1, 6.9 Hz, 1H) ppm. EIMS (*m/z*, %): 333 (M$^+$, 4.93), 302 [(M−31)$^+$, 20.15]. ESIMS (*m/z*, %): 351.1 [(M+NH$_4$)$^+$, 25.0], 334.1 [(M+H)$^+$, 38.0], 302.1 [(M−OMe)$^+$, 100]. IR (film): 2927, 1716, 1283, 1228, 757, 496 cm^{-1}. HR-EIMS for $C_{17}H_{19}NO_6^+$. Calcd: 333.1212. Found: 333.1232.

4.1.10. (4*R*)-4-(2-Ethoxycarbonyl-vinyl)-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (15). To a solution of $(COCl)_2$ (5.7 mL, 67.3 mmol) in dry DCM (250 mL) at −78 °C, was added a solution of DMSO (9.3 mL, 0.131 mol) in dry DCM (40 mL) during 15 min. Garner's alcohol (10.0 g, 43.2 mmol) in dry DCM (40 mL) was added dropwise to the above solution. The mixture was stirred at −78 °C for 2 h before Et_3N (50.0 mL, 356 mmol) was added, then warmed to rt. Satd NH_4Cl was added and the resulted mixture was extracted with DCM, the organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated; the residue was redissolved in ether (100 mL), filter through a celite to remove the $Et_3N \cdot HCl$. Ether was removed in vacuo and the residue was used directly for the next step. To a solution of the above residue in benzene (150 mL) at 0 °C, was added $Ph_3P{=}CHCOOEt$ (18.4 g, 52.5 mmol), and the mixture was stirred at rt overnight. Most of the benzene was removed and ether (150 mL) was added, filtered and concentrated, the residue was purified by flash column chromatography directly to give a pale yellow oil (10.7 g) in 83% yield. $[\alpha]_D^{20} = -61.4$ (*c* 0.95, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95–6.78 (m, 1H), 6.02–5.85 (m, 1H), 4.62–4.50 (m, 0.4H), 4.50–4.38 (m, 0.6H), 4.30–4.15 (m, 2H), 4.15–4.05 (m, 1H), 3.85–3.78 (m, 1H), 1.65–1.25 (m, 18H) ppm. ee%=97.3% (measured by HPLC).

4.1.11. (1*R*,4*R*)-4-[2-Ethoxycarbonyl-1-(4-methoxyphenyl)-ethyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (16). To the suspension of CuI (16.0 g, 84.0 mmol) in dry THF (100 mL) at −78 °C, was added fresh prepared *p*-methoxylphenylmagnesium bromide (0.5 M, 0.34 L, 0.17 mol) dropwise, the mixture was slowly warmed to −20 °C during 60 min. The mixture was cooled back to −78 °C, then a solution of TMSCl (25.4 mL, 0.20 mol) and ester **15** (5.0 g, 16.7 mmol) in dry THF (30 mL) was added. The mixture was stirred at −78 °C for 60 min and warmed to 0 °C during 2 h. Satd NH_4Cl was added and the resulted mixture was extracted with ether, the organic layer was washed by Satd $Na_2S_2O_3$ and brine, dried over anhydrous Na_2SO_4, concentrated, the residue was purified by flash column chromatography to give a colorless oil (6.98 g) in 100% yield. $[\alpha]_D^{20}=+38.5$ (c 2.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.10 (m, 2H), 6.88–6.80 (m, 2H), 4.10–3.65 (m, 9H), 2.85–2.70 (m, 2H), 1.60–1.30 (m, 15H), 1.25–1.05 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 158.3, 152.7/152.3$_{\text{(conformer)}}$, 132.1, 129.0, 128.6, 113.9, 113.6, 94.6/94.0, 80.1, 63.8/63.4, 61.8/61.5, 60.1, 55.0, 42.0, 33.7/32.5, 28.3, 26.6/25.9, 23.7/22.2, 13.9 ppm. EIMS (*m*/*z*, %): 407 (M^+, 0.43). IR (film): 2980, 1700, 1252, 1178, 854, 541 cm^{-1}. Anal. for $C_{22}H_{33}NO_6$. Calcd: C, 64.84; H, 8.16; N, 3.44. Found: C, 65.10; H, 8.29; N, 3.37. ee% > 96.4% (measured by HPLC).

4.1.12. (1*R*,2*S*,4*R*)-4-[2-Ethoxycarbonyl-1-(4-methoxyphenyl)-pent-4-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (17). To a solution of (i-Pr)$_2$NH (7.6 mL, 54.3 mmol) in dry THF (250 mL) at 0 °C, was added *n*-BuLi (1.6 M in hexane, 30.0 mL, 48.0 mmol) dropwise, the mixture was stirred for 15 min and cooled to −78 °C. HMPA (11.5 mL, 63.4 mmol) was added to the above solution, and the mixture was stirred for 30 min before ester **16** (13.0 g, 31.9 mmol) in dry THF (40 mL) was added. Allylic bromide (4.1 mL, 48.4 mmol) was added after another 30 min. The mixture was stirred at −78 °C for 30 min and warmed to rt during 60 min, satd NH_4Cl was added and the resulted mixture was extracted with ether. The organic layer was washed by brine, dried over Na_2SO_4, filtered and concentrated; the residue was purified by flash column chromatography to give a pale yellow oil (14.5 g) in 100% yield. $[\alpha]_D^{20}=-62.8$ (c 1.3, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10 (d, $J=8.4$ Hz, 2H), 6.82 (d, $J=8.4$ Hz, 2H), 5.68–5.52 (m, 1H), 4.92–4.79 (m, 2H), 4.25–4.10 (m, 3H), 4.03–3.90 (m, 2H), 3.80 (s, 3H), 3.40–3.10 (m, 2H), 2.17–1.95 (m, 2H), 1.60–1.45 (m, 15H), 0.80 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.4, 134.5, 131.0, 116.8, 113.3, 69.5, 60.3, 60.0, 55.2, 52.0, 48.5, 35.7, 28.3, 25.3, 24.4, 14.3 ppm. EIMS (*m*/*z*, %): 447 (M^+, 0.35). IR (film): 2980, 1697, 1391, 1180, 1037, 840, 566 cm^{-1}. Anal. for $C_{25}H_{37}NO_6$. Calcd: C, 67.09; H, 8.33; N, 3.13. Found: C, 67.35; H, 8.35; N, 3.05.

4.1.13. (1*R*,2*S*,4*R*)-4-[2-Hydroxymethyl-1-(4-methoxyphenyl)-pent-4-enyl]-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (18). To suspension of $LiAlH_4$ (1.83 g, 46.0 mmol) in dry THF (250 mL) at 0 °C, was added a solution of ester **17** (20.5 g, 46.0 mmol) in dry THF, the mixture was stirred at 0 °C for 1.5 h. Water (5.0 mL) was added dropwise to the reaction mixture, and the mixture was stirred vigorously until becoming white. The mixture was dried with anhydrous Na_2SO_4, filtered and concentrated; the residue was purified by flash column chromatography to give a colorless oil (17.3 g) in 93% yield. $[\alpha]_D^{20}=-70.1$ (c 1.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.09 (d, $J=8.4$ Hz, 2H), 6.79 (d, $J=8.4$ Hz, 2H), 5.76–5.70 (m, 1H), 4.98–4.84 (m, 2H), 4.60–4.43 (m, 1H), 4.08 (m, 1H), 3.99–3.90 (m, 1H), 3.80 (s, 3H), 3.80–3.72 (m, 1H), 3.60–3.50 (m, 1H), 3.40–3.28 (m, 1H), 2.86 (m, 1H), 2.28 (m, 1H), 2.05 (m, 1H), 1.68 (m, 1H), 1.50–1.30 (m, 12H), 0.90 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.2, 136.6, 131.9, 131.0, 116.4, 113.2, 94.4, 80.2, 68.9, 64.0, 58.3, 55.1, 42.8, 34.8, 28.3, 25.4, 24.5 ppm. EIMS (*m*/*z*, %): 407 (0.48), 308 (0.63). IR (film): 3462, 2978, 1694, 1513, 1249, 1178, 1040, 842, 563 cm^{-1}. Anal. for $C_{23}H_{35}NO_5$. Calcd: C, 68.12; H, 8.70; N, 3.45. Found: C, 68.07; H, 8.88; N, 3.33.

4.1.14. (1*R*,2*S*,4*R*)-4-[2-(2,2-Dimethyl-propionyloxymethyl)-1-(4-methoxy-phenyl)-pent-4-enyl]-2,2-dimethyloxazolidine-3-carboxylic acid *tert*-butyl ester (19). To a solution of alcohol **18** (6.50 g, 16.0 mmol) in dry DCM (100 mL) at 0 °C was added Et_3N (13.5 mL, 96.0 mmol), DMAP (195 mg, 1.60 mmol) and PivCl (7.9 mL, 64.0 mmol), then the mixture was warmed to rt and stirred overnight. The reaction mixture was diluted with DCM, washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated; the resulted residue was purified by flash column chromatography to give a pale yellow oil (8.16 g) in 100% yield. $[\alpha]_D^{20}=-42.1$ (c 2.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, $J=8.7$ Hz, 2H), 6.80 (d, $J=8.7$ Hz, 2H), 5.70 (m, 1H), 5.00–4.90 (m, 2H), 4.45 (m, 1H), 4.30 (m, 1H), 4.05–3.84 (m, 3H), 3.80 (s, 3H), 2.98 (m, 1H), 2.38 (m, 1H), 2.17 (m, 1H), 1.73 (m, 1H), 1.60–1.00 (m, 24H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3, 158.3, 153.1, 136.1, 131.3/130.9, 130.7, 116.8, 113.0, 94.0, 79.7, 68.5, 64.0, 58.1, 55.1, 50.7, 40.0, 38.9, 33.5, 28.2, 27.2, 26.0, 24.6 ppm. EIMS (*m*/*z*, %): 491 (0.20), 490 (MH^+, 0.61). IR (film): 2977, 1695, 1366, 1163, 838, 561 cm^{-1}. HR-MALDI for $(C_{28}H_{43}NO_6+Na)^+$. Calcd: 512.2988. Found: 512.2957.

4.1.15. 2,2-Dimethyl-propionic acid 2*S*-[2*R*-*tert*-butoxycarbonylamino-3-hydroxy-1*R*-(4-methoxyphenyl)-propyl]-pent-4-enyl ester (20). To a solution of **19** (7.33 g, 15.0 mmol) in methanol (800 mL), was added PTSA·H_2O (86 mg, 0.45 mmol), and the mixture was stirred at rt overnight. Satd $NaHCO_3$ (20 mL) was added to the reaction mixture, then methanol was removed in vacuo, and the residue mixture was extracted with EtOAc; the organic phase was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give a pale yellow oil (5.68) in 84% yield. $[\alpha]_D^{20}=-51.8$ (c 1.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (d, $J=8.7$ Hz, 2H), 6.80 (d, $J=8.7$ Hz, 2H), 5.72–5.58 (m, 1H), 5.00–4.82 (m, 2H), 4.40–4.25 (m, 1H), 4.20–4.00 (m, 2H), 3.80 (s, 3H), 3.48–3.25 (m, 2H), 3.00 (m, 1H), 2.30–2.10 (m, 2H), 2.10–1.95 (m, 2H), 1.78 (m, 1H), 1.50–1.18 (m, 18H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3, 158.6, 156.2, 135.9, 130.2, 129.7, 117.0, 113.9, 79.6, 64.3, 63.4, 55.2, 52.0, 45.3, 38.9, 38.0, 32.8, 28.3, 27.2 ppm. EIMS (*m*/*z*, %): 350 (1.31). IR (KBr film): 3437, 2978, 1725, 1168, 1036, 838, 539 cm^{-1}. HR-MALDI for $(C_{25}H_{39}NO_6+Na)^+$. Calcd: 472.2675. Found: 472.2697.

4.1.16. 2,2-Dimethyl-propionic acid 2*S*-[*R*-(4-methoxy-phenyl)-*R*-(2-oxo-oxazolidin-4-yl)-methyl]-pent-4-enyl ester (21). To a suspension of NaH (60%, 1.10 g, 27.3 mmol) in dry DMF (50) at 0 °C, was added the solution of alcohol **20** (4.9 g, 10.9 mmol) in dry DMF (50 mL) dropwise, and the mixture was stirred for 30 min. Satd NH_4Cl (20 mL) was added to the reaction mixture, then DMF was removed in vacuo, and the residue mixture was extracted with EtOAc; the organic phase was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give a colorless oil (3.10 g) in 76% yield. $[\alpha]_D^{20}=+3.91$ (*c* 1.2, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.10 (d, $J=8.4$ Hz, 2H), 6.90 (d, $J=8.4$ Hz, 2H), 5.70–5.60 (m, 2H), 5.10–4.90 (m, 2H), 4.50 (t, $J=8.4$ Hz, 1H), 4.40–4.33 (m, 1H), 4.11–4.00 (m, 2H), 3.93 (dd, $J=12.0$, 6.3 Hz, 1H), 3.80 (s, 3H), 2.80–2.76 (m, 1H), 2.15–2.00 (m, 2H), 1.77–1.70 (m, 1H), 1.25 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3, 159.0, 135.0, 130.1, 128.5, 117.7, 114.3, 68.8, 64.0, 55.2, 53.5, 49.9, 38.9, 32.8 27.2 ppm. EIMS (*m*/*z*, %): 291 (3.33), 290 (17.04), 289 (8.13). IR (film): 3280, 2974, 1751, 1162, 1034, 769, 553 cm^{-1}.

4.1.17. 4*R*-[2*S*-(2,2-Dimethyl-propionyloxymethyl)-1*R*-(4-methoxy-phenyl)-pent-4-enyl]-2-oxo-oxazolidine-3-carboxylic acid *tert*-butyl ester (22). To a solution of **21** (6.3 g, 16.8 mmol) in dry DCM (300 mL) at 0 °C, was added Et_3N (8.2 mL, 58.5 mmol), DMAP (100 mg, 0.82 mmol) and $(Boc)_2O$ (10.8 g, 49.5 mmol), the mixture was warmed to rt and stirred overnight. The reaction mixture was washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated; the residue was purified by flash column chromatography to give a colorless oil (7.40 g) in 93% yield. $[\alpha]_D^{20}=-99.9$ (*c* 2.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.01 (d, $J=8.4$ Hz, 2H), 6.80 (d, $J=8.1$ Hz, 2H), 5.60 (m, 1H), 5.00–4.75 (m, 3H), 4.54 (dd, $J=11.1$, 3.9 Hz, 1H), 4.30 (m, 1H), 4.15 (m, 1H), 4.02 (m, 1H), 3.75 (s, 3H), 2.70 (dd, $J=10.8$, 2.7 Hz, 1H), 2.30 (m, 1H), 1.94 (m, 1H), 1.75 (m, 1H), 1.52 (s, 9H), 1.20 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3, 159.0, 151.8, 150.2, 135.2, 130.3, 127.4, 117.4, 114.1, 84.1, 67.2, 62.9, 55.0, 54.6, 51.1, 38.9, 38.6, 33.7, 27.8, 27.2 ppm. ESIMS (*m*/*z*, %): 498.2 [$(M+Na)^+$, 10], 376.2 [$(MH-100)^+$, 40]. IR (KBr film): 2978, 1819, 1154, 1062, 776, 566 cm^{-1}. Anal. for $C_{26}H_{37}NO_7$. Calcd: C, 65.66; H, 7.84; N, 2.95. Found: C, 65.69; H, 7.64; N, 2.88.

4.1.18. 4*R*-[3-Carboxy-2*S*-(2,2-dimethyl-propionyloxy-methyl)-1*R*-(4-methoxy-phenyl)-propyl]-2-oxo-oxazolidine-3-carboxylic acid *tert*-butyl ester (23). O_3 was bulbed through the solution of **22** (1.50 g, 3.15 mmol) in DCM (40 mL) and methanol (10 mL) at −78 °C until pale blue (about 2 h), then Me_2S (10 mL) was added; the mixture was warmed to rt during 2 h and concentrated to a colorless oil which was used directly. To the solution of the above residue in ^{t}BuOH (50 mL) and H_2O (10 mL) at 0 °C, was added KH_2PO_4 (643 mg, 4.73 mmol) and 2-methyl-2-butene (1.34 mL, 12.6 mmol), $NaClO_2$ (860 mg, 9.46 mmol, in three portions); the mixture was warmed to rt and stirred overnight. Satd $Na_2S_2O_3$ (20 mL) was added to the reaction mixture and ^{t}BuOH was removed in vacuo, the residue aqueous was acidified to pH 3–4 and extracted by DCM, the organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give a white wax (1.30 g) in 84% yield. $[\alpha]_D^{20}=-80.3$ (*c* 2.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.04 (m, 2H), 6.80 (m, 2H), 4.84 (d, $J=8.4$ Hz, 1H), 4.63 (dd, $J=11.7$, 4.5 Hz, 1H), 4.35 (t, $J=8.6$ Hz, 1H), 4.27 (dd, $J=12.0$, 3.0 Hz, 1H), 4.03 (dd, $J=12.0$, 1.8 Hz, 1H), 3.78 (s, 3H), 2.84 (m, 1H), 2.74 (dd, $J=11.6$, 2.0 Hz, 1H), 2.15 (m, 2H), 1.56 (s, 9H), 1.23 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.4, 177.8, 159.4, 151.8, 150.2, 130.3, 126.6, 114.5, 84.5, 67.1, 63.8, 55.1, 54.2, 51.0, 39.0, 35.9, 34.5, 27.9, 27.2 ppm. ESIMS (*m*/*z*, %): 516.3 [$(M+Na)^+$, 10], 394.2 [$(MH-100)^+$, 50]. IR (film): 3224, 2979, 1813, 1726, 1156, 913, 734, 648 cm^{-1}. Anal. for $C_{25}H_{35}NO_9$. Calcd: C, 60.84; H, 7.15; N, 2.84. Found: C, 60.89; H, 7.22; N, 2.78.

4.1.19. 2,2-Dimethyl-propionic acid 6-methoxy-4-oxo-1*R*-(2-oxo-oxazolidin-4*R*-yl)-1*R*,2*R*,3,4-tetrahydro-naphthalen-2-ylmethyl ester (24). To a solution of acid **23** (1.25 g, 2.54 mmol) in DCM (5.0 mL) and CS_2 (30 mL) at rt, was added $(COCl)_2$ (0.67 mL, 7.61 mmol) and DMF (60 μL, 0.76 mmol), and the mixture was stirred at rt for 2 h. The solvent was removed in vacuo and the residue was redissovled in dry DCM (60 mL) and protected under N_2 as soon as possible, the solution was cooled to 0 °C before $AlCl_3$ (1.19 g, 8.89 mmol) was added, the reaction mixture was warmed to rt and stirred overnight. The mixture was poured into a mixture was ice and water and extracted with DCM, the organic phase was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give a white wax (0.70 g) in 74% yield. $[\alpha]_D^{20}=+62.4$ (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.41 (d, $J=2.7$ Hz, 1H), 7.15 (d, $J=8.4$ Hz, 1H), 7.04 (dd, $J=8.4$, 2.7 Hz, 1H), 5.85 (s, 1H), 4.52 (t, $J=8.4$ Hz, 1H), 4.17 (dd, $J=9.0$, 6.0 Hz, 1H), 4.00 (m, 1H), 3.89 (m, 2H), 3.75 (s, 3H), 2.87 (d, $J=9.0$ Hz, 1H), 2.80 (dd, $J=19.2$, 6.0 Hz, 1H), 2.55 (d, $J=19.2$ Hz, 1H), 2.42 (m, 1H), 1.06 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.6, 178.0, 159.4, 158.9, 132.5, 131.7, 121.8, 110.2, 69.1, 65.7, 55.4, 55.3, 44.6, 38.6, 35.5, 34.0, 26.9 ppm. EIMS (*m*/*z*, %): 375 (M^+, 0.62). IR (film): 3334, 2970, 1683, 1284, 1156, 1033, 769, 532 cm^{-1}. Anal. for $C_{20}H_{25}NO_6$. Calcd: C, 63.99; H, 6.71; N, 3.73. Found: C, 64.43; H, 7.03; N, 3.58. ee% = 99.13% (measured by HPLC).

4.1.20. 4*R*-[2*R*-(2,2-Dimethyl-propionyloxymethyl)-6-methoxy-4-oxo-1*R*,2*R*,3,4-tetrahydro-naphthalen-1-yl]-2-oxooxazolidine-3-carboxylic acid *tert*-butyl ester (26). To a solution of ketone **24** (1.00 g, 2.66 mmol) in dry DCM (30 mL) at 0 °C, was added Et_3N (1.2 mL, 8.56 mmol), DMAP (5 mg, 0.041 mmol) and $(Boc)_2O$ (0.70 g, 3.21 mmol), the mixture was warmed to rt and stirred overnight. The reaction mixture was washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated; the residue was purified by flash column chromatography to give a colorless oil (1.20 g) in 95% yield. $[\alpha]_D^{20}=+27.2$ (*c* 1.1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.55 (d, $J=2.7$ Hz, 1H), 7.18–7.10 (m, 2H), 4.59–4.53 (m, 1H), 4.39–4.33 (m, 1H), 4.16–4.10 (m, 1H), 4.05–3.90 (m, 2H), 3.84 (s, 3H), 3.23 (d, $J=8.7$ Hz, 1H), 2.92 (dd, $J=18.6$, 5.7 Hz, 1H), 2.69 (d, $J=18.3$ Hz, 1H), 2.58–2.51 (m, 1H), 1.24 (s, 9H), 1.11 (s, 9H) ppm. ^{13}C NMR (75 MHz,

$CDCl_3$): δ 194.7, 178.1, 159.6, 151.8, 148.4, 133.2, 131.9, 131.0, 122.2, 110.0, 84.1, 66.6, 66.0, 56.9, 55.6, 41.9, 38.7, 35.9, 34.0, 27.5, 27.0 ppm. ESIMS (*m*/*z*, %): 498.2 [(M+Na)$^+$, 15], 476.2 [(MH)$^+$, 100]. IR (film): 2978, 1816, 1728, 1284, 1156, 732, 550 cm^{-1}. Anal. for $C_{25}H_{33}NO_8$. Calcd: C, 63.14; H, 6.99; N, 2.95. Found: C, 63.23; H, 7.02; N, 2.88.

4.1.21. [2-Hydroxy-1*R*-(2*R*-hydroxymethyl-6-methoxy-4-oxo-1*R*,2*R*,3,4-tetrahydro-naphthalen-1-yl)-ethyl]-carbamic acid *tert*-butyl ester (27). To a solution of ketone **26** (1.10 g, 2.31 mmol) in methanol (50 mL), was added Cs_2CO_3 (1.65 g, 5.05 mmol), the mixture was stirred at rt overnight. Satd NaCl (30 mL) was added to the reaction mixture, and methanol was removed in vacuo, the residue was extracted with DCM; the organic layer was washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give a white wax (0.75 g) in 86% yield. $[\alpha]_D^{20}=+54.1$ (*c* 1.2, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.48 (d, *J*=2.7 Hz, 1H), 7.20 (d, *J*=8.4 Hz, 1H), 7.05 (dd, *J*=8.4, 2.7 Hz, 1H), 4.85 (d, *J*=9.6 Hz, 1H), 4.00–3.60 (m, 7H), 3.55–3.30 (m, 3H), 3.05–2.80 (m, 2H), 2.50 (m, 2H), 1.26 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.0, 158.9, 155.8, 133.4, 133.3, 132.4, 121.5, 109.4, 79.9, 64.8, 63.0, 55.5, 39.3, 38.6, 36.4, 29.6, 28.2 ppm. EIMS (*m*/*z*, %): 310 (1.10), 292 (3.39), 266 (1.09). ESIMS (*m*/*z*, %): 753 [(2M+Na)$^+$, 25], 731.4 [(2M+H)$^+$, 35], 310 [($MH_2-C_4H_9$)$^+$, 100], 266 [(MH−100)$^+$, 8]. IR (film): 3360, 2927, 1683, 1035, 875, 533 cm^{-1}.

4.1.22. 2,2-Dimethyl-propionic acid 2*R*-*tert*-butoxycarbonylamino-2*R*-[2-(2,2-dimethylpropionyloxymethyl)-6-methoxy-4-oxo-1*R*,2*R*,3,4-tetrahydro-naphthalen-1-yl]-ethyl ester (28). To a solution of diol **27** (0.66 g, 1.80 mmol) in dry DCM (30 mL) at 0 °C was added Et_3N (2.50 mL, 18.0 mmol), DMAP (5 mg, 0.041 mmol) and PivCl (1.80 mL, 14.5 mmol), then the mixture was warmed to rt and stirred overnight. The reaction mixture was diluted with DCM, washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated; the resulted residue was purified by flash column chromatography to give a pale yellow oil (0.74 g) in 76% yield. $[\alpha]_D^{20}=+21.3$ (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (s, 1H), 7.01 (m, 2H), 4.32–4.00 (m, 2H), 4.15–3.95 (m, 2H), 3.83 (d, *J*=6.9 Hz, 2H), 3.77 (s, 3H), 3.00–2.90 (m, 2H), 2.70–2.62 (m, 1H), 2.49 (m, 1H), 1.25 (s, 9H), 1.15 (s, 9H), 1.05 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.0, 177.7, 177.5, 158.7, 154.6, 133.4, 131.1, 131.0, 121.1, 109.2, 79.4, 66.4, 63.8, 59.8, 54.9, 53.1, 40.4, 38.3, 38.1, 36.2, 35.6, 27.6, 26.6, 26.5, 20.5, 13.6 ppm. ESIMS (*m*/*z*, %): 556.3 (M+Na)$^+$. IR (film): 3363, 2977, 1731, 1284, 1154, 1035, 877, 771, 537 cm^{-1}. HR-ESIMS for ($C_{29}H_{43}NO_8$+Na)$^+$. Calcd: 556.2886. Found: 556.2878.

4.1.23. (1*R*, 9*S*,12*R*, 13*R*)-12,13-Bis-(2,2-dimethylpropionyloxymethyl)-5-methoxy-8-oxo-11-azatricyclo[7.3.1.02,7]trideca-2(7),3,5-triene-11-carboxylic acid methyl ester (29). To a solution of **28** (0.66 g, 1.24 mmol) in DCM (10 mL), was added TFA (10 mL); after 2 h at rt, the mixture was concentrated to a pale yellow oil. To the solution of the above yellow oil in ethanol (30 mL), was added HCHO (36%, 0.50 mL, 6.00 mmol) and the mixture was refluxed for 2 h. The solvent was removed in vacuo and the residue was redissolved in dioxane (10 mL), water (5.0 mL) and sat. $NaHCO_3$ (5.0 mL), ClCOOMe (0.12 mL, 1.55 mmol) was added and the mixture was stirred at rt for 2 h. Dioxane was removed in vacuo and the residue was extracted with EtOAc, the organic layer was washed by water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The resulted residue was purified by flash column chromatography to give a colorless oil (0.72 g) in 100% yield. $[\alpha]_D^{20}=+51.2$ (*c* 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.50 (d, *J*=2.7 Hz, 1H), 7.19 (d, *J*=8.7 Hz, 1H), 7.14 (dd, *J*=8.7, 2.7 Hz, 1H), 4.64–4.55 (m, 2H), 4.47 (dd, *J*=12.0, 9.6 Hz, 1H), 3.96–3.80 (m, 5H), 3.70 (ddd, *J*=9.6, 4.5, 1.5 Hz, 1H), 3.26 (s, 3H), 3.21 (m, 2H), 2.67 (m, 1H), 2.60 (m, 1H), 1.27 (s, 9H), 1.15 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.7, 178.4, 178.3, 159.9, 155.8, 134.9, 132.0, 131.1, 122.6, 109.5, 64.8, 63.0, 62.1, 55.9, 52.7, 50.7, 45.4, 45.2, 39.2, 38.8, 31.9, 27.5, 27.3, 23.0 ppm. ESIMS (*m*/*z*, %): 526.4 (M+Na)$^+$, 542.4 (M+K)$^+$. IR (film): 2974, 1730, 1284, 1159, 764, 532 cm^{-1}. HR-ESIMS for ($C_{27}H_{37}NO_8$+Na)$^+$. Calcd: 526.2417. Found: 526.2412.

4.1.24. (1*R*,9*S*,12*R*,13*S*)-12,13-Bis-(2,2-dimethylpropionyloxymethyl)-5-methoxy-11-azatricyclo[7.3.1.02,7]-trideca-2(7),3,5-triene-11-carboxylic acid methyl ester (30). To a solution of ketone **29** (0.70 g, 1.39 mmol) in methanol (20 mL) at 0 °C, was added $NaBH_4$ (53 mg, 1.39 mmol); satd NH_4Cl was added after 10 min and the mixture was extracted with EtOAc; the organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the resulting residue was purified by flash column chromatography to give a white wax (642 mg) (contaminated by inseparable material). To a suspension of NaH (60%, 253 mg, 6.33 mmol) in dry THF (15.0 mL) at 0 °C, was added the solution of the above white wax in dry THF (5 mL), CS_2 (0.77 mL, 12.7 mmol) was added after 30 min. The mixture was warmed to rt and stirred for 60 min before MeI (0.79 mL, 12.7 mmol) was added, then stirred for another 2 h. Satd NH_4Cl was added and the mixture was extracted with ether. The organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give white wax (553 mg) and ketone **29** (154 mg), in 86% modified yield for two steps, and 78% conversion for the first step.

To the solution of the above product (553 mg) in dry toluene (30 mL), was added AIBN (30 mg, 0.19 mmol) and *n*-Bu_3SnH (0.50 mL, 1.86 mmol) under N_2, the mixture was refluxed for 3 h. Solvent was removed in vacuo and the residue was purified by flash column chromatography directly to give a white wax (450 mg) in 100% yield. $[\alpha]_D^{20}=-4.50$ (*c* 1.2, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 6.93 (d, *J*=8.7 Hz, 1H), 6.68 (dd, *J*=8.7, 2.7 Hz, 1H), 6.60 (d, *J*=2.4 Hz, 1H), 4.37 (dd, *J*=11.4, 5.7 Hz, 1H), 4.29 (dd, *J*=11.4, 9.0 Hz, 1H), 4.20 (dd, *J*=13.5, 3.0 Hz, 1H), 3.90 (dd, *J*=11.1, 7.2 Hz, 1H), 3.81 (dd, *J*=11.1, 8.1 Hz, 1H), 3.75 (s, 3H), 3.62 (m, 1H), 3.30–3.22 (m, 4H), 3.00 (s, 1H), 2.90–2.80 (m, 2H), 2.30 (m, 1H), 2.17 (m, 1H), 1.23 (s. 9H), 1.18 (s, 9H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 140.0, 138.0, 133.3, 130.3, 128.9, 128.5, 128.3, 127.3, 72.3, 60.8, 56.6, 55.2, 26.0, 22.4, 22.1, 20.6 ppm. EIMS (*m*/*z*, %): 489 (M$^+$, 2.17). IR (film): 2974, 1729, 1160, 1037, 733, 454 cm^{-1}. HR-MALDI for $(C_{27}H_{39}NO_7+Na)^+$. Calcd: 512.2624. Found: 512.2604.

4.1.25. (1*R*,9*S*,12*R*,13*S*)-5-Methoxy-11-azatricyclo-[7.3.1.02,7]trideca-2(7),3,5-triene-11,12,13-tricarboxylic acid trimethyl ester (31). The procedure was the same as the preparation of compound **34**. From material **30** (125 mg), **31** (61 mg) was obtained in 3% yield for four steps. $[\alpha]_D^{20}=+72.6$ (*c* 1.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.02 (d, $J=8.5$ Hz, 1H), 6.65 (dd, $J=8.5$, 2.6 Hz, 1H), 6.55 (d, $J=2.5$ Hz, 1H), 4.50 (d, $J=6.2$ Hz, 1H), 4.00 (br s, 1H), 3.87 (d, $J=6.0$ Hz, 1H), 3.80 (s, 3H), 3.67–3.50 (m, 9H), 3.45 (m, 1H), 3.05 (dd, $J=17.5$, 5.0 Hz, 1H), 2.87 (m, 1H), 2.80 (m, 1H), 2.67 (d, $J=17.6$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2, 170.2, 158.9, 135.7, 131.4, 125.4, 113.6, 112.3, 62.2, 55.1, 52.9, 52.0, 51.6, 42.7, 35.6, 31.8, 29.7, 28.2 ppm. EIMS (*m/z*, %): 378 (MH^+, 3.41), 3.77 (M^+, 20.30), 318 [$(M-59)^+$, 100]. IR (film): 2954, 1734, 1709, 1257, 1198, 778, 531 cm^{-1}.

4.1.26. (1*R*,9*S*,12*S*,13*S*)5-Methoxy-11-azatricyclo-[7.3.1.02,7]trideca-2(7),3,5-triene-11,12,13-tricarboxylic acid trimethyl ester (33). To a solution of diester **31** (5 mg) in methanol (5.0 mL) at rt, was added NaOMe (3 mg); sat. NH_4Cl was added after 10 min. The mixture was extracted with EtOAc, the organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give a colorless oil (5 mg) in 100% yield. $[\alpha]_D^{20}=+14.1$ (*c* 0.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.10 (m, 1H), 6.68 (m, 1H), 6.53 (m, 1H), 4.75 (d, $J=3.0$ Hz, 0.5H), 4.61 (d, $J=2.1$ Hz, 0.5H), 4.25 (dd, $J=13.5$, 1.5 Hz, 0.5H), 4.10 (dd, $J=13.5$, 1.5 Hz, 0.5H), 3.90 (d, $J=1.2$ Hz, 0.5H), 3.86 (d, $J=1.2$ Hz, 0.5H), 3.80 (s, 3H), 3.75 (s, 3H), 3.64–3.35 (m, 7H), 3.20 (m, 1H), 2.78–2.55 (m, 3H) ppm.

4.1.27. (1*R*,9*S*,12*R*,13*R*)-5-Methoxy-8-oxo-11-azatricyclo-[7.3.1.02,7]trideca-2(7),3,5-triene-11,12,13-tricarboxylic acid trimethyl ester (34). To a solution of ketone **29** (250 mg, 0.50 mmol) in dry DCM (20 mL) at −78 °C, was added DIBAL-H (1.0 in cyclohexane, 7.5 mL, 7.5 mmol), 1 N HCl (20 mL) was added after 60 min. The mixture was extracted with DCM, washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was used directly. To a solution of the above residue in DCM (20 mL), was added Dess–Martin Periodinane (2.50 g, 5.91 mmol), and the mixture was stirred at rt for 30 min before sat. $Na_2S_2O_3$ and sat. $NaHCO_3$ were added. The reaction mixture was extracted with DCM, the organic layer was washed by brine, dried over Na_2SO_4, filtered and concentrated to a residue which was redissoved in ^{t}BuOH (5.0 mL) and H_2O (1.0 mL); to this solution was added KH_2PO_4 (408 mg, 3.00 mmol), 2-methyl-2-butane (0.85 mL, 8.00 mmol) and $NaClO_2$ (543 mg, 6.00 mmol), the mixture was stirred at rt for 60 min before sat. $Na_2S_2O_3$ was added. The solvent was removed in vacuo, the residue was redissoved in $CHCl_3$ and dried over Na_2SO_4, filtered and concentrated. The residue was redissolved in Et_2O (30 mL) and excess CH_2N_2 (in ether) was added at 0 °C, the mixture was stirred at rt for 30 min and concentrated. The residue was purified by flash column chromatography directly to give a colorless oil (100 mg) in 50% yield for four steps. $[\alpha]_D^{20}=+51.2$ (*c* 1.3, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (d, $J=2.8$ Hz, 1H), 7.26 (d, $J=8.5$ Hz, 1H), 7.05 (dd, $J=8.5$, 2.8 Hz, 1H), 4.35 (dd, $J=12.3$, 2.6 Hz, 1H), 4.25 (d, $J=3.7$ Hz, 1H), 4.00 (s, 1H), 3.80 (s, 3H), 3.55 (s, 3H), 3.52 (s, 3H), 3.50–3.38 (m, 4H), 3.20–3.09 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.1, 171.4, 169.0, 159.7, 157.2, 133.1, 131.7, 131.0, 122.0, 108.7, 61.9, 55.4, 53.1, 52.5, 51.9, 48.0, 47.6, 42.8, 38.3 ppm. EIMS (*m/z*, %): 392 (MH^+, 2.81), 391 (M^+, 11.19), 332 [$(M-89)^+$, 100]. IR (film): 2955, 1713, 1234, 1037 cm^{-1}. HR-MALDI for $(C_{19}H_{21}NO_8+Na)^+$. Calcd: 414.1159. Found: 414.1112.

4.1.28. (1*R*,9*S*,12*S*,13*R*)5-Methoxy-8-oxo-11-azatricyclo-[7.3.1.02,7]trideca-2(7),3,5-triene-11,12,13-tricarboxylic acid trimethyl ester (36). The procedure was similar to the preparation of compound **33**. From material **34** (25 mg), **36** was obtained (20 mg) in 80% yield. (The physical data are shown in the preparation of **35** and **36**).

4.1.29. (1*R*,9*S*,12*S*,13*S*/*R*)-5-Methoxy-8-oxo-11-azatricyclo[7.3.1.02,7]trideca-2(7),3,5-triene-11,12,13-tricarboxylic acid trimethyl ester [35(13*S*) and 36(13*R*)]. To a solution of LDA (2.0 M in THF, 1.05 mL, 2.10 mmol) in dry THF (5.0 mL) at −78 °C, was added HMPA (0.6 mL, 3.31 mmol), after 30 min the solution of ketone **34** (55 mg, 0.14 mmol) in THF (5.0 mL) was added dropwise. The mixture was stirred at −78 °C for additional 30 min before acetic acid (0.14 mL, 2.10 mmol) was added, then warmed to rt and quenched by satd NH_4Cl. The reaction mixture was extracted by ether, the organic layer was washed by brine, dried over anhydrous Na_2SO_4, filtered and concentrated, the residue was purified by flash column chromatography to give **35** as a colorless oil (20 mg) in 36% yield, and **36** as a pale yellow oil (20 mg) in 36% yield. Date for **35**: $[\alpha]_D^{20}=+48.4$ (*c* 0.3, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 1H), 7.35 (m, 1H), 7.12 (m, 1H), 4.60 (s, 0.6H), 4.50 (s, 0.4H), 4.30–4.05 (m, 2H), 3.90–3.70 (m, 10H), 3.60–3.45 (m, 3H), 3.25–3.10 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.5, 170.8, 170.7, 159.5, 157.1/156.5, 137.0, 133.4/133.2, 129.1/128.8, 123.0/122.8, 109.5/109.4, 58.0, 57.2, 55.6, 53.2/53.1, 52.5/52.4, 42.7, 42.1, 41.9/41.6, 37.4/37.3 ppm. EIMS (*m/z*, %): 391 (M^+, 1.57), [332 $(M-59)^+$, 100]. ESIMS (*m/z*, %): 392.2 (MH^+). IR (film): 2957, 1740, 1710, 1447, 1234, 1025, 778, 531 cm^{-1}. HR-MALDI for $(C_{19}H_{21}NO_8+Na)^+$. Calcd: 414.1159. Found: 414.1153. Date for **36**: $[\alpha]_D^{20}=+36.3$ (*c* 0.7, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.45 (m, 1H), 7.30 (m, 1H), 7.10 (m, 1H), 4.86 (d, $J=2.0$ Hz, 0.7H), 4.72 (d, $J=2.0$ Hz, 0.3H), 4.45 (dd, $J=13.4$, 2.0 Hz, 0.3H), 4.35 (dd, $J=13.4$, 3.1 Hz, 0.7H), 4.12–4.04 (m, 1H), 3.85–3.55 (m, 6H), 3.55–3.45 (m, 7H), 3.13–3.00 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.7/196.1, 171.8/171.7, 170.3, 159.5/159.4, 156.9/156.3, 133.5/133.3, 133.1, 129.7/129.4, 122.8, 108.9/108.8, 60.5/59.8, 55.5, 53.2/53.1, 52.9, 52.4, 45.4/45.1, 44.1/44.0, 42.8/42.7, 38.1 ppm. EIMS (*m/z*, %): 392 (MH^+, 2.15), 391 (M^+, 9.47), [332 $(M-59)^+$, 100]. IR (film): 1739, 1224, 1031, 771, 530 cm^{-1}.

4.1.30. (1*R*,9*S*,12*S*,13*R*)-5-Methoxy-11-azatricyclo-[7.3.1.02,7]trideca-2(7),3,5-triene-11,12,13-tricarboxylic acid trimethyl ester (32). To a solution of **35** (20 mg, 0.051 mmol) in dry CH_2Cl_2 (5.0 mL) at 0 °C, was added $BF_3\cdot Et_2O$ (20 μL, 0.16 mmol) and 1,3-propanedithiol

(40 μL, 0.40 mmol). After stirring at rt for 48 h, the reaction was quenched with sat. $NaHCO_3$ and the mixture was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography to give the sulfer-ketal as a colorless oil (20 mg). To a solution of sulfer-ketal (20 mg) in EtOH (15 mL), was added Raney-nickel (about 0.5 g). After 40 min, Raney-Nickel was filtered through a pad of celite and the filtrate was concentrated. The residue was redissoved in EtOAc and washed with water and brine. The organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography to give a colorless oil (14 mg) in 73% yield for two steps. $[\alpha]_D^{20} = +3.13$ (*c* 0.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.17–7.12 (m, 1H), 6.74–6.70 (m, 1H), 6.61 (s, 1H), 4.56 (s, 0.5H), 4.43 (s, 0.5H), 4.10 (m, 0.5H), 3.95–3.90 (m, 2H), 3.80–3.70 (m, 9.5H), 3.60–3.40 (m, 3H), 3.20 (m, 1H), 2.98–2.82 (m, 1H), 2.80 (s, 1H), 2.68 (m, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.6, 171.2/171.1, 158.6, 156.8, 137.3/136.9, 130.5, 128.9/128.6, 113.0, 112.6/112.1, 59.4, 59.0, 55.2, 52.9/52.8, 52.2/52.0, 45.2/44.9, 41.2/41.1, 36.9/36.7, 35.2/35.1, 27.8/27.7 ppm. EIMS (*m*/*z*, %): 378 (MH^+, 1.12), 377 (M^+, 5.36), 318 [$(M-59)^+$, 100]. ESIMS (*m*/*z*, %): 400.10 $(M+Na)^+$, 378.10 $(M+H)^+$, 318 $(M-59)^+$. IR (KBr film): 2956, 1741, 1706, 1226, 1120, 776, 530 cm^{-1}.

4.1.31. (1*R*,9*S*,12*S*,13*R*)-5-hydroxy-11-azatricyclo-[7.3.1.02,7]trideca-2(7),3,5-triene-12,13-dicarboxylic acid hydrochloride salt (3). Compound **32** (14 mg, 0.037 mmol) was refluxed with HCl (6 N, 10 mL) for 4 days. The mixture was concentrated in vacuo to give **3** and *epi*-**3** as a white solid (10 mg) in 85% yield. $[\alpha]_D^{20} = +30.5$ (*c* 0.3, H_2O). ^1H NMR (300 MHz, $CDCl_3$): δ 7.00 (d, $J=7.8$ Hz, 0.6H), 6.80 (d, $J=8.1$ Hz, 0.4H), 6.60–6.43 (m, 2H), 4.10 (m, 1H), 3.95–3.68 (m, 1.4H), 3.45 (d, $J=12.6$ Hz, 0.6H), 3.23–3.00 (m, 2H), 2.95–2.50 (m, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.9, 177.4, 172.9, 172.1, 158.2, 157.8, 143.0, 140.5, 139.2, 133.3, 133.1, 129.3, 127.5, 117.9, 117.6, 116.8, 63.7, 60.3, 51.0, 49.2, 44.4, 41.6, 37.7, 37.5, 36.4, 32.0, 29.3, 28.9 ppm. ESIMS (*m*/*z*, %): 278.1 $(M+H)^+$. IR (KBr): 3375, 3131, 1720, 1403, 1214, 824 cm^{-1}. HR-ESIMS for $(C_{14}H_{15}NO_5+H)^+$. Calcd: 278.1028. Found: 278.1021.

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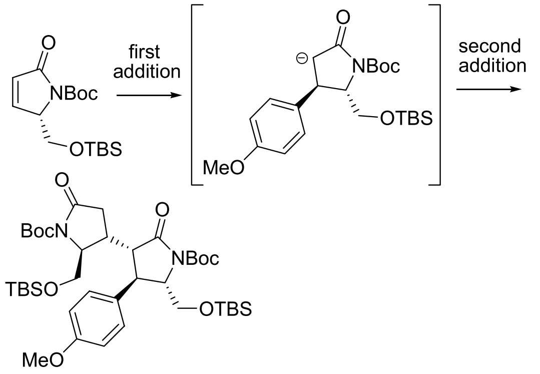
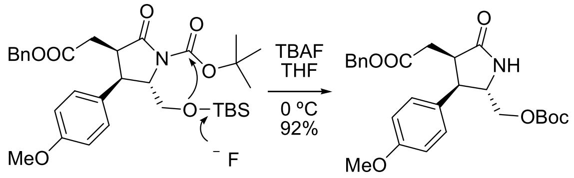
References and notes

1. Burke, T. R., Jr.; Barchi, J. J., Jr.; George, C.; Wolf, G.; Shoelson, S. E.; Yan, X. *J. Med. Chem.* **1995**, *38*, 1386–1396.
2. Josien, H.; Lavielle, S.; Brunissen, A.; Saffroy, M.; Torrens, Y.; Beaujouan, J. C.; Glowinski, J.; Chassaing, G. *J. Med. Chem.* **1994**, *37*, 1586–1601.
3. (a) Jiao, D.; Russell, K. C.; Hruby, V. J. *Tetrahedron* **1993**, *49*, 3511–3520. (b) Mosberg, H. I.; Lomize, A. L.; Wang, C. G.; Kroona, H.; Heyl, D. L.; Sobczykkojiro, K.; Ma, W. L.; Mousigian, C.; Porreca, F. *J. Med. Chem.* **1994**, *37*, 4371–4383. (c) Obrecht, D.; Lehmann, C.; Ruffieux, R.; Schonholzer, P.; Muller, K. *Helv. Chim. Acta* **1995**, *78*, 1567–1587.
4. (a) Birge, R. B.; Hanafusa, H. *Science* **1993**, *262*, 522. (b) Tong, L.; Warren, T. C.; King, J.; Betageri, R.; Jakes, S. *J. Mol. Biol.* **1996**, *256*, 601.
5. Wang, X.-Z.; Yao, Z.-J.; Liu, H.; Zhang, M.; Yang, D.; George, C.; Burke, R. T., Jr. *Tetrahedron* **2003**, *59*, 6087.
6. Ye, B.; Yao, Z.; Burke, T. R., Jr. *J. Org. Chem.* **1997**, *62*, 5428.
7. Liu, F.; Jiao, J.; Zha, H.; Yao, Z. *Chin. J. Chem.* **2004**, *22*, 1022–1028.
8. Liu, F.; Zha, H-Y. ; Yao, Z.-J. *J. Org. Chem.* **2003**, *68*, 6679.
9. (a) Otsuka, M.; Masuda, T.; Haupt, A.; Masaji, O. *J. Am. Chem. Soc.* **1990**, *112*, 838–845. (b) Acevedo, C. M.; Kogut, E. F.; Lipton, M. A. *Tetrahedron* **2001**, *57*, 6353–6359.
10. Herdeis, C.; Hubmann, H. P. *Tetrahedron: Asymmetry* **1994**, *3*, 351–354.
11. Formation of the two-time Michael addition product:



12. If **8** was treated with TBAF, Boc immigration occurred in high conversion.



13. CCDC 264692 & 264693 contain the supplementary crystallographic data for compounds **25** and **11**, respectively, in this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
14. The Wittig reagent was prepared by treating the corresponding phosphonium salt with aq. NaOH solution. If the excess NaOH was not washed off completely, the olefin product of the Witting reaction maybe partly racemize because of the base.

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Tetrahedron

1,3-Dipolar cycloadditions of MeOPEG-bounded azides

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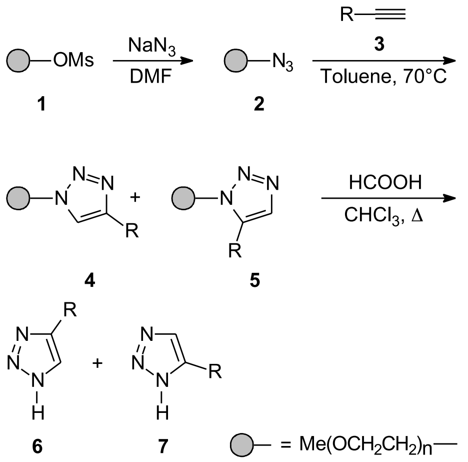
Abstract—1,3-Dipolar cycloadditions of MeOPEG-supported azide **2** with a variety of dipolarophiles have been studied. 1-MeOPEG-supported 1,2,3-triazoles **4** and **5**, 1,2,3,4-tetrazoles **12** and aziridine **14** were obtained in nearly quantitative yields. The removal of the MeOPEG moiety from the 1,2,3-triazole nucleus was achieved by acidic cleavage of the cycloadduct mixtures **4** and **5** giving 4- and 5-substituted-1,2,3-triazoles **6** and **7**.

1. Introduction

1,3-Dipolar cycloaddition between organic azides and a variety of unsaturated derivatives represent a versatile method for the direct synthesis of substituted azoles.[1] Recently, it have been recognised that 1,2,3-triazoles, which are formed via azide-acetylene cycloaddition[2] are the ideal representative of 'click chemistry'.[3] Furthermore, a number of examples are concerned with the synthesis of the 1,2,3,4-tetrazole ring by azide cycloaddition onto the cyano group.[4] Due to their biological activity as anti-HIV[5] and anti-microbial[6] agents, as well as selective β_3 adrenergic receptor agonist,[7] new methods for the regio- and/or stereoselective synthesis of both 1,2,3-triazoles and 1,2,3,4-tetrazoles should be highly valuable. In this context, the two following points should be considered: (i) the polymer-supported synthesis of small heterocyclic molecules is the subject of intense research activity,[8] since it represent one of the most promising ways to generate small molecular libraries in the field of combinatorial chemistry,[9] (ii) very few examples of cycloaddition reactions involving resin-bounded azides to acetylenes have been reported,[10,11] while no examples are available onto the ethylenic dipolarophile or the cyano group. In the above-mentioned papers, however, the azide was prepared using Merrifield's,[10a] polystyrene[10b] or Wang's[11] resin. These are insoluble in organic media. Recently, soluble polymers have gained popularity amongst organic chemists due to several advantageous features. Analytical simplicity, high reactivity and low costs of the starting materials actually compete with that of usual solution chemistry,[12] while the easy reaction work up is one of the major advantages of solid-supported synthesis. Thus, after having demonstrated the feasibility of poly(ethylene glycol)-supported azide (MeOPEG-supported azide) cycloadditions towards acetylenic dipolarophiles,[13] we present here a systematic study on the behaviour of these kind of azides towards acetylenes, ethylenes and the cyano group as dipolarophiles.

2. Results and discussion

First, of all, we devised the monomethylether of poly(ethylene glycol) with a M_W of 5000 (MeOPEG) as the suitable soluble support. MeOPEG-supported azide **2** were easily obtained as previously reported[13] from MeOPEG-mesylate[14] (Scheme 1). Heating **2** in dry toluene in the presence of a large excess (10 mol equiv) of acetylenic



a: R = Ph, **b:** R = COOMe, **c:** CH_2Cl, **d:** CH_2Br, **e:** CH_2OH, **f:** CH_2NH_2

Scheme 1.

Keywords: Azides; 1,3-Dipolar cycloadditions; Azoles; Polymer-supported synthesis.

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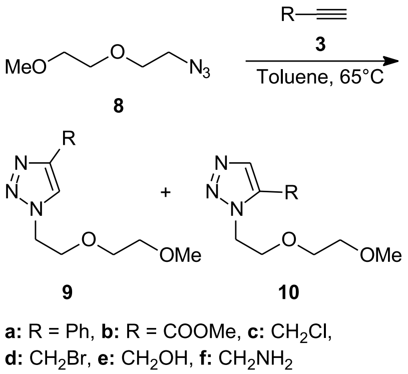
Table 1. Cycloaddition between MeOPEG-supported azide **2** and 1-(1-ethoxy-2-azido)-2-methoxyethane **8** with akynyl dipolarophiles **3**

R	Product yield (%)[a]			Product ratio[b]	
	4+5	**9**	**10**	**4**: **5**	**9**: **10**
Ph	98	58	16	80:20	50:50
COOMe	98	66	17	83:17	80:20
CH_2Cl	96	49	26	70:30	65:35
CH_2Br	96	46	25	70:30	65:35
CH_2OH	95	34	28	57:43	55:45
CH_2NH_2	97	30	24	55:45	55:45

[a] Isolated yields.

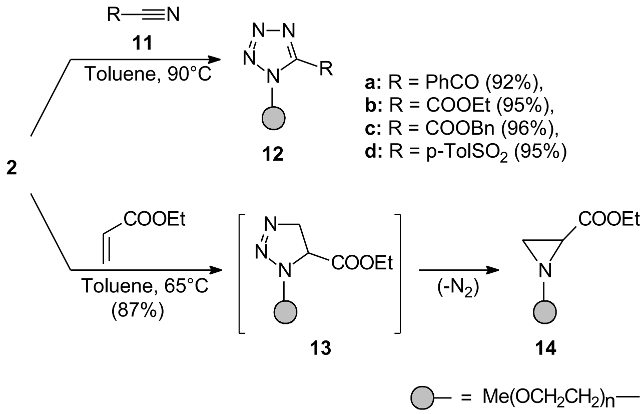
[b] Determined by ^1H NMR spectroscopy.

dipolarophile **3** gave the desired MeOPEG-supported-1,2,3-triazole cycloadducts **4** and **5**, as mixture of inseparable regioisomers. Product yields and ratios are summarised in Table 1. MeOPEG-supported-1,2,3-triazoles **4** and **5** were obtained as white solids, in near quantitative yields, simply by addition of diethyl ether to the crude reaction mixtures. Structural assignments of the MeOPEG-supported cycloadducts rely upon ^1H NMR spectroscopic analysis and are fully consistent with those of 4- and 5-substituted-1,2,3-triazoles reported in the literature.[15] In particular, the proton on the C_5 of the 1,2,3-triazole ring of **4a-f** appears in the range δ 7.79–8.02, thus accounting for the depicted regiochemistry resulting from cycloaddition. As can be inferred from Scheme 1, the removal of the MeOPEG pendant from the mixture of supported cycloadducts **4**+**5** was accomplished under acidic conditions, affording 1-unsubstituted 1,2,3-triazoles **6** and **7** with 71–85% overall yield. Finally, the latter products were obtained as pure regioisomers through silica gel column chromatography. In order to ensure the effect of the MeOPEG moiety on regioselectivity, we submitted 1-(1-ethoxy-2-azido)-2-methoxyethane **8**[16] to a reaction with acetylenic dipolarophiles **3** (Scheme 2). The results summarised in Table 1 show that regioselectivity ratio of 1-methoxyethoxy ethyl-1,2,3-triazoles **9**: **10** are generally similar to that observed for MeOPEG-supported materials and reflect the usual balance of HOMO and LUMO-dipole control of azide cycloadditions.[17] The only exception to this behaviour arises with phenylacetylene **3a** and may be ascribed to steric repulsion between the phenyl ring of the dipolarophile and the MeOPEG residue in the formation of MeOPEG-supported-5-phenyl-1,2,3-triazole **7a**.



Scheme 2.

We have also developed the first example of cycloaddition between MeOPEG-supported azide **2** and a series of electron-poor (activated) nitriles **11** (Scheme 3). Clean cycloaddition reactions were performed by heating **2** in dry toluene in the presence of 20 mol equiv of the appropriate cyano dipolarophile, while the usual reaction workup gave MeOPEG-supported-1,2,3,4-tetrazoles **12** in near quantitative yields. Finally, the same protocol using ethyl acrylate gave the MeOPEG-supported-2-ethoxycarbonyl aziridine **14** (Scheme 3). Structural assignments of the latter product rely upon ^1H NMR spectroscopic analysis, the data obtained agrees with that of the known 1-benzyl-2-ethoxycarbonyl aziridine.[18] The formation of the MeOPEG-supported aziridine **14** is not surprising since its formation follows the known thermal degradative behaviour of the initially-formed 4,5-dihydro-1,2,3-triazole cycloadduct **13**.[19]



Scheme 3.

3. Conclusions

The behaviour of MeOPEG-supported azide **2** towards a variety of acetylenes, ethylenes and the cyano group as dipolarophiles illustrates the advantages concerned with soluble polymer-supported synthesis. All cycloadditions were satisfactory in terms of product yields and easy workup procedures. The removal of the MeOPEG pendant was achieved onto 1,2,3-triazole derivatives **4** and **5**, giving 1-unsubstituted-1,2,3-triazoles **6** and **7**. As a concluding remark, the presence of the MeOPEG mojety had little or no influence on cycloaddition regioselectivity compared with that of non-polymeric azides.

4. Experimental

Melting points were measured with a Büchi apparatus in open capillary tubes and are uncorrected. IR Spectra were recorded with a Perkin–Elmer 1725X spectrophotometer. Mass spectra were determined with a VG-70EQ apparatus. ^{1}H NMR and ^{13}C NMR Spectra were taken with a Bruker AC 300 or AMX 300 instruments in $CDCl_3$ solutions at room temperature unless otherwise stated. Chemical shifts are given as ppm from tetramethylsilane, *J* values are given in Hz.

Before use, all MeOPEG-bounded materials were melted at 90 °C at 1 mmHg for 1 h to remove moisture.

Compounds **6a**,[20] **6b**,[21] **6c**,[22] **6e**,[23] **6f**[24] and **7a**,[20] **7b**[25] are known in the literature. MeOPEG-azide **2**[12] and 1-(1-ethoxy-2-azido)-2-methoxyethane **8**[16] was synthesised according to literature procedures.

4.1. Yields and ^{1}H NMR purity determination of MeOPEG-bounded compounds

The yields of MeOPEG-bounded materials were determined by weight of pure compounds. It was assumed that the average M_W of MeOPEG monomethylether residue is 5000 Da, while the M_W actually encompasses the range between 4500 and 5500 Da. The purity of MeOPEG-bounded compounds were determined by ^{1}H NMR analyses with pre-saturation of the MeOPEG methylene signals at δ 3.64. In order to ensure complete relaxation of the proton nuclei and integration accuracy, we set relaxation delay (RD) to 6 s and aquisition time (AQ) to 4 s. The integrations of the PEG fragment CH_3OCH_2, which were found between δ 3.30 and 3.37, were used as internal standards with an estimated integration error of ±4%.

4.1.1. MeOPEG-supported 1,2,3-triazoles 4 and 5. A solution of **2** (5.00 g, 1.0 mmol) and the appropriate alkynyl derivative **3** (10.0 mmol) in dry toluene (40 mL) was heated to 70 °C for 48 h. The solvent was partly removed under reduced pressure and Et_2O (40 mL) was added. The white solid was collected by filtration affording a mixture of the MeOPEG-supported cycloadducts **4**+**5** (see Table 1 for yields and yield ratio).

The following data are selected from the ^{1}H NMR analyses of the mixtures **5**+**6** in $CDCl_3$ solutions; signals due to the PEG fragment CH_3OCH_2 have been omitted.

4a: δ 4.53 (2H, t, $J=7.0$ Hz, CH_2–N<), 7.20–7.40 (4H, m, aromatics), 7.97 (1H, s, C_5–*H*); **5a**: δ 4.47 (2H, t, $J=7.0$ Hz, CH_2–N<), 7.20–7.40 (4H, aromatics), 7.65 (1H, s, C_4–*H*).

4b: δ 3.93 (3H, s, COOCH_3), 4.54 (2H, t, $J=7.0$ Hz, CH_2–N<), 8.02 (1H, s, C_5–*H*); **5b**: δ 3.90 (3H, s, COOCH_3), 4.90 (2H, t, $J=7.0$ Hz, CH_2–N<), 7.65 (1H, s, C_4–*H*).

4c: δ 4.45 (2H, t, $J=6.9$ Hz, CH_2–N<), 4.67 (2H, s, CH_2Cl), 7.79 (1H, s, C_5–*H*); **5c**: δ 4.56 (2H, t, $J=6.9$ Hz, CH_2–N<), 4.74 (2H, s, CH_2Cl), 7.58 (1H, s, C_4–*H*).

4d: δ 4.53 (2H, t, $J=6.9$ Hz, CH_2–N<), 4.58 (2H, s, CH_2Br), 7.84 (1H, s, C_5–*H*); **5d**: δ 4.60 (2H, t, $J=6.9$ Hz, CH_2–N<), 4.67 (2H, s, CH_2Br), 7.65 (1H, s, C_4–*H*).

4e: δ 4.52 (2H, t, $J=7.0$ Hz, CH_2–N<), 4.73 (2H, s, CH_2OH), 7.97 (1H, s, C_5–*H*); **5e**: δ 4.56 (2H, t, $J=7.0$ Hz, CH_2–N<), 4.67 (2H, s, CH_2OH), 7.65 (1H, s, C_4–*H*).

4f: δ 4.28 (2H, t, $J=7.4$ Hz, CH_2NH_2), 4.50 (2H, t, $J=6.9$ Hz, CH_2–N<), 7.87 (1H, s, C_5–*H*); **5f**: δ 4.31 (2H, s, CH_2NH_2), 4.56 (2H, t, $J=7.0$ Hz, CH_2–N<), 7.56 (1H, s, C_4–*H*).

4.1.2. MeOPEG-supported 1,2,3,4-tetrazoles 12. A solution of **2** (5.00 g, 1.0 mmol) and the appropriate nitrile **11** (20.0 mmol) in dry toluene (40 mL) was heated to 90 °C for 48 h. The solvent was partly removed under reduced pressure and Et_2O (40 mL) was added. The white solid was collected by filtration giving the MeOPEG-supported cycloadducts **12**.

12a (4.76 g, 92%). δ_H 3.25 (2H, t, $J=7.0$ Hz, CH_3OCH_2–), 3.34 (3H, s, CH_3OCH_2–), 4.54 (2H, t, $J=7.0$ Hz, –CH_2–N<), 7.50–7.75 (5H, aromatics).

12b (4.96 g, 95%). δ_H 1.46 (3H, t, $J=6.6$ Hz, CH_3–CH_2OCO), 3.25 (2H, t, $J=7.0$ Hz, CH_3OCH_2–), 3.34 (3H, s, CH_3OCH_2–), 4.26 (2H, q, $J=6.6$ Hz, CH_3–CH_2OCO), 4.56 (2H, t, $J=7.0$ Hz, –CH_2–N<).

12c (4.88 g, 96%). δ_H 3.25 (2H, t, $J=7.0$ Hz, CH_3OCH_2–), 3.34 (3H, s, CH_3OCH_2–), 4.35 (2H, s, Ph-CH_2OCO), 4.56 (2H, t, $J=7.0$ Hz, –CH_2–N<), 7.10–7.60 (5H, aromatics).

12d (4.99 g, 95%). δ_H 2.38 (3H, s, CH_3–C_6H_4), 3.25 (2H, t, $J=7.0$ Hz, CH_3OCH_2–), 3.34 (3H, s, CH_3OCH_2–), 4.58 (2H, t, $J=7.0$ Hz, –CH_2–N<), 7.50–8.00 (4H, aromatics).

4.1.3. MeOPEG-supported 2-ethoxycarbonylaziridine 14. A solution of **2** (5.00 g, 1.0 mmol) and ethyl acrylate (2.00 g, 20.0 mmol) in dry toluene (40 mL) was heated to 70 °C for 16 h. The solvent was partly removed under reduced pressure and Et_2O (40 mL) was added. The white solid was collected by filtration giving the MeOPEG-supported aziridine **14** (4.45 g, 87%) δ 1.24 (3H, t, $J=7.1$ Hz, CH_3CH_2O–), 1.51 (1H, dd, $J=3.3$, 1.3 Hz, –*H*CH–), 2.07 (1H, dd, $J=6.7$, 3.3 Hz, –*H*CCOOEt), 2.23 (1H, dd, $J=6.7$, 1.3 Hz, –HC*H*–), 3.30 (3H, s, CH_3OCH_2–), 3.35 (2H, t, $J=7.0$ Hz, CH_3OCH_2–), 4.11 (2H, q, $J=7.1$ Hz, CH_3CH_2O–).

4.1.4. 1-*H*-1,2,3-Triazoles 6 and 7. A solution of **4**+**5** (7.50 g) in chloroform (4.5 mL) and 95% formic acid (4.5 mL) was stirred at room temperature for 1 h, and then refluxed for 6 h. Et_2O (40 mL) was added and the white solid was filtered off. The solvent was partly evaporated under reduced pressure, chloroform (20 mL) was added and the mixture was washed firstly with water (2×10 mL), then with 5% aqueous sodium hydrogencarbonate (2×10 mL). The organic layer was dried over sodium sulfate, the solvent was evaporated and the residue was chromatographed on a silica gel column with ethyl acetate/dichloromethane 4:1 affording 1-*H*-1,2,3-triazoles **6** and **7**.

6a (0.11 g, 42%).

6b (0.13 g, 68%).

6c (89 mg, 52%).

6d (0.12 g, 52%) as white solid mp 64 °C (from diisopropyl ether). ^{1}H NMR (DMSO-d_6) δ 3.64 (2H, s), 8.37 (1H, s), 11.12 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 37.4 (t), 127.85 (d), 128.50 (s); MS *m/z* (EI) 162 (M$^+$). Anal. Calcd for $C_3H_4BrN_3$: C, 22.24; H, 2.49; N, 25.94%. Found: C, 22.29; H, 2.54; N, 26.04.

6e (68 mg, 47%).

6f (55 mg, 39%).

7a (0.11 g, 42%).

7b (27 mg, 17%).

7c (48 mg, 28%) as white solid mp 57 °C (from diisopropyl ether). ^{1}H NMR (DMSO-d_6) δ 3.76 (2H, s), 7.89 (1H, s), 11.05 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 43.74 (t), 126.15 (s), 129.13 (d); MS *m/z* (EI) 117 (M$^+$). Anal. Calcd for $C_3H_4ClN_3$: C, 30.66; H, 3.43; N, 35.75%. Found: C, 30.61; H, 3.47; N, 35.82.

7d (66 mg, 28%) as yellow thick oil. ^{1}H NMR (DMSO-d_6) δ 3.66 (2H, s), 7.83 (1H, s), 11.15 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 37.9 (t), 126.00 (s), 128.88 (d); MS *m/z* (EI) 162 (M$^+$). Anal. Calcd for $C_3H_4BrN_3$: C, 22.24; H, 2.49; N, 25.94%. Found: C, 22.21; H, 2.54; N, 25.93.

7e (56 mg, 38%) as colourless thick oil. IR (nujol) 3385 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.80 (1H, br s), 3.90 (2H, s), 7.90 (1H, s), 10.90 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 54.74 (t), 125.90 (s), 128.76 (d); MS *m/z* (EI) 99 (M$^+$). Anal. Calcd for $C_3H_5N_3O$: C, 36.36; H, 5.09; N, 42.41%. Found: C, 36.40; H, 5.12; N, 42.47.

7f (45 mg, 32%) as colourless thick oil. IR (nujol) 3280, 3160 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.24 (2H, br s), 3.16 (2H, s), 7.81 (1H, s), 10.86 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 46.26 (t), 126.16 (s), 129.10 (d); MS *m/z* (EI) 98 (M$^+$). Anal. Calcd for $C_3H_6N_4$: C, 36.73; H, 6.16; N, 57.11%. Found: C, 36.78; H, 6.20; N, 57.17.

4.1.5. Dipolar cycloaddition between 1-(1-ethoxy-2-azido)-2-methoxyethane 8 and acetylenes 3. A solution of **8** (0.36 g, 2.5 mmol) and the appropriate alkynyl derivative **3** (10.0 mmol) in dry toluene (25 mL) was heated to 65 °C for 48 h. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with ethyl acetate/hexane 2:1 affording 1,2,3-triazoles **9** and **10**.

9a (0.36 g, 58%) as colourless thick oil. ^{1}H NMR δ 3.38–3.75 (8H, m), 3.80 (3H, s), 7.0–7.3 (5H, m), 8.07 (1H, s); MS *m/z* (EI) 247 (M$^+$). Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.30; N, 16.99%. Found: C, 63.19; H, 6.27; N, 17.05.

9b (0.38 g, 66%) as pale yellow thick oil. ^{1}H NMR δ 3.30–3.70 (8H, m), 3.78 (3H, s), 3.93 (3H, s), 8.11 (1H, s); MS *m/z* (EI) 229 (M$^+$). Anal. Calcd for $C_9H_{15}N_3O_4$: C, 47.16; H, 6.60; N, 18.33%). Found: C, 47.11; H, 6.64; N, 18.38.

9c (0.27 g, 49%) as colourless thick oil. ^{1}H NMR δ 3.35–3.70 (10H, m), 3.76 (3H, s), 8.04 (1H, s); MS *m/z* (EI) 219 (M$^+$). Anal. Calcd for $C_8H_{14}ClN_3O_2$: C, 43.74; H, 6.42; N, 19.13%). Found: C, 43.79; H, 6.38; N, 19.18.

9d (0.30 g, 46%) as pale yellow thick oil. ^{1}H NMR δ 3.40–3.80 (10H, m), 3.92 (3H, s), 7.96 (1H, s); MS *m/z* (EI) 264 (M$^+$). Anal. Calcd for $C_8H_{14}BrN_3O_2$: C, 36.38; H, 5.34; N, 15.91%. Found: C, 36.43; H, 5.38; N, 15.98.

9e (0.17 g, 34%) as colourless thick oil. ^{1}H NMR δ 2.90 (1H, br s), 3.44–3.80 (10H, m), 3.90 (3H, s), 8.11 (1H, s); MS *m/z* (EI) 201 (M$^+$). Anal. Calcd for $C_8H_{15}N_3O_3$: C, 47.75; H, 7.51; N, 20.88%. Found: C, 47.71; H, 7.47; N, 20.95.

9f (0.15 g, 30%) as colourless thick oil. ^{1}H NMR δ 3.12–3.85 (12H, m), 3.94 (3H, s), 7.91 (1H, s); MS *m/z* (EI) 200 (M$^+$). Anal. Calcd for $C_8H_{16}N_4O_2$: C, 47.99; H, 8.05; N, 27.98%. Found: C, 48.03; H, 8.09; N, 28.04.

10a (0.10 g, 16%) as colourless thick oil. ^{1}H NMR δ 3.40–3.80 (8H, m), 3.82 (3H, s), 7.0–7.3 (5H, m), 7.69 (1H, s); MS *m/z* (EI) 247 (M$^+$). Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.30; N, 16.99%. Found: C, 63.11; H, 6.25; N, 16.93.

10b (95 mg, 17%) as pale yellow thick oil. ^{1}H NMR δ 3.30–3.75 (8H, m), 3.78 (3H, s), 3.95 (3H, s), 7.65 (1H, s); MS *m/z* (EI) 229 (M$^+$). Anal. Calcd for $C_9H_{15}N_3O_4$: C, 47.16; H, 6.60; N, 18.33%. Found: C, 47.20; H, 6.65; N, 18.40.

10c (0.14 g, 26%) as colourless thick oil. ^{1}H NMR δ 3.35–3.78 (10H, m), 3.88 (3H, s), 7.51 (1H, s); MS *m/z* (EI) 219 (M$^+$). Anal. Calcd for $C_8H_{14}ClN_3O_2$: C, 43.74; H, 6.42; N, 19.13%. Found: C, 43.79; H, 6.38; N, 19.18.

10d (0.16 g, 25%) as pale yellow thick oil. ^{1}H NMR δ 3.40–3.80 (10H, m), 3.90 (3H, s), 7.58 (1H, s); MS *m/z* (EI) 264 (M$^+$). Anal. Calcd for $C_8H_{14}BrN_3O_2$: C, 36.38; H, 5.34; N, 15.91%. Found: C, 36.42; H, 5.31; N, 15.86.

10e (0.14 g, 28%) as colourless thick oil. ^{1}H NMR δ 2.80 (1H, br s), 3.40–3.85 (10H, m), 3.90 (3H, s), 7.61 (1H, s); MS *m/z* (EI) 201 (M$^+$). Anal. Calcd for $C_8H_{15}N_3O_3$: C, 47.75; H, 7.51; N, 20.88%. Found: C, 47.80; H, 7.52; N, 20.94.

10f (0.12 g, 24%) as colourless thick oil. ^{1}H NMR δ 3.10–3.85 (12H, m), 3.90 (3H, s), 7. 95 (1H, s); MS *m/z* (EI) 200 (M$^+$). Anal. Calcd for $C_8H_{16}N_4O_2$: C, 47.99; H, 8.05; N, 27.98%. Found: C, 48.01; H, 8.03; N, 27.94

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References and notes

1. (a) Sharp, J. T. In *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Product Products*; Padwa, A., Pearson, W. H., Eds.; Wiley: New York, 2002; Chapter 4, pp 473–538. (b) Lwowsky, W. In Padwa, A., Ed.; 1,3-Dipolar Cycloaddition Chemistry; Wiley-Interscience: New York, 1984; Vol. 1, Chapter 4, pp 621–627.
2. (a) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897. (b) Katritzky, A. R.; Zhang, Y.; Singh, S. K. *Heterocycles* **2003**, *60*, 1225. (c) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057. (d) Wang, Z.-X.; Qin, H.-L. *Chem. Commun.* **2003**, 2450. (e) Demko, Z. P.; Sharpless, K. B. *Org. Lett.* **2002**, *4*, 2525.
3. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
4. (a) Demko, Z. P.; Sharpless, K. B. *Org. Lett.* **2001**, *3*, 4091. (b) Demko, Z. P.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2110. (c) Demko, Z. P.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2113.
5. (a) Alvarez, R.; Velazquez, S.; San, F.; Aquaro, S.; De, C.; Perno, C.; Karlsson, A.; Balzarin, J.; Camarasa, M. J. *J. Med. Chem.* **1994**, *37*, 4185. (b) Velazquez, S.; Alvarez, R.; Perez, C.; Gago, F.; De, C.; Balzarini, J.; Camarasa, M. J. *Antivir. Chem. Chemoter.* **1998**, *9*, 481.
6. Genin, M. J.; Allwine, D. A.; Anderson, D. J.; Barbachyn, M. R.; Emmert, D. E.; Garmon, S. A.; Graber, D. R.; Grega, K. C.; Hester, J. B.; Hutchinson, D. K.; Morris, J.; Reischer, R. J.; Ford, C. W.; Zurenko, G. E.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H. *J. Med. Chem.* **2000**, *43*, 953.
7. Brockunier, L. L.; Parmee, E. R.; Ok, H. O.; Candelore, M. R.; Cascieri, M. A.; Colwell, L. F.; Deng, L.; Feeney, W. P.; Forrest, M. J.; Hom, G. J.; MacIntyre, D. E.; Tota, L.; Wywratt, M. J.; Fisher, M. H.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2111.
8. (a) Früchtel, J. S.; Jung, G. *Angew. Chem., Int. Ed.* **1996**, *35*, 17. (b) Allin, S. M.; Sharma, P. K. *Synthesis* **1997**, 1217. (c) Ellmann, J. A. *Chem. Rev.* **1996**, *96*, 555. (d) Krcnák, V.; Holladay, M. W. *Chem. Rev.* **2002**, 102.
9. Nefzi, A.; Otresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449.
10. (a) Löber, S.; Rodriguez-Loaiza, P.; Gmeiner, P. *Org. Lett.* **2003**, *5*, 1753. (b) Löber, S.; Gmeiner, P. *Tetrahedron* **2004**, *60*, 8699.
11. Blass, B. E.; Coburn, K. R.; Faulkner, A. L.; Seibel, W. L.; Srivastava, A. *Tetrahedron Lett.* **2003**, *44*, 2153.
12. (a) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489. (b) Toy, P. H.; Janda, K. D. *Acc. Chem. Res.* **2000**, *33*, 546.
13. Garanti, L.; Molteni, G. *Tetrahedron Lett.* **2003**, *44*, 1133.
14. Benaglia, M.; Ciniquini, M.; Cozzi, F. *Tetrahedron Lett.* **1999**, *40*, 2019.
15. Zalkow, L. H.; Hill, R. H. *Tetrahedron* **1975**, *31*, 831.
16. Neumayer, D. A.; Belot, J. A.; Feezel, R. L.; Reedy, C.; Stern, C. L.; Marks, T. J.; Liable-Sands, L. M.; Rheingold, A. L. *Inorg. Chem.* **1998**, *37*, 5625.
17. Houk, K. N.; Sims, J.; Watts, C. R.; Luskus, L. J. *J. Am. Chem. Soc.* **1973**, *95*, 7301.
18. Pierre, J. L.; Baret, P.; Arnaud, P. *Bull. Soc. Chim. Fr.* **1971**, 3619.
19. (a) Bourgois, J.; Bourgois, M.; Texier, F. *Bull. Soc. Chim. Fr.* **1978**, 485. (b) Schultz, A. G. In Curran, D. P., Ed.; Advances in Cycloadditions; JAI: London, 1988; Vol. 1, p 53.
20. Trifonov, R. E.; Ostrovskii, V. A. *Croat. Chem. Acta* **1999**, *72*, 953.
21. Woerner, F. P.; Reimlinger, H. *Chem. Ber.* **1970**, *103*, 1908.
22. Szekeres, H. J.; Atack, J. R.; Chambers, M. S.; Cook, S. M.; Macaulay, A. J.; Pillai, G. V.; MacLeod, A. M. *Biorg. Med. Chem. Lett.* **2004**, *14*, 2871.
23. Banert, K. *Chem. Ber.* **1989**, *122*, 1963.
24. Banert, K. *Chem. Ber.* **1989**, *122*, 911.
25. Huisgen, R.; Knorr, R.; Möbius, L.; Szeimies, G. *Chem. Ber.* **1965**, *98*, 4014.

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Regioselective reactivity of some 5,7-dimethoxyindoles

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Abstract—The reactivity of some 5,7-dimethoxyindoles with respect to electrophiles has been investigated. The favoured sites for reaction are C3 and C4 and regioselectivity can be controlled by the judicious arrangement of electron-withdrawing substituents. Results of formylation, acylation, the Mannich reaction, bromination and nitration are described.

1. Introduction

The reactivity of indoles towards electrophiles is of fundamental importance in heterocyclic chemistry. While it is well established that the most nucleophilic site of an indole nucleus is the C3 position, the targeted reactivity at other sites can expand its synthetic applications immensely. 4,6-Dimethoxyindoles have been shown to exhibit enhanced reactivity, especially at C7.[1–4] Here, we examine the behaviour of some simple 5,7-dimethoxyindoles, including methyl 5,7-dimethoxyindole-2-carboxylate, towards a range of electrophiles.

2. Results and discussion

2.1. Synthesis of 5,7-dimethoxyindoles

The preparation of 5,7-dimethoxyindole **1** was first reported in 1953 by Süs and co-workers[5] via the decarboxylation of the corresponding 2-carboxylic acid. However, in 1970 Crohare and co-workers[6] reported an alternative synthesis, which was followed shortly after in the same year by yet another synthesis by Mulligan and LaBerge, involving reductive cyclisation of 3′,5′-dimethoxy-2′-nitro-2-nitrostyrene.[7] The last method has been used in this study. The parent 5,7-dimethoxyindole **1** was methylated, benzylated, acetylated and phenylsulfonated on nitrogen to yield the corresponding *N*-substituted compounds **2–5**. Methyl 5,7-dimethoxyindole-2-carboxylate **6** has been synthesized from the related carboxylic acid following a Reissert indole synthesis.[6] However, the Hemetsberger methodology[8,9] was found to be the most effective for the synthesis of the carboxylate **6**. 3,5-Dimethoxybenzaldehyde was condensed with the ethyl azidoacetate to give methyl 2-azido-3-(3,5-dimethoxyphenyl)propenoate in high yield and purity. This intermediate could then be pyrolized to the corresponding indole in refluxing xylene. However, it was found that the use of 1,2-dichlorobenzene gave a cleaner reaction. The indole **6** was isolated in 87% yield. Either ethyl azidoacetate or methyl azidoacetate could be used in this reaction because the presence of sodium methoxide and methanol ensured that the condensed product was always the methyl ester **6**. In general, ethyl azidoacetate[10,11] was utilised and was prepared according to the method reported by Allen and co-workers for the preparation of methyl azidoacetate.[12] The indole **6** was *N*-methylated to give compound **7** and a bis-alkylation with α,α′-dibromo-*m*-xylene gave compound **8** in good yield.

4,6-Dimethoxyindoles have been synthesized very effectively by the cyclisation of an acylaminoketone.[1] Consequently, this approach was also investigated for the preparation of some 3-aryl-5,7-dimethoxyindoles. 2,4-Dimethoxyaniline was reacted with 4-bromophenacyl bromide and the resulting relatively unstable aminoketone was treated directly with trifluoroacetic anhydride to give the trifluoroacetamide **9**. However, all attempts to cyclise this compound, using either trifluoroacetic acid or polyphosphoric acid, failed. The 5,7-dimethoxyindoles **1–8** could potentially undergo reaction at C3 and C4. Therefore, achievement of regioselectivity became a major point of interest and a key synthetic goal (Scheme 1).

2.2. Formylation

Vilsmeier–Haack formylation of 5,7-dimethoxyindole **1** with one equivalent of phosphoryl chloride in DMF at 0 °C

Keywords: Indoles; Formylation; Acylation; Regioselectivity.

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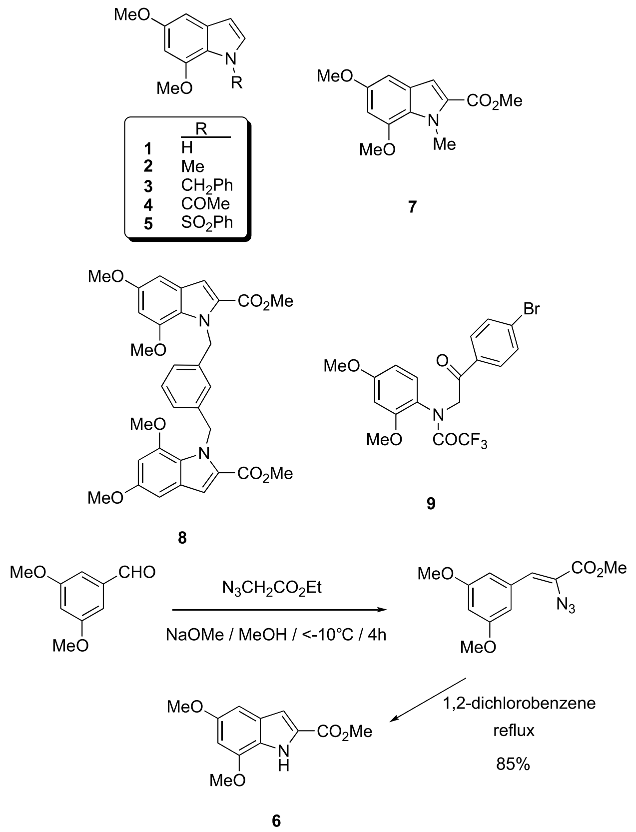


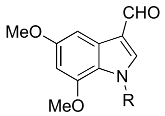
	R
1	H
2	Me
3	CH_2Ph
4	COMe
5	SO_2Ph

Scheme 1.

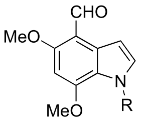
gave the 3- and 4-carbaldehydes, **10** and **11**, respectively, in 66 and 33% yield. Use of excess phosphoryl chloride and a little warming gave the 3,4-dicarbaldehyde **12** in 93% yield. Interestingly, the dialdehyde **12** underwent reaction with ammonia to give the pyrroloisoquinoline **13** in quantitative yield, and also with nitromethane and potassium hydroxide to give the benzindole **14** in 63% yield. In general, the indole-3-carbaldehydes **15** and **18** were formed in preference to the 4-carbaldehydes **16** and **19** in formylation reactions of the *N*-methyl and *N*-benzylindoles **2** and **3**, respectively. Selectivity for the 3-carbaldehyde was increased with a lowering of temperature, and was more effective with the *N*-methylindole **2** than the *N*-benzylindole **3**. Replacement of phosphoryl chloride with triflic anhydride gave reduced amounts of the 3-carbaldehyde and led to the only reaction in which the 4-carbaldehyde predominated: this was the reaction of 5,7-dimethoxyindole **1** at 0 °C. The 3,4-dialdehydes **17** and **20** could also be obtained in almost quantitative yields from the indoles **2** and **3**, respectively.

In comparison, it was noted that the corresponding Vilsmeier–Haack reactions on the known 5,7-dimethylindole[14,15] gave only the 5,7-dimethylindole-3-carbaldehyde[15] in high yield, and no 4-substituted product was detected. A similar Vilsmeie–Haack reaction of 5,7-dimethylindole using *N*,*N*-dimethylacetamide and phosphoryl chloride gave an 82% yield of the previously unreported 3-acetylindole **21**.

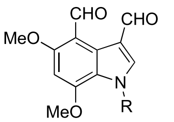
Surprisingly, regioselective formylation was observed in the reaction of the *N*-acetylindole **4**, with the acetyl group being hydrolysed off during the reaction work-up, and the corresponding 4-carbaldehyde **11** was obtained in 87% yield. Presumably, the 3-position is relatively deactivated by the electron-withdrawing *N*-acetyl group. Attempted formylation of the *N*-phenylsulfonylindole **5** led to extensive decomposition.



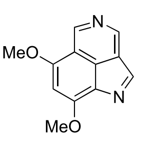
	R
10	H
15	Me
18	CH_2Ph



	R
11	H
16	Me
19	CH_2Ph



	R
12	H
17	Me
20	CH_2Ph



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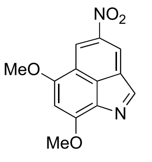

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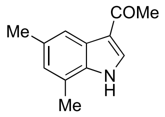

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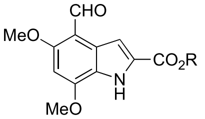
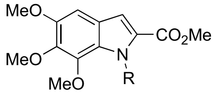
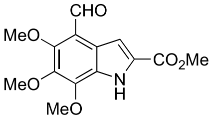


	R
22	Me
23	H



	R
24	H
27	Me
30	**CO_2Bu^t**



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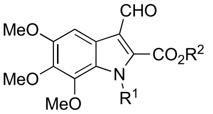


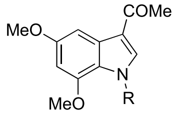
	R^1	R^2
25	H	Me
28	Me	Me
29	Me	H

A more satisfactory regioselective reaction arises in the case of the indole ester **6**. When it was treated with a slight excess of the Vilsmeier–Haack reagent at 0 °C for 2 h the indole-4-carbaldehyde **22** was obtained in quantitative yield. Attempts to achieve diformylation of indole **6** either directly with excess reagent or via the formylation of aldehyde **22** failed. Even when phosphoryl chloride was used as the solvent and the reaction temperature was increased to near the boiling point, diformylation was not observed. Hydrolysis of formylindole ester **22** was easily achieved by heating it in aqueous ethanolic potassium hydroxide solution, and the acid **23** was obtained as a pale yellow powder in 95% yield.

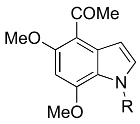
It is worth noting that the corresponding formylation of the known[16] methyl 5,6,7-trimethoxyindole-2-carboxylate **24** produced a 70:30 ratio of 3-formylindole **25** to 4-formylindole **26**, in an overall yield of 84%. The related *N*-methylindole[17] **27** gave formylation only at C3: the 3-formyl ester **28** was produced only in 15% yield and could not be fully characterized, but the major product in 69% yield was the related acid **29**, as a result of facile ester hydrolysis on work-up. The *N*-*t*-Boc indole derivative **30** could not be formylated successfully without loss of the Boc substituent.

2.3. Acylation

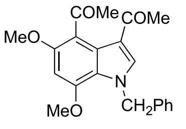
The results of acetylation of indoles **1**–**3** were similar to those of formylation, and both 3- and 4-acetyl-indoles were obtained. Reaction of phosphoryl chloride and *N*,*N*-dimethylacetamide at room temperature with indoles **1** and **2** gave mainly the 3-acetyl derivatives **31** (41%) and **33** (79%), respectively, with smaller amounts of the 4-acetyl compounds **32** (29%) and **34** (7%), respectively. Greater selectivity was observed for the *N*-methylindole **2**. Effective reaction of the *N*-benzylindole **3** required warming to 40–60 °C for production of the major 3-acetyl derivative **35** (60%) and the minor 4-acetyl derivative **36** (20%). No 3,4-diacetyl compound could be formed under these conditions, but reaction of indole **3** with acetyl chloride and tin (IV) chloride gave a the 3,4-diacetyl derivative **37** in 34% yield.



	R
31	H
33	Me
35	CH_2Ph



	R
32	H
34	Me
36	CH_2Ph



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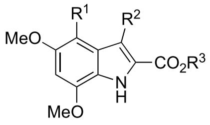
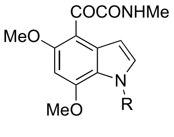


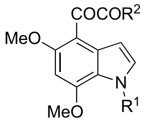
	R^1	R^2	R^3
38	COMe	H	Me
39	$COCF_3$	H	Me
40	$COCF_3$	H	H
41	$COCF_3$	CHO	Me

The indole ester **6** failed to react under modified Vilsmeier–Haack acetylation conditions, even at 60 °C for several days. However, when it was treated with acetyl chloride and antimony (V) chloride, the 4-acetylindole **38** was isolated in moderate yield, and the 3-acetyl isomer could not be detected. Treatment of indole **6** with an excess of trifluoroacetic anhydride in THF for 3 days at room temperature gave the 4-trifluoroacetyl derivative **39** in 88% yield. Substitution occurred exclusively at C4 and no substitution at C3 was observed. The trifluoroacetyl derivative **39** was treated with base in an attempt to transform the compound into either the 4-carboxylic acid or, if hydrolysis could not be controlled, the 2,4-dicarboxylic acid. After heating with aqueous ethanolic potassium hydroxide for 20 min the reaction was complete and work-up produced the 4-trifluoroacetyl-2-carboxylic acid derivative **40** in 82% yield. This was surprising, since it was expected that the trifluoroacetyl group would be hydrolyzed more easily than the ester group.

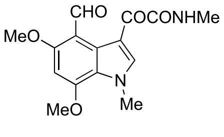
The trifluoroacetyl derivative **39** could be formylated by reaction with the Vilsmeier–Haack reagent in neat phosphoryl chloride at 60 °C for 2 days, to give the 3-formyl-4-trifluoroacetylindole **41** in 85% yield. It is significant that the trifluoroacetyl group survived intact and that formylation occurred, since attempted formylation of 4-formylindole **22** at C3 was unsuccessful. This result suggests that the trifluoroacetyl group of compound **39** might be twisted out of the plane of the indole nucleus, thereby decreasing conjugation with the ring and consequently decreasing the electron withdrawing effect of the trifluoroacetyl group at C4 with respect to C3. A comparison of the ^{1}H NMR spectra of the 4-trifluoroacetylindole **39** and 4-formylindole **22** reveals a significant difference in the heights of the two methoxy signals of indole **39** compared with the two methoxy signals of indole **22** which are almost equal in height. This suggests that there is some steric hindrance between the trifluoroacetyl group and the adjacent 5-methoxy group of 4-trifluoroacetylindole **39**, causing the trifluoroacetyl group to be twisted out of the plane of the indole nucleus.



	R
42	H
43	Me
44	CH_2Ph



	R^1	R^2
45	Me	NH_2
46	CH_2Ph	NMe_2



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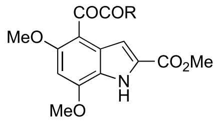


	R
48	NH_2
49	NH-[3,5-$(MeO)_2C_6H_3$]

Indole **6** was heated in dichloromethane with trichloroacetyl chloride for 2 days without reaction, except for very minor decomposition. This result suggests that C4 of indole **6** is too hindered for the bulky trichloroacetyl chloride to approach. The related reaction occurs smoothly at C7 in 4,6-dimethoxyindoles.[2]

Oxalyl chloride is known to react readily with simple and activated indoles.[1,2,18,19] In the case of 3-substituted-4,6-dimethoxyindoles, mixtures of 2- and 7-substituted glyoxyloyl chlorides are obtained, with regioselectivity being strongly influenced by the solvent. Reactions of indoles **1**–**3** with oxalyl chloride gave only 3-substituted products in high yields. Ether was the most effective solvent, allowing precipitation of the glyoxyloyl chloride, which could then be converted into the corresponding methyl amides **42**–**44** by reaction with methylamine. Additionally, the primary amide **45** and tertiary amide **46** were prepared and characterized. The amide **43** could also be formylated to give the 4-formyl analog **47** in low yield. In contrast, reaction of the indole ester **6** with oxalyl chloride gave a chloro-oxalyl derivative, which was not isolated, but quenched with ammonia and 3,5-dimethoxyaniline, respectively, to give the 4-substituted primary amide **48** and the secondary amide **49** in high yields and purity. The reaction of indole **6** with oxalyl chloride again exemplifies the directing ability of the methoxy groups and the deactivation of the 3-position by the ester group. While Crohare and co-workers were able to react 5,7-dimethoxyindole **1** with oxalyl chloride selectively at C3,[7] the inclusion of an ester group at C2 allows for selective reaction at C4.

2.4. Mannich reaction

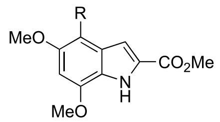
Indole **6** underwent the Mannich reaction to give 4-(*N*,*N*-dimethylaminomethyl)indole **50** in 78% yield. Once again, substitution occurred exclusively at C4. In contrast, Crohare and co-workers prepared 5,7-dimethoxygramine in 36% yield from 5,7-dimethoxyindole **1**, with no mention of any other products.[7]

2.5. Bromination

Kruse and Meyer have previously investigated the bromination of ethyl 5-methoxyindole-2-carboxylate[20] and their results indicated that the 3-bromoindole is the kinetic product, formed quickly but reversibly, and was the major product when the reaction was quenched after only 30 s or less. Extended reaction time (24 h) allowed for the isolation of the 4-bromoindole in high yield. These results have been complemented by the more recent work of Murakami and co-workers.[21] Exposures of indole **6** to bromine for between 30 s and 3 days gave almost quantitative yields of the 4-bromoindole **51**, the 3-bromoindole not being observed. In an attempt to observe the 3-bromoindole, the reaction was quenched after 1–2 s, and just a trace of the 3-bromoindole could be observed by ^{1}H NMR spectroscopy.

2.6. Nitration

When indole **6** was treated with nitric acid absorbed on silica gel[22] a mixture of multiple products formed, however, the 4-nitroindole **52** was easily retrieved via chromatography in 40% yield. Reduction of 4-nitroindole **52** gave the 4-aminoindole **53** in 59% yield, and this compound could serve as a promising precursor to pyrroloquinones.



	R
50	CH_2NMe_2
51	Br
52	NO_2
53	NH_2

3. Conclusions

Activated 5,7-dimethoxyindoles undergo preferential electrophilic substitution at C3 rather than C4, unless an electron withdrawing group is present at N1 or C2, when substitution at C4 is favoured. This behaviour is illustrated by examples of formylation, acylation, aminomethylation, bromination and nitration. Good selectivity can be achieved and some sequential substitution processes provide possibilities for further structural elaboration.

4. Experimental

4.1. General information

Melting points (uncorrected) were measured using a Mel-Temp melting point apparatus. Microanalyses were performed by the Microanalysis Unit of the Australian National University, Canberra or the University of Otago, New Zealand. Infrared spectra were recorded as Nujol mulls on a Perkin–Elmer 298 or a Perkin–Elmer 580B spectrometer. Ultraviolet-visible spectra were recorded in methanol (unless otherwise stated) on a Hitachi UV-3200 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained in the designated solvents on a Bruker AC300F (300 MHz) spectrometer or at 500 MHz with a Bruker AM-500 spectrometer. ^{1}H NMR data are reported as follow: chemical shift measured in parts per million (ppm) downfield from TMS (δ), multiplicity, observed coupling constant (J) in Hertz (Hz), proton count. Multiplicities are reported as singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), quintet (quin) and multiplet (m). ^{13}C NMR chemical shifts are reported in ppm downfield from TMS and identifiable carbons are given. The EI and ES mass spectra were recorded on an AEI MS 12 mass spectrometer at 70 eV ionizing potential and 8000 V accelerating voltage with an ion source temperature of 210 °C. MALDI (matrix assisted laser desorption) mass spectra were recorded on a Finnigan MAT Lasermat 2000 for high molecular weight compounds. The principal ion peaks *m*/*z* are reported together with their percentage intensities relative to the base peak (where possible). Kieselgel 60H (Merck, Art 7736) was employed for flash chromatography and thin-layer chromatography (t.l.c.) was performed on DC Aluminium Foil Kieselgel F_{254} (Merck, Art 5554). Preparative thin layer chromatography was performed on 3 mm plates using Merck silica gel 7730 $60GF_{254}$. Solvents and reagents were purified by literature methods. Petroleum ether refers to the hydrocarbon fraction of boiling range 60–80 °C. Compounds were detected by short and long ultraviolet light and with iodine vapor.

4.2. Preparation of indoles

4.2.1. 5,7-Dimethoxyindole (1).[13] 3,5-Dimethoxybenzaldehyde (5.00 g, 30 mmol), nitromethane (5.0 mL, 92 mmol), and ammonium acetate (2.50 g, 32 mmol) were dissolved in glacial acetic acid (20 mL) and gently refluxed for 1.5 h. The reaction mixture was cooled to room temperature and for a short period in ice. The product was filtered and washed with cold 95% ethanol to yield pure 3,5-dimethoxy-β-nitrostyrene (4.09 g, 65%) as yellow needles, mp 133 °C (lit.[13] 133.5–134.5 °C). ν_{max}: 3114, 1604, 1514, 1347, 1212, 1063, 970, 835 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$): δ 3.79 and 3.83 (2s, 6H, OMe), 6.58 (t, $J=2.4$ Hz, 1H, aryl H), 6.66 (d, $J=2.4$ Hz, 2H, aryl H), 7.54 (d, $J=14$ Hz, 1H, CH), 7.92 (d, $J=14$ Hz, 1H, $CHNO_2$). Mass spectrum: *m*/*z* 210 (M+1, 12%), 209 (M, 100), 162 (25).

To a solution of 3,5-dimethoxy-β-nitrostyrene (4.0 g, 19 mmol) in acetic anhydride (80 mL) at 60 °C was added with stirring powdered anhydrous copper (II) nitrate (8.00 g, 43 mmol) portionwise over 70 min. The reaction was complete when brown fumes first appeared. The reaction mixture was poured over ice and allowed to stand overnight. The precipitated yellow solid was filtered and washed with water to yield 3,5-dimethoxy-2-nitro,β-nitrostyrene (3.94 g, 81%) as a yellow powder, mp 189–190 °C (lit.[6] 179–181 °C). ν_{max}: 1595, 1522, 1347, 1214, 1178 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.90 and 3.92 (2s, 6H, OMe), 6.58 (d, $J=2.5$ Hz, 1H, aryl H), 6.65 (d, $J=2.5$ Hz, 1H, aryl H), 7.46 (d, $J=14$ Hz, 1H, CH), 7.93 (d, $J=14$ Hz, 1H, $CHNO_2$). Mass spectrum: *m/z* 255 (M+1, 13%), 254 (M, 100), 208 (72), 141 (91), 136 (74), 120 (75).

Iron powder (32.0 g) was added to a solution of 3,5-dimethoxy-2-nitro,β-nitrostyrene (4.00 g, 15.7 mmol) in 80% aqueous acetic acid (130 mL). When the initial exothermic reaction was complete (approximately 30 min), the reaction mixture was heated to 80 °C for 15 min and then stirred at room temperature for 1 h. The reaction mixture was diluted with water and extracted with dichloromethane. The organic layer was washed successively with water and saturated sodium bicarbonate solution, dried ($MgSO_4$), and concentrated to yield a black solid. Flash column chromatography (50:50 dichloromethane–petroleum ether) afforded 5,7-dimethoxyindole **1** (1.48 g, 53%) as an off-white crystalline solid, mp 82–83 °C (lit.[6] 81–83 °C). ν_{max}: 3353, 1597, 1311, 1148, 1045, 932, 815, 537 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.85 and 3.93 (2s, 6H, OMe), 6.35 (d, $J=2.2$ Hz, 1H, H6), 6.45 (t, $J=2.7$ Hz, 1H, H3), 6.69 (d, $J=2.2$ Hz, 1H, H4), 7.14 (t, $J=2.8$ Hz, 1H, H2), 8.25 (bs, 1H, NH). Mass spectrum: *m/z* 178 (M+1, 12%), 177 (M, 100), 162 (58), 34 (42), 119 (26).

4.2.2. 5,7-Dimethoxy-1-methylindole (2). Dimethyl sulfoxide (50 mL) was added to powdered potassium hydroxide (2.54 g, 45.2 mmol) and the mixture was stirred at room temperature for at least 5 min. 5,7-Dimethoxyindole **1** (2.0 g, 11.3 mmol) was then added and the mixture was stirred at room temperature for 1 h. The reaction mixture was cooled briefly in ice, iodomethane (1.64 mL, 26.3 mmol) was added, and the reaction mixture stirred at room temperature for a further 1 h. The reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was washed with water, dried ($MgSO_4$), and concentrated to yield 5,7-dimethoxy-1-methylindole **2** (2.12 g, 98%) as a white powder, mp 49–52.5 °C. (Found: C, 69.1; H, 6.9; N, 7.3. $C_{11}H_{13}NO_2$ requires C, 69.3; H, 7.1; N, 7.1%). λ_{max}: 230 nm (ε 32,000 cm^{-1} M^{-1}), 262 (14,000), 268 (15,000), 295 (10,000), 301 (9000). ν_{max}: 1593, 1497, 1315, 1217, 1153, 1042, 820, 805 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.87 and 3.92 (s, 6H, OMe), 4.02 (s, 3H, NMe), 6.35 (d, $J=2.3$ Hz, 1H, H6), 6.37 (d, $J=3.1$ Hz, 1H, H3), 6.67 (d, $J=2.0$ Hz, 1H, H4), 6.90 (d, $J=3.1$ Hz, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 36.2 (NMe), 55.3 and 55.6 (OMe), 93.8, 94.4 and 100.7 (aryl CH), 121.9 (aryl C), 129.9 (aryl CH), 130.2, 148.1 and 154.5 (aryl C). Mass spectrum: *m/z* 192 (M+1, 10%), 191 (M, 100), 176 (83), 148 (46).

4.2.3. 1-Benzyl-5,7-dimethoxyindole (3). Dimethyl sulfoxide (60 mL) was added to powdered potassium hydroxide (6.32 g, 0.113 mol) and the mixture was stirred at room temperature for at least 5 min. 5,7-Dimethoxyindole **1** (5.00 g, 28.2 mmol) was then added and the mixture stirred at room temperature for a further 45 min. Benzyl bromide (6.7 mL, 56 mmol) was added, and the reaction mixture was cooled briefly in ice then stirred at room temperature for 2 h. The reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was washed with water, dried ($MgSO_4$), and concentrated to yield 1-benzyl-5,7-dimethoxyindole **3** as dark yellow crystals (6.51 g, 86%), mp 86.5–87 °C (from petroleum ether). (Found: C, 76.1; H, 6.7; N, 5.2. $C_{17}H_{17}NO_2$ requires C, 76.4; H, 6.4; N, 5.2%). λ_{max}: 208 nm (ε 46,000 cm^{-1} M^{-1}), 261 (9400), 292 (5600), 300 (4900). ν_{max}: 1583, 1494, 1316, 1267, 1205, 1148, 1051, 825, 726 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.79 and 3.83 (2s, 6H, OMe), 5.56 (s, 2H, CH_2), 6.30 (d, $J=2.2$ Hz, 1H, H6), 6.40 (d, $J=3.0$ Hz, 1H, H3), 6.66 (d, $J=2.2$ Hz, 1H, H4), 6.99 (d, $J=3.0$ Hz, 1H, H2), 7.07 (m, 2H, aryl H), 7.25 (m, 3H, aryl H). ^{13}C NMR spectrum ($CDCl_3$): δ 52.3 (CH_2), 55.3 and 55.6 (OMe), 93.9, 94.8 and 101.8 (aryl CH), 121.5 (aryl C), 126.6 (2C, aryl CH), 127.0 (aryl CH), 128.4 (2C, aryl CH), 129.3 (aryl CH), 130.4, 139.7, 147.9 and 154.6 (aryl C). Mass spectrum: *m/z* 268 (M+1, 18%), 267 (M, 96), 146 (24), 131 (34), 91 (100).

4.2.4. 1-Acetyl-5,7-dimethoxyindole (4). Dry dimethyl sulfoxide (20 mL) was added to powdered potassium hydroxide (0.95 g, 16.9 mmol) and the reaction mixture was stirred at room temperature for 10 min. 5,7-Dimethoxyindole **1** (0.80 g, 4.52 mmol) was added, and the stirring was continued for 1 h. The solution was decanted from the excess potassium hydroxide (which was washed with 5 mL dry dimethyl sulfoxide) and transferred to another reaction flask containing acetic anhydride (3.95 mL, excess). The reaction mixture was cooled briefly in ice, then placed under a nitrogen atmosphere and stirred at room temperature overnight. The reaction mixture was diluted with water and extracted with diethyl ether. The organic phase was washed with water, dried (Na_2SO_4), and concentrated to give a brown oil (1.25 g). The oil was separated using flash column chromatography (dichloromethane) followed by preparative thin layer chromatography (50:50 dichloromethane–petroleum ether) affording 1-acetyl-5,7-dimethoxyindole **4** (0.75 g, 76%) as a pale tan solid, mp 52–55 °C. (Found: C, 65.9; H, 6.0; N, 6.2. $C_{12}H_{13}NO_3$ requires C, 65.7; H, 6.0; N, 6.4%). λ_{max}: 209 nm (ε 23,000 cm^{-1} M^{-1}), 244 (17,000), 313 (7900). ν_{max}: 1684, 1589, 1271, 1222, 1152 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.64 (s, 3H, COMe), 3.85 and 3.92 (2s, 6H, OMe), 6.47 (d, $J=2.3$ Hz, 1H, H6), 6.50 (d, $J=3.6$ Hz, 1H, H3), 6.64 (d, $J=2.3$ Hz, 1H, H4), 7.59 (d, $J=3.6$ Hz, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 25.8 (CO*Me*), 55.7 (2C, OMe), 95.2, 97.2 and 108.0 (aryl CH), 119.5 (aryl C), 128.2 (aryl CH), 134.5, 148.5 and 157.4 (aryl C), 169.5 (*C*OMe). Mass spectrum: *m/z* 220 (M+1, 6%), 219 (M, 48), 177 (100), 162 (73), 134 (61), 131 (25), 119 (36), 103 (29).

4.2.5. 5,7-Dimethoxy-1-phenylsulfonylindole (5). Dry dimethyl sulfoxide (4 mL) was added to powdered potassium hydroxide (0.23 g, 4.16 mmol) and the reaction mixture was stirred at room temperature for 10 min. 5,7-Dimethoxyindole **1** (0.20 g, 1.13 mmol) was added, and the stirring was continued for 1 h. The solution was decanted from the excess potassium hydroxide and transferred to another reaction flask containing freshly distilled phenylsulfonyl chloride (1.44 mL, 11.3 mmol). The reaction mixture was cooled briefly in ice, then placed under a nitrogen atmosphere and stirred at room temperature for 5 h. The reaction mixture was then diluted with water and extracted with diethyl ether. The organic phase was washed successively with water and saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), and concentrated to give a brown oil (0.11 g). Separation using radial chromatography (dichloromethane) afforded the phenylsulfonylindole **5** as a cream solid (74 mg, 21%). The product was too unstable to be characterised fully. ^{1}H NMR spectrum ($CDCl_3$): δ 3.62 and 3.79 (2s, 6H, OMe), 6.31 (d, $J=2.0$ Hz, 1H, H6), 6.57 (d, $J=3.6$ Hz, 1H, H3), 6.60 (d, $J=2.3$ Hz, 1H, H4), 7.50 (m, 3H, ArH), 7.79 (d, $J=3.8$ Hz, 1H, H2), 7.83 (m, 2H, ArH). Mass spectrum: *m/z* 318 (M+1, 3%), 317 (M, 12), 177 (62), 162 (41), 141 (100), 134 (54). HRMS (ES) Found: M+Na$^+$, 340.0618. $C_{16}H_{15}NO_4S$ requires M+Na$^+$, 340.0614.

4.2.6. Methyl 5,7-dimethoxyindole-2-carboxylate (6). A solution of sodium methoxide was prepared via the portionwise addition of sodium (1.18 g, 51.3 mmol) to anhydrous methanol (20 mL), with stirring under a nitrogen atmosphere. The methoxide solution was cooled in a salt-ice slurry and stirred during the dropwise addition of a solution of 3,5-dimethoxybenzaldehyde (1.03 g, 6.2 mmol) and methyl azidoacetate (7.1 g, 62 mmol) in anhydrous methanol (20 mL), under a nitrogen atmosphere. The resulting mixture was stirred in ice for a further 3 h with cooling and then poured onto crushed ice. The resulting precipitate was filtered, washed with water, and dried to give methyl 2-azido-3-(3,5-dimethoxyphenyl)propenoate (1.42 g, 87%) as a pale yellow powder, mp 94–97 °C. (Found: C, 55.6; H, 5.4; N, 14.1. $C_{12}H_{13}N_3O_4$. 0.2 $C_{12}H_{13}NO_4$ requires C, 55.7; H, 5.1; N, 14.4%). λ_{max}: 239 nm (ε 8310 cm^{-1} M^{-1}), 311 (19,400); ν_{max}: 2108, 1717, 1698, 1597, 1437, 1384, 1293, 1245, 1208, 1155, 1089, 1053 cm^{-1}; ^{1}H NMR spectrum ($CDCl_3$): δ 3.81 (s, 6H, OMe), 3.91 (s, 3H, CO_2Me), 6.47 (t, 1H, $J=2.3$ Hz, H4′), 6.83 (s, 1H, C*H*=C), 6.98 (d, 2H, $J=2.3$ Hz, H2′ and H6′). ^{13}C NMR spectrum ($CDCl_3$): 52.89 (CO_2*Me*), 55.35 (OMe), 101.83 (C4′), 108.44 (C2′ and C6′), 125.62 and 134.63 (aryl C and CH=*C*), 160.45 (C3′ and C5′), 163.88 (*C*O_2Me). Mass spectrum: *m/z* 263 (M, 9%), 235 (31), 203 (100), 176 (95), 174 (60), 160 (24), 146 (31), 133 (21), 121 (22), 116 (31); HRMS (ES) Found: M$^+$, 263.0906. $C_{12}H_{13}N_3O_4$ requires M$^+$, 263.0906.

A solution of methyl 2-azido-3-(3′,5′-dimethoxyphenyl)propenoate (6.73 g, 25.6 mmol) in 1,2-dichlorobenzene (100 mL) was added dropwise over a period of 2 h to 1,2-dichlorobenzene (25 mL), heated at reflux. The solvent was then distilled under reduced pressure, the remaining residue was boiled in *n*-hexane, and then filtered hot to give the indole ester **6** (5.09 g, 85%) as a colourless granular solid, mp 125–128 °C. (Found: C, 61.3; H, 5.6; N, 6.0. $C_{12}H_{13}NO_4$ requires C, 61.3; H, 5.6; N, 6.0%). λ_{max}: 240 nm (ε 21,200 cm^{-1} M^{-1}), 275 (9040, sh), 294 (19,400), 326 (4400). ν_{max}: 3321, 1704, 1591, 1539, 1436, 1310, 1244, 1206, 1148, 1096 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$): δ 3.83, 3.92, and 3.93 (3s, 9H, OMe), 6.42 (d, 1H, $J=1.9$ Hz, H6), 6.64 (d, 1H, $J=1.9$ Hz, H4), 7.10 (d, 1H, $J=2.3$ Hz, H3), 8.95 (bs, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 51.82, 55.40 and 55.60 (OMe), 93.66 (C6), 97.32 (C4), 108.48 (C3), 123.74, 126.77, 127.82, 146.80 and 155.44 (aryl C), 162.10 (*C*O_2Me). Mass spectrum: *m/z* 235 (M, 60), 203 (100), 174 (46), 160 (22), 117 (22).

4.2.7. Methyl 5,7-dimethoxy-1-methylindole-2-carboxylate (7). A solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (1.00 g, 4.25 mmol) in dimethyl sulfoxide (10.0 mL) was stirred with powdered potassium hydroxide (0.63 g, 11 mmol) for 1 h at room temperature. Methyl iodide (0.45 mL, 7.2 mmol) was added and stirring continued for 2.5 h before addition of iced water. The resulting precipitate was filtered, washed with water, dried, and purified via flash column chromatography (dichloromethane) to give indole **7** (0.995 g, 94%) as a white solid, mp 76–79 °C. (Found: C, 62.73; H, 6.14; N, 5.72. $C_{13}H_{15}NO_4$ requires C, 62.64; H, 6.07; N, 5.62%). λ_{max}: 242 nm (ε 23,900 cm^{-1} M^{-1}), 274 (7710, sh), 283 (9290, sh), 297 (19,100), 334 (4280). ν_{max}: 1711, 1627, 1588, 1531, 1475, 1426, 1393, 1318, 1240, 1204, 1165, 1144, 1086, 1047, 815, 800, 732 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$): δ 3.82, 3.88 and 3.92 (3s, 9H, OMe), 4.32 (s, 3H, NMe), 6.58 and 6.39 (d, 2H, $J=2.3$ Hz, H4 and H6), 7.12 (s, 1H, H3). ^{13}C NMR spectrum ($CDCl_3$): δ 34.99 (NMe), 51.82, 55.83, and 55.92 (OMe), 94.18 (C6), 98.39 (C4), 110.66 (C3), 126.29, 127.87, 128.47, 149.12 and 155.36 (aryl C), 162.83 (*C*O_2Me). Mass spectrum: *m/z* 250 (20%), 249 (M, 100), 234 (76), 206 (38), 191 (32), 174 (27), 160 (21), 129 (22).

4.2.8. Dimethyl 1,3-phenylenebis(5,7-dimethoxyindol-1-ylmethyl-2-carboxylate) (8). A solution of indole 6 (0.25 g, 1.06 mmol) in dimethyl sulfoxide (5.0 mL) was stirred with powdered potassium hydroxide (74 mg, 1.3 mmol) for 70 min at room temperature. α,α'-Dibromo-*m*-xylene (0.14 g, 0.53 mmol) was added and stirring continued for 24 h. The solution was then acidified with dilute hydrochloric acid and the resulting precipitate was filtered, washed with water, and dried. Purification via gravity column chromatography (dichloromethane) gave indole **8** (0.27 g, 89%) as a pale yellow glassy solid, mp 69–73 °C. (Found: C, 67.1; H, 5.8; N, 5.0. $C_{32}H_{32}N_2O_8$ requires C, 67.1; H, 5.6; N, 4.9%). λ_{max}: 242 nm (ε 41,000 cm^{-1} M^{-1}), 277 (19,000, sh), 297 (33,000), 335 (8500). ν_{max}: 1717, 1582, 1536, 1478, 1457, 1322, 1239, 1208, 1156, 1093 cm^{-1}. ^{1}H NMR spectrum ($CDCl_3$): δ 3.54, 3.76, and 3.84 (3s, 9H, OMe), 5.98 (s, 4H, CH_2), 6.28 (d, 2H, $J=2.3$ Hz, H4), 6.51 (s, 1H, xylenyl H2), 6.59 (d, 2H, $J=2.3$ Hz, H6), 6.81 (dd, 2H, $J=7.9$, 7.5 Hz, xylenyl H4 and H6), 7.07 (t, 1H, $J=7.9$, 7.5 Hz, xylenyl H5), 7.13 (s, 2H, H3). ^{13}C NMR spectrum ($CDCl_3$): δ 50.14 (CH_2), 51.79, 55.60, and 55.95 (OMe), 94.33 (C4), 98.79 (C6), 111.79 (C3), 124.25, 125.01, and 128.44 (xylenyl CH), 125.90, 128.09, 128.13, 140.73, 148.82 and 155.56 (aryl C), 162.42 (*C*O_2Me). Mass spectrum: *m/z* 573 (M+1, 1%), 440 (100), 426 (71), 371 (23), 332 (34), 321 (21), 266 (24), 252 (44), 228 (43).

4.2.9. *N*-Trifluoroacetyl-1-(4-bromophenyl)-2-(2,4-dimethoxyphenylamino)-ethanone (9). A mixture of 2,4-dimethoxyaniline (5.00 g, 32.6 mmol), 4-bromophenacyl bromide (10.89 g, 39.2 mmol), sodium hydrogen carbonate (5.49 g, 65.3 mmol) in absolute ethanol (100 mL) was refluxed for 4 h. The reaction mixture was allowed to cool to room temperature and the solvent removed under reduced pressure. The resulting oil was redissolved in dry acetone and filtered twice to remove sodium hydrogen carbonate.

The solvent was removed under reduced pressure, affording a red-brown oil. This was redissolved in dry distilled tetrahydrofuran (130 mL) followed by the addition of trifluoroacetic anhydride (10.1 mL, 71.5 mmol) and pyridine (5.82 mL, 72.0 mmol). The reaction mixture was stirred at room temperature for 7 h. The solvent was removed under reduced pressure, to afford a dark brown oil, which was chromatographed on silica (70:30 dichloromethane–petroleum ether) to yield the amidoketone **9** (6.22 g, 43%) as colourless prisms, mp 141–142 °C (from ethyl acetate and petroleum ether). (Found: C, 48.6; H, 3.1; N, 3.0. $C_{18}H_{15}BrF_3NO_4$ requires C, 48.5; H, 3.4; N, 3.1%). λ_{max}: 206 nm (ε 42,000 cm^{-1} M^{-1}), 231 (14,000), 257 (21,000). ν_{max}: 1704, 1687, 1588, 1213, 1195 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.81 and 3.84 (2s, 6H, OMe), 4.31 and 5.65 (d, $J=17.4$ Hz, 2H, CH_2); 6.44 (dd, $J=2.6$, 8.7 Hz, 1H, H5), 6.48 (d, $J=2.6$ Hz, 1H, H3); 7.46 (bd, $J=8.7$ Hz, 1H, H6), 7.61 and 7.79 (m, 4H, aryl H). ^{13}C NMR spectrum ($CDCl_3$): δ 55.5 and 55.7 (OMe), 56.5 (CH_2), 99.1 and 103.8 (aryl CH), 116.2 (q, $J=288$ Hz, CF_3); 120.8 and 129.0 (aryl C), 129.4 (2C, aryl CH), 131.5 (aryl CH), 132.1 (2C, aryl CH), 133.6 and 156.0 (aryl C), 158.0 (q, $J=36$ Hz, $\mathit{C}OCF_3$), 161.5 (ArC), 191.1 (CO). Mass spectrum: *m*/*z* 447 (M, ^{81}Br, 27%), 445 (M, ^{79}Br, 28), 262 (56), 183 (40), 165 (100), 150 (34).

4.2.10. Methyl 2-azido-3-(3′,4′,5′-trimethoxyphenyl)propenoate.[16] A solution of sodium methoxide was prepared via the portionwise addition of sodium (10.3 g, 0.448 mmol) to anhydrous methanol (160 mL) with stirring under a nitrogen atmosphere. The methoxide solution was cooled in a salt-ice slurry and stirred during the dropwise addition of a solution of 3,4,5-trimethoxybenzaldehyde (14.50 g, 73.9 mmol) and ethyl azidoacetate (63.6 g, 493 mmol) in anhydrous methanol (175 mL) over 2 h. The resulting mixture was further stirred for 6.5 h with cooling and then poured onto crushed ice. The resulting precipitate was filtered, washed with water, and dried to give the title compound (18.11 g, 84%) as a pale yellow powder, mp 86–89 °C. ^1H NMR spectrum ($CDCl_3$): δ 3.89 (3s, 9H, OMe), 3.91 (s, 3H, OMe), 6.84 (s, 1H, CH), 7.09 (s, 2H, H2′ and H6′).

4.2.11. Methyl 5,6,7-trimethoxyindole-2-carboxylate (24).[16] A solution of methyl 2-azido-3-(3′,4′,5′-trimethoxyphenyl)-propenoate (17.84 g, 60.8 mmol) in 1,2-dichlorobenzene (250 mL) was added dropwise over 3 h to stirred 1,2-dichlorobenzene (25 mL) held at reflux. The resulting solution was heated at reflux for a further 40 min and then distilled under reduced pressure. The residue was triturated with light petroleum and the resulting precipitate was filtered, washed with light petroleum, and dried to give the title compound (14.73 g, 91%) as a pale yellow powder, mp 106–110 °C (lit.[16] 101–103 °C). ^1H NMR spectrum ($CDCl_3$): δ 3.89 (s, 3H, OMe), 3.92 (s, 6H, OMe), 4.06 (s, 3H, OMe), 6.81 (s, 1H, H4), 7.10 (d, 1H, $J=2.3$ Hz, H3), 8.98 (bs, 1H, NH).

4.2.12. Methyl 5,6,7-trimethoxy-*N*-methylindole-2-carboxylate (27).[17] A solution of methyl 5,6,7-trimethoxyindole-2-carboxylate **24** (0.50 g, 1.88 mmol) in dimethyl sulfoxide (15.0 mL) was stirred with powdered potassium hydroxide (0.224 g, 4.0 mmol) at room temperature for 75 min. Methyl iodide (0.13 mL, 2.1 mmol) was added and stirring continued for 1 h before the red solution was poured into water. The resulting precipitate was filtered, washed with water, and dried to give the title compound (0.48 g, 92%) as an off-white powder, mp 92–94 °C (lit.[17] syrup). λ_{max}: 239 nm (ε 14,700 cm^{-1} M^{-1}), 304 (20,800). ν_{max}: 1709, 1483, 1473, 1451, 1241, 1227, 1146, 1113, 1083, 994 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.88 (s, 6H, OMe), 3.92 and 3.99 (2s, 6H, OMe), 4.29 (s, 3H, NMe), 6.79 (s, 1H, H4), 7.14 (s, 1H, H3). ^{13}C NMR spectrum ($CDCl_3$): δ 33.47 (NMe), 51.35, 56.02, 61.20 and 61.67 (OMe), 98.01 (C4), 110.45 (C3), 122.54, 127.98, 128.17, 140.72, 141.80 and 149.52 (aryl C), 162.21 ($\mathit{C}O_2Me$). Mass spectrum: *m*/*z* 279 (M, 100), 264 (89), 221 (31), 206 (22).

4.2.13. Methyl 1-(*tert*-butyloxycarbonyl)-5,6,7-trimethoxyindole-2-carboxylate (30). A mixture of methyl 5,6,7-trimethoxyindole-2-carboxylate **24** (1.00 g, 3.77 mmol), di-*tert*-butyl dicarbonate (1.65 g, 7.56 mmol), and 4-dimethylaminopyridine (91 mg, 0.76 mmol) in acetonitrile (20.0 mL) was stirred at 40 °C for 1.5 h under a nitrogen atmosphere. The solvent was removed and the residue purified via flash column chromatography (20% light petroleum/dichloromethane) to give indole **30** (1.31 g, 95%) as a white powder, mp 109–112 °C. (Found: C, 59.5; H, 6.2; N, 4.1. $C_{18}H_{23}NO_7$ requires C, 59.2; H, 6.3; N, 3.8%). λ_{max}: 239 nm (ε 12,000 cm^{-1} M^{-1}), 276 (8860, sh), 299 (19,900). ν_{max}: 1760, 1715, 1489, 1431, 1371, 1256, 1234, 1157, 1124, 1060 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 1.66 (s, 9H, cme_3), 3.88, 3.89, 3.92 and 3.96 (4s, 12H, OMe), 6.79 and 7.09 (s, 2H, H3 and H4). ^{13}C NMR spectrum ($CDCl_3$): δ 27.79 ($C\mathit{Me}_3$), 52.39, 56.61, 61.66 and 61.72 (OMe), 85.45 ($\mathit{C}Me_3$), 98.44 (C4), 111.62 (C3), 122.82, 126.96, 127.69, 140.27, 142.39, 151.00 and 151.27 (CO and aryl C), 161.47 ($\mathit{C}O_2Me$). Mass spectrum: *m*/*z* 365 (M, 5%), 265 (47), 250 (31), 233 (36), 218 (25), 57 (100).

4.3. Formylation of indoles

4.3.1. Formylation of 5,7-dimethoxyindole (1). An ice-cold solution of phosphoryl chloride (0.32 mL, 4.05 mmol) in dimethylformamide (1 mL) was added dropwise to a stirred ice-cooled solution of indole **1** (1.32 g, 4.00 mmol) in dimethylformamide (1 mL). The reaction mixture was allowed to come to room temperature and then made strongly basic with sodium hydroxide solution (20%). The resulting yellow precipitate was filtered, washed with water, dried and purified by column chromatography (dichloromethane) to give two products.

(i) 5,7-Dimethoxyindole-3-carbaldehyde **10** (0.55 g, 66%), mp 130 °C. (Found: C, 64.3; H, 5.7; N, 6.6. $C_{11}H_{11}NO_3$ requires C, 64.4, H, 5.4; N, 6.8%). λ_{max}: 242 nm (ε 8300 cm^{-1} M^{-1}), 247 (18,000), 264 (12,000), 308 (7100). ν_{max}: 1623, 1283, 1234, 1157, 785 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.85 and 3.88 (2s, 6H, OMe), 6.41 (d, $J=2.1$ Hz, 1H, H6), 7.34 (d, $J=2.1$ Hz, 1H, H4), 7.73 (d, $J=3.2$ Hz, 1H, H2), 9.55 (bs, 1H, NH), 9.98 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 55.49 and 55.85 (OMe), 94.68, 96.39 and 135.01 (C4, C6 and C2), 119.82, 122.41 and 125.64 (C3, C3a and C7a), 146.45 and 157.63 ($\mathit{C}OMe$), 185.54 (CHO). Mass spectrum: *m*/*z* 206 (M+1, 13%), 205 (M, 100), 190 (37), 162 (34).

(ii) 5,7-Dimethoxyindole-4-carbaldehyde **11** (0.27 g, 33%), mp 176–177 °C. (Found: C, 64.3; H, 5.7; N, 6.6. $C_{11}H_{11}NO_3$ requires C, 64.4; H, 5.4; N, 6.8%). λ_{max}: 222 nm (ε 8300 cm^{-1} M^{-1}), 238 (7700), 254 (8900), 247 (14,000). ν_{max}: 1632, 1575, 1324, 1171 cm^{-1}. ^{1}H NMR spectrum (CDCl$_3$): δ 3.83 and 3.92 (2s, 6H, OMe), 6.20 (s, 1H, H6), 7.11 (t, J=2.6 Hz, 1H, H3), 7.16 (t, J=2.8 Hz, 1H, H2); 9.79 (bs, 1H, NH), 10.40 (s, 1H, CHO). ^{13}C NMR spectrum (DMSO-d_6): δ 56.2 and 57.3 (OMe), 89.8, 103.0 and 152.7 (C6, C2 and C3), 109.7, 121.4 and 126.9 (C3a, C4 and C7a), 152.7 and 161.9 (*C*OMe), 187.5 (CHO). Mass spectrum: *m/z* 206 (M+1, 15%), 205 (M, 100), 204 (37), 189 (24), 188 (24).

4.3.2. Formylation of 5,7-dimethoxyindole (1) with excess reagent: 5,7-dimethoxyindole-3,4-dicarbaldehyde (12). An ice-cold solution of phosphoryl chloride (0.64 mL, 8.1 mmol) in dimethylformamide (1 mL) was added dropwise to a stirred ice-cooled solution of indole **1** (1.32 g, 4.00 mmol) in dimethylformamide (1 mL). The reaction mixture was allowed to come to room temperature and then warmed to 40 °C for 30 min. On cooling it was made strongly basic with sodium hydroxide solution (20%). The resulting yellow precipitate was filtered off, washed with water, and dried to give dialdehyde **12** (0.88 g, 94%), mp 249.5–251.5 °C (from methanol). (Found: C, 61.8; H, 4.8; N, 5.9. $C_{12}H_{11}NO_4$ requires C, 61.8; H, 4.8; N, 6.0%). λ_{max}: 212 nm (ε 4900 cm^{-1} M^{-1}), 245 (7500), 290 (8300), 330 (8600). ν_{max}: 1647, 1625, 1573, 1221, 1149 cm^{-1}. ^{1}H NMR spectrum (DMSO-d_6): δ 3.98 and 4.09 (s, 6H, OMe), 6.73 (s, 1H, H6), 8.03 (d, J=3.4 Hz, 1H, H2), 10.52 and 10.66 (2s, 2H, CHO), 12.66 (br s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6): δ 56.3 and 57.2 (OMe), 91.1 (aryl CH), 111.0, 120.1, 122.4 and 123.2 (aryl C), 133.9 (aryl CH), 152.9 and 163.3 (aryl C), 188.3 and 189.4 (CHO). Mass spectrum: *m/z* 233 (M, 100%), 232 (M−1, 31), 218 (22), 205 (73), 190 (46), 174 (27), 162 (43), 148 (34), 134 (22), 119 (23).

4.3.3. 6,8-Dimethoxypyrrolo[4,3,2-de]isoquinoline (13). A mixture of 5,7-dimethoxyindole-3,4-dicarbaldehyde **12** (3.00 g, 12.9 mmol), concentrated aqueous ammonia solution (3.0 mL, excess) and 95% methanol (360 mL) was heated under reflux for 3 h. After cooling to room temperature, the solvent was removed under reduced pressure and the product dried under vacuum to yield compound **13** (2.74 g, 99%) as a bright yellow powder, mp 175–177 °C. (Found: C, 67.4; H, 4.9; N, 12.9. $C_{12}H_{10}N_2O_2$ requires C, 67.3; H, 4.7; N, 13.1%). λ_{max}: 211 nm (ε 17,000 cm^{-1} M^{-1}), 223 (21,000), 268 (14,000), 302 (4400), 332 (4300), 344 (5500), 393 (4400). ν_{max}: 1646, 1615, 1581, 1225, 1186, 938, 836 cm^{-1}. ^{1}H NMR spectrum (CDCl$_3$): δ 4.04 and 4.62 (2s, 6H, OMe), 6.37 (s, 1H, H7), 8.59 (s, 1H, H2), 9.14 and 9.33 (2s, 2H, H3 and H5). ^{13}C NMR spectrum (CDCl$_3$): δ 56.3 and 61.2 (OMe), 101.0 (aryl CH), 115.1, 127.2, 128.5 and 134.9 (aryl C), 143.4, 144.7 and 151.5 (aryl CH), 159.1 and 160.8 (aryl C). Mass spectrum: *m/z* 214 (M, 100%), 213 (65), 169 (49), 141 (42).

4.3.4. 6,8-Dimethoxy-4-nitrobenz[*cd*]indole (14). A mixture of 5,7-dimethoxyindole-3,4-dicarbaldehyde **12** (0.50 g, 2.14 mmol), nitromethane (0.12 mL, 2.22 mmol), potassium hydroxide (0.13 g, 2.23 mmol) in 95% methanol (75 mL) was heated under reflux for 3 h. The reaction mixture was allowed to cool to room temperature, and diluted with water. The product was extracted with dichloromethane, dried (Na$_2$SO$_4$), and concentrated to give a non-homogeneous yellow-black solid. Preparative thin layer chromatography (95:5 chloroform–methanol) afforded compound **14** (0.35 g, 63%) as a bright yellow powder, mp 188.5–190.5 °C. (Found: C, 60.7; H, 3.7; N, 10.7. $C_{13}H_{10}N_2O_4$ requires C, 60.5; H, 3.9; N, 10.8%). λ_{max}: 206 nm (ε 13,000 cm^{-1} M^{-1}), 245 (16,000), 281 (16,000), 341 (5200), 364 (5100), 390 (4200). ν_{max}: 2948, 1639, 1497, 1344, 1291, 1225, 1178, 749 cm^{-1}. ^{1}H NMR spectrum (CDCl$_3$): δ 4.06 and 4.62 (2s, 6H, OMe), 6.42 (s, 1H, H7), 8.59 (s, 1H, H2), 8.84 (d, J=1.6 Hz, 1H, H5), 9.09 (d, J=1.6 Hz, 1H, H3). ^{13}C NMR spectrum (CDCl$_3$): δ 56.6 and 61.5 (OMe), 101.5 (aryl CH), 117.4 (aryl C), 120.5 and 120.7 (aryl CH), 127.6, 132.9, 133.2 and 145.8 (aryl C), 152.1 (aryl CH), 159.8 and 161.3 (aryl C). Mass spectrum: *m/z* 259 (M+1, 30%), 258 (M, 100), 257 (M−1, 65), 229 (88), 228 (43), 183 (55), 182 (48), 139 (31).

4.3.5. Formylation of 5,7-dimethoxy-1-methylindole (2). To a stirred solution of 5,7-dimethoxy-1-methylindole **2** (0.40 g, 2.10 mmol) in dry dimethylformamide (5.0 mL) at 0 °C was added dropwise an ice-cold solution of phosphoryl chloride (0.29 mL, 3.11 mmol) in dry dimethylformamide (2.0 mL). The reaction mixture was stirred in ice for 10 min, then poured over ice and basified with 10% aqueous sodium hydroxide solution. After standing overnight at room temperature the solid was filtered, washed with water and dried. Preparative thin layer chromatography (dichloromethane) afforded two products.

(i) 5,7-Dimethoxy-1-methylindole-4-carbaldehyde **16** (34 mg, 7%) as a white powder, mp 140.5–142 °C. (Found: C, 66.0; H, 6.1; N, 6.3. $C_{12}H_{13}NO_3$ requires C, 65.7; H, 6.0; N, 6.4%). λ_{max}: 213 nm (ε 34,000 cm^{-1} M^{-1}), 228 (27,000), 252 (24,000), 258 (25,000), 356 (27,000). ν_{max}: 1630, 1577, 1337, 1256, 1213, 786 cm^{-1}. ^{1}H NMR spectrum (CDCl$_3$): δ 3.95, 3.98 and 4.00 (3s, 9H, OMe and NMe), 6.26 (s, 1H, H6), 7.03 (d, J=3.1 Hz, 1H, H3), 7.24 (d, J=3.1 Hz, 1H, H2), 10.52 (s, 1H, CHO). ^{13}C NMR spectrum (CDCl$_3$): δ 36.5 (NMe), 55.6 and 57.1 (OMe), 89.1 and 102.5 (aryl CH), 110.4, 121.6 and 128.8 (aryl C), 133.4 (aryl CH), 153.6 and 161.7 (aryl C), 188.7 (CHO). Mass spectrum: *m/z* 220 (M+1, 23%), 219 (M, 100), 218 (M−1, 47), 202 (29), 176 (32), 173 (29), 162 (39).

(ii) 5,7-Dimethoxy-1-methylindole-3-carbaldehyde **15** (0.29 g, 63%) as off-white needles, mp 127–128 °C (from ethyl acetate and petroleum ether). (Found: C, 65.5; H, 6.3; N, 6.1. $C_{12}H_{13}NO_3$ requires C, 65.7; H, 6.0; N, 6.4%). λ_{max}: 219 nm (ε 52,000), 246 (34,000), 251 (41,000), 265 (26,000), 315 (18,000). ν_{max}: 1650, 1275, 1202, 1150, 1117 cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 3.88 and 3.89 (2s, 6H, OMe), 4.04 (s, 3H, NMe), 6.39 (d, J=2.1 Hz, 1H, H6), 7.35 (d, J=2.5 Hz, 1H, H4), 7.45 (s, 1H, H2), 9.90 (s, 1H, CHO). ^{13}C NMR (DMSO-d_6): δ 37.1 (NMe), 55.3 and 55.8 (OMe), 94.3 and 96.2 (aryl CH), 116.9, 122.0 and 127.0 (aryl C), 141.5 (aryl CH), 147.9 and 156.9 (aryl C), 184.2 (CHO). Mass spectrum: *m/z* 219 (M, 100%), 204 (53), 176 (34), 161 (25).

4.3.6. Formylation of 5,7-dimethoxy-1-methylindole (2) with excess reagent: 5,7-dimethoxy-1-methylindole-3,4-dicarbaldehyde (17). Phosphoryl chloride (3.65 mL, 39.2 mmol) was added to an ice-cold solution of 5,7-dimethoxy-1-methylindole 2 (1.50 g, 7.8 mmol) in dry dimethylformamide (15 mL) with stirring and cooling in ice, and the reaction mixture was heated with stirring at 70 °C for 10 min. After cooling to room temperature, the reaction mixture was poured over ice and basified using 10% aqueous sodium hydroxide. After standing overnight, the precipitate was filtered, washed with water and dried to give dicarbaldehyde 17 (1.85 g, 95%) as yellow crystals, mp 218–219 °C (from methanol). (Found: C, 63.0; H, 5.5; N, 5.6. $C_{13}H_{13}NO_4$ requires C, 63.2; H, 5.3; N, 5.7%). λ_{max}: 295 nm (ε 14,000 cm^{-1} M^{-1}), 336 (14,000). ν_{max}: 1650, 1569, 1253, 1217, 1120, 1055, 857 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.96, 3.99 and 4.02 (3s, 9H, OMe and NMe), 6.31 (s, 1H, H6), 7.76 (s, 1H, H2), 10.54 and 10.81 (2s, 2H, CHO). ^{13}C NMR (DMSO-d_6: δ 37.7 (NMe), 56.6 and 57.2 (OMe), 91.6 (aryl CH), 110.8, 118.3, 122.2 and 124.7 (aryl C), 139.2 (aryl CH), 154.5 and 163.4 (aryl C), 188.1 and 189.0 (CHO). Mass spectrum: *m*/*z* 248 (M+1, 28%), 247 (M, 100), 219 (74), 204 (61), 176 (51), 162 (39).

4.3.7. Formylation of 1-benzyl-5,7-dimethoxyindole (3). Formylation of the benzylindole was carried out by the Vilsmeier–Haack method used for 5,7-dimethoxy-1-methylindole **2** starting from 1-benzyl-5,7-dimethoxyindole **3** (0.20 g, 0.75 mmol) in dry dimethylformamide (3.0 mL) and phosphoryl chloride (0.10 mL, 1.07 mmol) in dry dimethylformamide (1.5 mL). Preparative thin layer chromatography (dichloromethane) afforded 1-benzyl-5,7-dimethoxyindole-4-carbaldehyde **19** (77 mg, 34%) as a cream powder, mp 136.5–140 °C. (Found: C, 73.0; H, 6.0; N, 4.5. $C_{18}H_{17}NO_3$ requires C, 73.2; H, 5.8; N, 4.7%). λ_{max}: 209 nm (ε 24,000 cm^{-1} M^{-1}), 251 (12,000), 352 (11,000). ν_{max}: 1640, 1572, 1329, 1247, 1213, 1163 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.93 and 3.95 (2s, 6H, OMe), 5.54 (s, 2H, CH_2), 6.52 (s, 1H, H6), 7.08 (m, 3H, aryl H and H3), 7.25 (m, 3H, aryl H), 7.55 (d, $J=2.6$ Hz, 1H, H2), 10.39 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 52.6 (CH_2), 55.5 and 57.0 (OMe), 89.6 and 103.4 (aryl CH), 110.5 and 121.2 (aryl C), 126.3, 2C (aryl CH), 127.2 (aryl CH), 128.5, 2C (aryl CH), 129.2 (aryl C), 133.1 (aryl CH), 139.1, 153.3 and 161.7 (aryl C), 188.8 (CHO). Mass spectrum: *m*/*z* 295 (M, 73%), 91 (100).

1-Benzyl-5,7-dimethoxyindole-3-carbaldehyde **18** (0.14 g, 61%) was obtained as a cream powder, mp 144–145.5 °C. (Found: C, 73.1; H, 6.0; N, 4.6. $C_{18}H_{17}NO_3$ requires C, 73.2; H, 5.8; N, 4.7%). λ_{max}: 217 nm (ε 29,000 cm^{-1} M^{-1}), 245 (16,000), 251 (19,000), 265 (13,000), 313 (8100). ν_{max}: 1650, 1277, 1193, 1170, 1143 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.77 and 3.79 (2s, 6H, OMe), 5.60 (s, 2H, CH_2), 6.45 (d, $J=2.1$ Hz, 1H, H6), 7.26 (m, 6H, aryl H and H4), 8.28 (s, 1H, H2), 10.39 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 53.4 (CH_2), 55.4 and 55.8 (OMe), 94.8 and 97.1 (aryl CH), 118.5 and 122.2 (aryl C), 127.0, 2C (aryl CH), 127.7 (aryl C), 127.8 (aryl CH), 128.8, 2C (aryl CH), 137.4 (aryl C), 139.0 (aryl CH), 147.8 and 157.5 (aryl C), 184.6 (CHO). Mass spectrum: *m*/*z* 296 (M+1, 43%), 295 (M, 96), 204 (22), 190 (25), 174 (39), 159 (43), 131 (34), 91 (100).

4.3.8. Formylation of 1-benzyl-5,7-dimethoxyindole (3) with excess reagent: 1-benzyl-5,7-dimethoxyindole-3,4-dicarbaldehyde (20). Phosphoryl chloride (7.85 mL, 84.2 mmol) was added to an ice-cold solution of 1-benzyl-5,7-dimethoxyindole **3** (4.50 g, 16.8 mmol) in dry dimethylformamide (15 mL) with stirring and cooling in ice, and the reaction mixture was heated with stirring at 70 °C for 15 min. After cooling to room temperature, the reaction mixture was poured over ice and basified using concentrated aqueous sodium hydroxide solution. After standing overnight, the precipitate was filtered, washed with water and dried to give dicarbaldehyde **20** (5.40 g, 99%) as a white powder, mp 260.5–261 °C (from dichloromethane). (Found: C, 70.7; H, 5.5; N, 4.2. $C_{19}H_{17}NO_4$ requires C, 70.6; H, 5.3; N, 4.3%). λ_{max}: 295 nm (ε 10,000 cm^{-1} M^{-1}), 337 (12,000). ν_{max}: 1663, 1640, 1596, 1570, 1248, 1208, 1058, 857 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.96 and 3.97 (2s, 6H, OMe), 5.64 (s, 2H, CH_2), 6.67 (s, 1H, H6), 7.13 (m, 2H, aryl H), 7.29 (m, 3H, aryl H), 8.35 (s, 1H, H2), 10.48 and 10.62 (2s, 2H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 54.0 (CH_2), 55.7 and 57.1 (OMe), 90.8 (aryl CH), 112.2, 120.1, 122.3 and 125.8 (aryl C), 126.7, 2C (aryl CH), 127.9 (aryl CH), 128.8, 2C (aryl CH), 137.1 (aryl C), 137.8 (aryl CH), 153.8 and 163.6 (aryl C), 188.9 and 191.2 (CHO). Mass spectrum: *m*/*z* 323 (M, 23%), 91 (100).

4.3.9. Formylation of 1-acetyl-5,7-dimethoxyindole (4). Phosphoryl chloride (0.41 mL, 4.45 mmol) was added to a frozen solution of 1-acetyl-5,7-dimethoxyindole **4** (0.65 g, 2.97 mmol) in dry dimethylformamide (13 mL) under a dry nitrogen atmosphere at −94 °C. The reaction mixture was allowed to come to room temperature gradually over 2 h then stirred at room temperature for a further 3 h. The reaction mixture was poured over ice and basified with 10% aqueous sodium hydroxide solution. After standing overnight the solid was filtered, washed with water and dried, affording 4-carbaldehyde **11** (0.53 g, 87%) as a white powder. Confirmation of this product was obtained by comparison with an authentic sample using ^1H NMR spectroscopy and thin layer chromatography.

4.3.10. Formylation of 5,7-dimethylindole. Combination of ice-cold solutions of phosphoryl chloride (0.19 mL, 2.07 mmol) in dimethylformamide (1 mL) and 5,7-dimethylindole[14,15] (0.20 g, 1.38 mmol) in dimethylformamide (1 mL), followed by stirring at 0 °C for 15 min and treatment with sodium hydroxide solution (20%) gave 5,7-dimethylindole-3-carbaldehyde (0.22 g, 92%) as a pale yellow powder, mp 139 °C (lit.[15] 121–122 °C).

4.3.11. Formylation of methyl 5,7-dimethoxyindole-2-carboxylate (6). Dimethylformamide (5.0 mL) was cooled in an iced water bath, treated with phosphoryl chloride (3.0 mL, 32 mmol), and stirred for 20 min. This solution was then added dropwise, over 8 min, to a similarly cooled solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (3.00 g, 12.8 mmol) in dimethylformamide (15.0 mL) with stirring. The resulting solution was stirred further with cooling for 2 h before a small amount of chilled water was added and the mixture basified to high pH with 5 M sodium hydroxide. The mixture was then stirred at ambient temperature for 30 min before the resulting precipitate was filtered, washed with water, and dried to give the 4-formylindole ester **22** (3.34 g, 100%) as a cream coloured powder, mp 270–276 °C. (Found: C, 59.2; H, 5.0; N, 5.3. $C_{13}H_{13}NO_5$ requires C, 59.3; H, 5.0; N, 5.3%). λ_{max}: 243 nm

(ε 24,200 cm^{-1} M^{-1}), 325 (20,400), 354 (13,100), 370 (8850, sh). ν_{max}: 3431, 3127, 1721, 1624, 1573, 1527, 1473, 1378, 1345, 1260, 1222 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.94, 3.99, 4.07 (3s, 9H, OMe), 6.44 (s, 1H, H6), 7.95 (d, J=2.3 Hz, 1H, H3), 9.10 (bs, 1H, NH), 10.53 (s, 1H, CHO). ^{13}C NMR spectrum (DMSO-d_6): δ 57.51, 56.64, and 52.21 (OMe), 93.02 (C6), 109.02 (C3), 109.70, 123.89, 125.41, 130.34, 153.84, 161.52 (aryl C), 162.82 (CO_2Me), 187.48 (CHO). Mass spectrum: m/z 263 (M, 100%), 231 (68), 230 (64), 202 (100), 174 (42), 160 (41).

4.3.12. 4-Formyl-5,7-dimethoxyindole-2-carboxylic acid (23). Methyl 4-formyl-5,7-dimethoxyindole-2-carboxylate **22** (1.00 g, 3.80 mmol) was refluxed in aqueous/ethanolic (1:1) potassium hydroxide (0.5 M, 40 mL) for 35 min. After cooling, the yellow solution was acidified with 5 M hydrochloric acid and the resulting precipitate filtered through a frit. The precipitate was washed with water and dried to give the title compound (0.90 g, 95%) as a pale yellow powder, mp >275 °C (decomp.). (Found: C, 55.2; H, 4.9; N, 5.4. $C_{12}H_{11}NO_5$. 0.66 H_2O requires C, 55.2; H, 4.8; N, 5.4%). λ_{max}: 242 nm (ε 21,000 cm^{-1} M^{-1}), 262 (9000, sh), 324 (17,000), 352 (13,000, sh), 369 (8200, sh). ν_{max}: 3597, 3458, 1690, 1639, 1573, 1264, 1229, 1215 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.96 and 4.04 (2s, 6H, OMe), 6.68 (s, 1H, H6), 7.60 (d, J=1.9 Hz, 1H, H3), 10.37 (s, 1H, CHO), 11.97 (bs, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6) 56.57 and 57.52 (OMe), 92.74 (C6), 108.75 (C3), 109.87, 123.69, 125.64, 131.87, 153.75, 162.60, 162.69 (CO_2H and aryl C), 187.49 (CHO). Mass spectrum: m/z 249 (M, 100%), 231 (36), 230 (45), 202 (45), 188 (20), 160 (28).

4.3.13. Formylation of methyl 5,6,7-trimethoxyindole-2-carboxylate (24). Dimethylformamide (2.0 mL) was cooled in a salt-ice slurry and treated with phosphoryl chloride (0.3 mL, 3.2 mmol) and stirred for 15 min. The resulting solution was then added dropwise, over 5 min, to a similarly cooled and stirred solution of methyl 5,6,7-trimethoxyindole-2-carboxylate **24** (0.600 g, 2.26 mmol) in dimethylformamide (5.0 mL). The resulting solution was stirred at ambient temperature for 22 h and then heated for 2 h in an oil bath at 75 °C. After cooling, the mixture was poured onto crushed ice and basified to high pH with 5 M sodium hydroxide and then extracted with ethyl acetate. The organic extract was washed twice with brine, dried ($MgSO_4$), and concentrated to give a pale yellow powder. Purification via flash column chromatography (2% methanol/dichloromethane) gave two isomers.

(i) Methyl 3-formyl-5,6,7-trimethoxyindole-2-carboxylate **25** (0.392 g, 59%) was obtained as a white powder, mp 184–188 °C. (Found: C, 57.2; H, 5.0; N, 4.9. $C_{14}H_{15}NO_6$ requires C, 57.3; H, 5.2; N, 4.8%). λ_{max}: 233 nm (ε 16,000 cm^{-1} M^{-1}), 251 (20,000), 277 (7700, sh), 335 (13,000). ν_{max}: 3132, 3112, 1721, 1649, 1463, 1450, 1271, 1247, 1216, 1118 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.92, 3.95, 4.03 and 4.09 (4s, 12H, OMe), 7.61 (s, 1H, H4), 9.47 (bs, 1H, NH), 10.68 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 52.70, 56.23, 61.25 and 61.37 (OMe), 98.48 (C4), 119.74, 121.37, 124.50, 130.37, 138.56, 140.89, 152.83 (aryl C), 160.70 (CO_2Me), 188.44 (CHO). Mass spectrum: m/z 293 (M, 32%), 279 (100), 264 (85), 246 (27), 221 (35), 206 (31), 204 (29), 149 (21).

(ii) Methyl 4-formyl-5,6,7-trimethoxyindole-2-carboxylate **26** (0.169 g, 25%) was obtained as a pale yellow powder, mp 140–143 °C. (Found: C, 57.5; H, 5.1; N, 4.7. $C_{14}H_{15}NO_6$ requires C, 57.3; H, 5.2; N, 4.8%). λ_{max}: 232 nm (ε 14,000 cm^{-1} M^{-1}), 258 (16,000), 277 (5100, sh), 344 (18,000). ν_{max}: 3337, 3227, 1716, 1697, 1649, 1568, 1281, 1261, 1215, 1201, 1138, 1100, 1038, 924, 766 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.93 (s, 6H, OMe), 4.02 and 4.20 (2s, 6H, OMe), 7.91 (d, J=2.3 Hz, 1H, H3), 9.25 (bs, 1H, NH), 10.47 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 52.07, 61.00, 61.60 and 63.33 (OMe), 109.96 (C3), 117.20, 120.98, 127.52, 129.35, 141.38, 144.97 and 157.37 (aryl C), 162.00 (CO_2Me), 189.31 (CHO). Mass spectrum: m/z 293 (M, 100%), 278 (41), 261 (25), 260 (22), 246 (68), 232 (29), 218 (23), 190 (21), 188 (32), 174 (29), 160 (45), 144 (27), 132 (21), 117 (20), 104 (25).

4.3.14. Formylation of methyl 5,6,7-trimethoxy-1-methylindole-2-carboxylate. Dimethylformamide (1.0 mL) was cooled in a salt-ice slurry and treated with phosphoryl chloride (0.2 mL, 2 mmol) and stirred for 15 min. The resulting solution was then added dropwise, over 3 min, to a similarly cooled and stirred solution of methyl 5,6,7-trimethoxy-*N*-methylindole-2-carboxylate **27** (0.250 g, 0.895 mmol) in dimethylformamide (3.0 mL). The resulting solution was stirred at ambient temperature for 6 h and then heated for 30 min in an oil bath at 75 °C. After re-cooling in a salt-ice slurry, the mixture was treated with chilled water, basified to high pH with 5 M sodium hydroxide, and then extracted with ethyl acetate. The aqueous layer was kept aside (see below), while the organic extract was washed with brine, dried ($MgSO_4$), and concentrated to give methyl 3-formyl-5,6,7-trimethoxy-*N*-methylindole-2-carboxylate **28** (42 mg, 15%) as an off-white powder. ^1H NMR spectrum ($CDCl_3$): δ 3.92 and 3.94 (2s, 6H, OMe), 4.01 (s, 6H, OMe), 4.28 (s, 3H, NMe), 7.75 (s, 1H, H4), 10.48 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 34.46 (NMe), 52.37, 56.10, 61.17 and 61.79 (OMe), 98.94 (C4), 119.48, 121.48, 126.37, 133.52, 140.67, 142.21 and 152.29 (aryl C), 161.13 (CO_2Me), 188.21 (CHO).

4.3.15. 3-Formyl-5,6,7-trimethoxy-*N*-methylindole-2-carboxylic acid (29). The basified aqueous layer from the above reaction was acidified to low pH with 5 M hydrochloric acid and extracted with ethyl acetate. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated to give the title compound (0.190 g, 69%) as a yellow powder, mp >173 °C (dec.). (Found: C, 57.3; H, 5.2; N, 4.6. $C_{14}H_{15}NO_6$ requires C, 57.3; H, 5.2; N, 4.8%). λ_{max}: 256 nm (ε 18,000 cm^{-1} M^{-1}), 333 (13,000). ν_{max}: 1698, 1591, 1513, 1470, 1413, 1372, 1245, 1115, 1041, 1020 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.94, 3.97 and 4.04 (s, 9H, OMe), 4.52 (s, 3H, NMe), 7.21 (s, 1H, H4), 10.14 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 56.17, 61.27 and 61.84 (OMe), 35.34 (NMe), 94.21 (C4), 160.17 (CO_2Me), 115.64, 124.19, 126.60, 131.93, 141.80, 142.60 and 152.90 (aryl C), 186.42 (CHO). Mass spectrum: m/z 293 (M, 100%), 278 (27), 249 (22), 234 (62), 206 (21), 191 (65), 176 (43), 173 (39), 163 (31), 155 (35), 149 (25), 148 (24), 147 (21), 146 (20).

4.4. Acylation of indoles

4.4.1. Acetylation of 5,7-dimethylindole. Combination of ice-cold solutions of phosphoryl chloride (1.28 mL, 13.7 mmol), dimethylacetamide (3.8 mL, 40.9 mmol) and 5,7-dimethylindole (0.20 g, 1.38 mmol), followed by heating at 40–60 °C for 43 h and treatment with sodium hydroxide solution (20%) gave 3-acetyl-5,7-dimethylindole **21** (0.21 g, 82%) as a pale brown powder, mp 223–224 °C. (Found: C, 77.0; H, 7.3; N, 7.2. $C_{12}H_{13}NO$ requires C, 77.0; H, 7.0; N, 7.5%). λ_{max}: 210 nm (ε 6100 cm^{-1} M^{-1}), 244 (16,000), 300 (12,000). ν_{max}: 3264, 1637, 1530, 1306, 1148, 927 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 2.35 (s, 3H, COMe), 2.43 and 2.44 (2s, 6H, Me), 6.83 (s, 1H, H6), 7.81 (s, 1H, H4), 8.21 (d, $J=2.5$ Hz, 1H, H2), 11.77 (br s, 1H, NH). Mass spectrum: *m/z* 187 (M, 45%), 172 (100), 144 (18).

4.4.2. Acetylation of 5,7-dimethoxyindole (1). Phosphoryl chloride (1.05 mL, 11.3 mmol) was added to ice-cold dimethylacetamide (3.2 mL, 33.9 mmol) with stirring and cooling in ice. 5,7-Dimethoxyindole **1** (0.20 g, 1.13 mmol) was added with stirring, and the reaction mixture was stirred at room temperature for 2 h, then poured over ice and basified with 10% aqueous sodium hydroxide solution. After standing overnight, the precipitate was filtered, washed with water and dried. Preparative thin layer chromatography (dichloromethane) yielded two products.

(i) 4-Acetyl-5,7-dimethoxyindole **32** (72 mg, 29%) as a white powder, mp 198.5–201 °C. (Found: C, 65.5; H, 6.2; N, 6.1. $C_{12}H_{13}NO_3$ requires C, 65.7; H, 6.0; N, 6.4%). λ_{max}: 208 nm (ε 25,000), 224 (16,000), 252 (11,000), 336 (13,000). ν_{max}: 3258, 1612, 1562, 1311, 1212, 1173, 1099 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 2.52 (s, 3H, COMe), 3.94 and 4.02 (2s, 6H, OMe), 6.57 (s, 1H, H6), 6.91 (t, $J=2.6$, 2.6 Hz, 1H, H3), 7.27 (t, $J=2.6$, 2.6 Hz, 1H, H2), 11.28 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 32.9 (CO*Me*), 55.5 and 57.2 (OMe), 90.1 and 104.9 (aryl CH), 112.9 and 121.9 (aryl C), 126.2 (aryl CH), 128.5, 149.7 and 157.8 (aryl C), 199.3 (*C*OMe). Mass spectrum: *m/z* 219 (M, 85%), 204 (100), 189 (57), 146 (47), 118 (49), 103 (41).

(ii) 3-Acetyl-5,7-dimethoxyindole **31** (0.10 g, 41%) as a white powder, mp 174.5–176 °C. (Found: C, 65.5; H, 6.2; N, 6.2. $C_{12}H_{13}NO_3$ requires C, 65.7; H, 6.0; N, 6.4%). λ_{max}: 213 nm (ε 28,000 cm^{-1} M^{-1}), 243 (15,000), 248 (17,000), 262 (10,000), 308 (7500). ν_{max}: 3204, 1613, 1297, 1221, 1209, 1140 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.51 (s, 3H, COMe), 3.87 and 3.88 (2s, 6H, OMe), 6.40 (d, $J=2.0$ Hz, 1H, H6), 7.45 (d, $J=2.0$ Hz, 1H, H4), 7.75 (d, $J=3.1$ Hz, 1H, H2), 9.16 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 27.4 (CO*Me*), 55.4 and 55.8 (OMe), 95.1 and 95.7 (aryl CH), 118.6, 122.0 and 126.5 (aryl C), 130.9 (aryl CH), 146.3 and 157.3 (aryl C), 193.9 (*C*OMe). Mass spectrum: *m/z* 219 (M, 69%), 204 (100), 189 (25), 146 (20).

4.4.3. Acetylation of 5,7-dimethoxy-1-methylindole (2). Phosphoryl chloride (0.98 mL, 10.5 mmol) was added to ice-cold dimethylacetamide (2.92 mL, 31.4 mmol) with stirring and cooling in ice. 5,7-Dimethoxy-1-methylindole **2** (0.20 g, 1.05 mmol) was added with stirring, and the reaction mixture was stirred at room temperature for 2 h, then poured over ice and basified with 10% aqueous sodium hydroxide solution. After standing overnight, the precipitate was filtered, washed with water and dried. Preparative thin layer chromatography (dichloromethane) yielded two products.

(i) 4-Acetyl-5,7-dimethoxy-1-methylindole **34** (16 mg, 7%) as a white powder, mp 83–84 °C. λ_{max}: 213 nm (ε 6000 cm^{-1} M^{-1}), 219 (5100), 256 (3500), 343 (3300). ν_{max}: 1620, 1567, 1330, 1239, 1211, 1108, 972 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.64 (s, 3H, COMe), 3.94 and 3.98 (2s, 3H and 6H, OMe and NMe), 6.32 (s, 1H, H6), 6.98 (d, $J=3.1$ Hz, 1H, H3), 7.07 (d, $J=3.1$ Hz, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 33.0 (CO*Me*), 36.6 (NMe), 55.5 and 57.1 (OMe), 90.2 and 102.8 (aryl CH), 112.6, 122.0 and 130.1 (aryl C), 132.5 (aryl CH), 151.4 and 157.3 (aryl C), 199.2 (*C*OMe). Mass spectrum: *m/z* 233 (M, 80%), 218 (100), 203 (33), 160 (30), 132 (44). HRMS (ES) Found: $M+Na^+$, 256.0941. $C_{13}H_{15}NO_3$ requires $M+Na^+$, 256.0944.

(ii) 3-Acetyl-5,7-dimethoxy-1-methylindole **33** (0.19 g, 79%) was obtained as a white powder, mp 164–165 °C. (Found: C, 67.1; H, 6.7: N, 5.8. $C_{13}H_{15}NO_3$ requires C, 66.9; H, 6.5; N, 6.0%). λ_{max}: 218 nm (ε 30,000 cm^{-1} M^{-1}), 250 (19,000), 264 (11,000), 315 (9700). ν_{max}: 1635, 1615, 1586, 1280, 1214, 1144, 1111 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.44 (s, 3H, COMe), 3.85, 3.87 and 3.98 (3s, 9H, OMe and NMe), 6.34 (d, $J=2.0$ Hz, 1H, H6), 7.44 (s, 1H, H2), 7.46 (d, $J=2.0$ Hz, 1H, H4). ^{13}C NMR spectrum ($CDCl_3$): δ 27.3 (CO*Me*), 37.2 (NMe), 55.3 and 55.6 (OMe), 95.0 and 95.9 (aryl CH), 116.4, 122.2 and 128.5 (aryl C), 136.3 (aryl CH), 147.9 and 157.0 (aryl C), 192.8 (*C*OMe). Mass spectrum: *m/z* 234 (M+1, 33%), 233 (M, 92), 218 (100), 203 (37), 190 (42), 175 (64), 160 (63), 109 (39).

4.4.4. Acetylation of 1-benzyl-5,7-dimethoxyindole (3). Phosphoryl chloride (0.28 mL, 3.00 mmol) was added to ice-cold dimethylacetamide (0.85 mL, 9.14 mmol) with stirring and cooling in ice. 1-Benzyl-5,7-dimethoxyindole **3** (80 mg, 0.30 mmol) was added with stirring, and the reaction mixture was stirred at 50–60 °C for 3.5 h, then poured over ice and basified with 10% aqueous sodium hydroxide solution. The turbid yellow solution was heated at 100 °C briefly, then cooled, diluted with water and extracted with dichloromethane. The extract was washed with water, dried (Na_2SO_4), and concentrated to give a brown oil. Preparative thin layer chromatography (dichloromethane) yielded two products.

(i) 4-Acetyl-1-benzyl-5,7-dimethoxyindole **36** (19 mg, 20%) as a white powder, mp 111–112 °C. λ_{max}: 216 nm (ε 29,000 cm^{-1} M^{-1}), 250 (17,000), 260 (11,000), 314 (8300). ν_{max}: 1633, 1575, 1335, 1247, 727 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.65 (s, 3H, COMe), 3.86 and 3.93 (2s, 6H, OMe), 5.55 (s, 2H, CH_2), 6.31 (s, 1H, H6), 7.02 (m, 2H, aryl H), 7.12 and 7.16 (d, $J=3.0$ Hz, 2H, H2 and H3), 7.25 (m, 3H, aryl H). ^{13}C NMR spectrum ($CDCl_3$): δ 33.0 (CO*Me*), 52.5 (CH_2), 55.4 and 57.0 (OMe), 90.6 and 103.7 (aryl CH), 112.7 and 121.5 (aryl C), 126.3, 2C (aryl

CH), 127.1 (aryl CH), 128.5, 2C (aryl CH), 130.4 (aryl C), 132.2 (aryl CH), 139.4, 151.1 and 157.3 (aryl C), 199.4 (*C*OMe). Mass spectrum: *m/z* 309 (M, 84%), 295 (21), 294 (100), 91 (54). HRMS (ES) Found: $M+Na^+$, 332.1283. $C_{19}H_{19}NO_3$ requires $M+Na^+$, 332.1257.

(ii) 3-Acetyl-1-benzyl-5,7-dimethoxyindole **35** (56 mg, 60%) was obtained as a white powder, mp 151–152 °C. (Found: C, 73.6; H, 6.3; N, 4.3. $C_{19}H_{19}NO_3$ requires C, 73.8; H, 6.2; N, 4.5%). λ_{max}: 216 nm (ε 36,000 cm^{-1} M^{-1}), 250 (21,000), 265 (13,000), 316 (11,000). ν_{max}: 1636, 1534, 1495, 1294, 1211, 1146, 975, 832 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.46 (s, 3H, COMe), 3.79 and 3.89 (2s, 6H, OMe), 5.59 (s, 2H, CH_2), 6.38 (d, $J=2.2$ Hz, 1H, H6), 7.11 (m, 2H, aryl H); 7.30 (m, 3H, aryl H), 7.51 (d, $J=2.2$ Hz, 1H, H4), 7.58 (s, 1H, H2). ^{13}C NMR ($CDCl_3$): δ 27.4 (CO*Me*), 53.2 (CH_2), 55.4 and 55.8 (OMe), 95.3 and 96.5 (aryl CH), 117.4 and 122.0 (aryl C), 126.7, 2C (aryl CH), 127.6 (aryl CH), 128.7, 2C (aryl CH), 135.6 (aryl CH), 138.0, 147.7 and 157.3 (aryl C), 193.1 (*C*OMe). Mass spectrum: *m/z* 309 (M, 45%), 173 (24), 145 (33), 91 (100).

4.4.5. Friedel–Crafts acetylation of 1-benzyl-5,7-dimethoxyindole (3): 3,4-diacetyl-1-benzyl-5,7-dimethoxyindole (37). A solution of anhydrous tin (IV) chloride (2.2 mL, 18.8 mmol) in dry benzene (10 mL) was added dropwise with stirring to an ice-cold suspension of 1-benzyl-5,7-dimethoxyindole **3** (0.50 g, 1.87 mmol) in dry benzene (40 mL) containing acetyl chloride (1.33 mL, 18.7 mmol). The reaction mixture was heated at 40–50 °C for 20 min. Water (100 mL) was added to the reaction mixture, which was then stirred at room temperature for 2 h. The benzene layer was separated and the aqueous layer was extracted with dichloromethane. The dichloromethane and benzene solutions were combined, dried (Na_2SO_4), and concentrated to yield a black oil. Flash column chromatography (98:2 chloroform–methanol) yielded 3,4-diacetyl-1-benzyl-5,7-dimethoxyindole **37** (0.22 g, 34%) as an off-white powder, mp 190–191.5 °C. (Found: C, 71.6; H, 6.3; N, 3.8. $C_{21}H_{21}NO_4$ requires C, 71.8; H, 6.0; N, 4.0%). λ_{max}: 203 nm (ε 45,000), 248 (22,000), 317 (11,000). ν_{max}: 1696, 1647, 1579, 1285, 1204, 737 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.41 and 2.69 (2s, 6H, COMe), 3.82 and 3.84 (2s, 6H, OMe), 5.60 (s, 2H, CH_2), 6.44 (s, 1H, H6), 7.09 (m, 2H, aryl H), 7.31 (m, 3H, aryl H), 7.62 (s, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 27.1 and 33.0 (CO*Me*), 53.4 (CH_2), 55.6 and 57.6 (OMe), 93.2 (aryl CH), 117.5, 118.4, 122.6 and 123.5 (aryl C), 126.5, 2C (aryl CH), 127.7 (aryl CH), 128.8, 2C (aryl CH), 137.0 (aryl CH), 137.7, 147.9 and 153.0 (aryl C), 191.1 and 203.6 (*C*OMe). Mass spectrum: *m/z* 351 (M, 16%), 336 (50), 91 (100).

4.4.6. Methyl 4-acetyl-5,7-dimethoxyindole-2-carboxylate (38). A solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (0.30 g, 1.28 mmol) and acetyl chloride (0.4 mL, 6 mmol) in dichloromethane (10.0 mL) was stirred under a nitrogen atmosphere with cooling *via* a salt-ice slurry. Antimony pentachloride (0.4 mL, 3 mmol) was added dropwise over 2 min and stirring continued for 80 min with cooling. The dark mixture was then poured onto crushed ice and extracted with dichloromethane. The organic extract was washed with water, dried ($MgSO_4$), concentrated and the residue was purified by gravity column chromatography (dichloromethane) to give compound **38** (0.212 g, 60%) as a pale yellow powder, mp 217–220 °C. (Found: C, 60.5; H, 5.5; N, 5.2. $C_{14}H_{15}NO_5$ requires C, 60.6; H, 5.5; N, 5.1%). λ_{max}: 242 nm (ε 22,400 cm^{-1} M^{-1}), 264 (11,700, sh), 317 (16,800), 346 (11,300, sh), 362 (5960, sh). ν_{max}: 3309, 1703, 1634, 1580, 1532, 1439, 1251, 1210, 1172, 984 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.65 (s, 3H, Me), 3.92, 3.96 and 4.02 (3s, 9H, OMe), 6.46 (s, 1H, H6), 7.82 (d, $J=2.6$ Hz, 1H, H3), 9.02 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 32.75 (CO*Me*), 51.88, 55.62 and 57.08 (OMe), 92.74 (C6), 110.63 (C3), 113.23, 123.55, 127.57, 128.71, 150.23 and 158.11 (aryl C), 162.13 (*C*O_2Me), 198.51 (*C*OMe). Mass spectrum: *m/z* 277 (M, 40%), 262 (33), 230 (100).

4.4.7. Methyl 4-trifluoroacetyl-5,7-dimethoxyindole-2-carboxylate (39). Trifluoroacetic anhydride (0.32 mL, 2.3 mmol) was added dropwise with stirring to an ice-cooled solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (0.50 g, 2.13 mmol) in anhydrous tetrahydrofuran (10.0 mL), under a nitrogen atmosphere. The mixture was stirred at ambient temperature for 3 days. Water was then added and the resulting precipitate filtered, washed with water, and dried to give compound **39** (0.62 g, 88%) as a cream powder, mp 201–205 °C. (Found: C, 51.0; H, 3.8; N, 4.2. $C_{14}H_{12}F_3NO_3$ requires C, 50.8; H, 3.7; N, 4.2%). λ_{max}: 250 nm (ε 18,200 cm^{-1} M^{-1}), 274 (6720, sh), 340 (14,700), 359 (14,200, sh). ν_{max}: 3305, 1720, 1655, 1573, 1301, 1256, 1216, 1193, 1156, 1132, 981 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.94, 3.97 and 4.06 (3s, 9H, OMe), 6.43 (s, 1H, H6), 7.64 (d, $J=2.3$ Hz, 1H, H3), 9.15 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 52.06, 55.89 and 57.00 (OMe), 92.03 (C6), 106.76 (aryl C), 109.29 (C3), 114.79 and 118.62 (CO*C*F_3), 123.42, 128.03, 129.36, 152.68 and 160.19 (aryl C), 161.77 (*C*O_2Me), 180.85 and 180.37 (*C*OCF_3). Mass spectrum: *m/z* 331 (M, 28%), 262 (53), 230 (100).

4.4.8. 4-Trifluoroacetyl-5,7-dimethoxyindole-2-carboxylic acid (40). Methyl 4-trifluoroacetyl-5,7-dimethoxyindole-2-carboxylate **39** (0.250 g, 0.755 mmol) was heated under reflux in aqueous ethanolic (1:1) potassium hydroxide (0.5 M, 10 mL) for 20 min. After cooling, the orange solution was acidified with 10% aqueous hydrochloric acid and the resulting precipitate filtered through a frit, washed with water, and dried to give the acid **40** (0.164 g, 69%) as an off-white powder, mp >250 °C (decomp.). (Found: C, 49.4; H, 3.3; N, 4.7. $C_{13}H_{10}F_3NO_5$ requires C, 49.2; H, 3.2; N, 4.4%). λ_{max}: 248 nm (ε 17,800 cm^{-1} M^{-1}), 277 (6930, sh), 340 (13,900), 361 (13,300, sh). ν_{max}: 3465, 1692, 1661, 1584, 1298, 1167, 1142 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.96 and 4.07 (2s, 6H, OMe), 6.74 (s, 1H, H6), 7.35 (s, 1H, H3), 12.21 (bs, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6): δ 56.62 and 57.37 (OMe), 92.89 (C6), 104.70 (aryl C), 108.13 (C3), 115.01 and 118.85 (CO*C*F_3), 123.69, 127.34, 131.80, 154.48 and 160.92 (aryl C), 162.16 (CO_2H), 178.90 and 179.37 (*C*OCF_3). Mass spectrum: *m/z* 317 (M, 37%), 248 (62), 230 (100), 144 (20).

4.4.9. Methyl 4-trifluoroacetyl-3-formyl-5,7-dimethoxyindole-2-carboxylate (41). A solution was prepared via the dropwise addition of dimethylformamide (0.36 mL, 4.7 mmol) to phosphoryl chloride (1.0 mL) in a salt-ice slurry and then stirred for 15 min. This solution was then added dropwise over 5 min, to a similarly cooled and stirred solution of ester **39** (0.150 g, 0.453 mmol) in phosphoryl chloride (3.0 mL). The mixture was stirred at room temperature for 3 h and then with heating at 60 °C for 2 days before it was poured onto crushed ice, and treated with solid sodium hydrogen carbonate over 2 h. The resulting mixture was diluted with water and extracted with dichloromethane. The organic extract was washed with water, dried ($MgSO_4$), and concentrated to give a yellow residue. Purification via flash column chromatography (1% methanol/dichloromethane) gave compound **41** (0.159 g, 98%) as a yellow powder, mp 170–175 °C. (Found: C, 50.3; H, 3.3; N, 3.9. $C_{15}H_{12}F_3NO_6$ requires C, 50.2; H, 3.4; N, 3.9%). λ_{max}: 256 nm (ε 14,000 cm^{-1} M^{-1}), 284 (8500, sh), 317 (11,000), 349 (6600). ν_{max}: 3310, 1741, 1715, 1661, 1271, 1204, 1137 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.89 (s, 3H, OMe), 4.04 (s, 6H, OMe), 6.59 (s, 1H, H6), 9.61 (bs, 1H, NH), 10.47 (s, 1H CHO). ^{13}C NMR spectrum ($CDCl_3$): δ 52.99, 55.87 and 57.67 (OMe), 94.29 (C6), 108.71 (aryl C), 114.43 and 118.28 (CO*C*F_3), 119.09, 121.54, 122.30, 131.70, 148.84 and 156.74 (aryl C), 160.04 (*C*O_2Me), 182.29 and 182.79 (*C*OCF_3), 186.34 (CHO). Mass spectrum: *m*/*z* 359 (M, 15%), 290 (37), 258 (100).

4.5. Reactions of indoles with oxalyl chloride and amines: formation of glyoxylic amides

4.5.1. *N*-Methyl-2-(5,7-dimethoxyindol-3-yl)glyoxylamide (42). 5,7-Dimethoxyindole **1** (0.20 g, 1.13 mmol) was dissolved in anhydrous diethyl ether (10 mL), oxalyl chloride (0.25 mL, 2.87 mmol) was added and the mixture stirred for 1.75 h at room temperature under a nitrogen atmosphere. The resulting red precipitate was filtered off, added to methylamine (10 mL, 24% aqueous solution) and stirred for 1 h. The yellow precipitate was filtered off, washed with water and dried to yield the 3′-glyoxylamide **42** (0.17 g, 57%) as a bright yellow powder, mp 232 °C (lit.[7] 228–229 °C) (from ethyl acetate). λ_{max}: 275 nm (ε 13,000 cm^{-1} M^{-1}), 340 (9200). ν_{max}: 3350, 1612, 1223, 1144 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.97 (d, $J=5.6$ Hz, 3H, N-Me), 3.91 and 3.93 (2s, 6H, OMe), 6.43 (d, $J=2.0$ Hz, 1H, H6), 7.47 (d, $J=2.0$ Hz, 1H, H4), 7.49 (bs, 1H, NHMe), 8.94 (bs, 1H, NH), 8.98 (d, $J=3.1$ Hz, 1H, H2). ^{13}C NMR (DMSO-d_6): δ 25.6 (NMe), 55.3 and 55.5 (OMe), 95.1 and 95.5 (aryl CH), 112.6, 121.2 and 127.8 (aryl C), 137.1 (aryl CH), 146.8 and 157.1 (aryl C), 164.1 (CONH), 181.9 (CO). Mass spectrum: *m*/*z* 262 (M, 24%), 204 (100), 189 (27). Treatment of the filtrate with excess methylamine (24% aqueous solution) gave a further crop of the 3′-glyoxylamide 42 (59 mg, 20%).

4.5.2. *N*-Methyl-2-(5,7-dimethoxy-1-methylindol-3-yl)glyoxylamide (43). 5,7-Dimethoxy-1-methylindole 2 (0.30 g, 1.57 mmol) was dissolved in anhydrous diethyl ether (10 mL), oxalyl chloride (0.34 mL, 3.90 mmol) was added, and the reaction mixture was stirred for 15 min at room temperature under a nitrogen atmosphere. The resulting red precipitate was filtered off, added to methylamine (10 mL, 24% aqueous solution) and stirred for 1 h. The yellow precipitate was filtered off, washed with water and dried to yield the 3′-glyoxylamide **43** (0.34 g, 78%) as pale yellow needles, mp 170 °C (from ethyl acetate and petroleum ether). (Found: C, 61.0; H, 6.1; N, 9.9. $C_{14}H_{16}N_2O_4$ requires C, 60.9; H, 5.8; N, 10.1%). λ_{max}: 216 nm (ε 36,000 cm^{-1} M^{-1}), 258 (17,000), 277 (11,000), 348 (9000). ν_{max}: 3367, 1618, 1294, 775 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.95 (d, $J=5.1$ Hz, 3H, NH*Me*; 3.89 (s, 6H, OMe), 4.04 (s, 3H, NMe), 6.39 (d, $J=2.1$ Hz, 1H, H6), 7.49 (d, $J=2.0$ Hz, 1H, H4), 7.52 (bs, 1H, N*H*Me), 8.75 (s, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 25.8 (NHMe), 37.7 (NMe), 55.4 and 55.7 (OMe), 95.5 and 96.4 (aryl CH), 111.6, 121.5 and 130.2 (aryl C), 142.2 (aryl CH), 148.1 and 157.9 (aryl C), 163.5 (CONH), 179.7 (CO). Mass spectrum: *m*/*z* 276 (M, 16%), 218 (100).

4.5.3. *N*-Methyl-2-(1-benzyl-5,7-dimethoxyindol-3-yl)glyoxylamide (44). 1-Benzyl-5,7-dimethoxyindole **3** (0.30 g, 1.12 mmol) was dissolved in anhydrous diethyl ether (10 mL), oxalyl chloride (0.24 mL, 2.75 mmol) was added, and the reaction mixture was stirred for 15 min at room temperature under a nitrogen atmosphere. The resulting red precipitate was filtered off, added to methylamine (10 mL, 24% aqueous solution) and stirred for 1 h. The yellow precipitate was filtered off, washed with water and dried to yield the 3′-glyoxylamide **44** (0.35 g, 89%) as pale yellow needles, mp 212.5–214 °C (from ethyl acetate and petroleum ether). (Found: C, 68.2; H, 5.9; N, 7.8. $C_{20}H_{20}N_2O_4$ requires C, 68.2; H, 5.7; N, 8.0%). λ_{max}: 278 (ε 9900 cm^{-1} M^{-1}), 348 (8400). ν_{max}: 3364, 1613, 1296, 1204, 1163, 776 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.94 (d, $J=5.1$ Hz, 3H, NMe), 3.79 and 3.89 (2s, 6H, OMe), 5.58 (s, 2H, CH_2), 6.39 (d, $J=2.1$ Hz, 1H, H6), 7.15 (m, 2H, aryl H), 7.26 (m, 3H, aryl H), 7.51 (bs, 1H, NH), 7.53 (d, $J=2.6$ Hz, 1H, H4), 8.91 (s, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 25.8 (NHMe), 53.5 (CH_2), 55.4 and 55.7 (OMe), 95.6 and 96.8 (aryl CH), 112.2 and 121.0 (aryl C), 126.8, 2C (aryl CH), 127.6 (aryl CH), 128.6, 2C, (aryl CH), 130.3 and 137.4 (aryl C), 141.8 (aryl CH), 147.8 and 157.9 (aryl C), 163.4 (CONH), 179.9 (CO). Mass spectrum: *m*/*z* 352 (M, 10%), 294 (40), 91 (100).

4.5.4. 2-(5,7-Dimethoxy-1-methylindol-3-yl)glyoxylamide (45). 5,7-Dimethoxy-1-methylindole 2 (1.30 g, 6.82 mmol) was dissolved in anhydrous diethyl ether (40 mL), oxalyl chloride (1.49 mL, 17.1 mmol) was added, and the reaction mixture was stirred for 15 min at room temperature under a nitrogen atmosphere. The red precipitate was filtered off, added to concentrated aqueous ammonia solution (10 mL) and stirred for 1 h. The yellow precipitate was filtered off, washed with water and dried to yield glyoxylamide 45 (1.48 g, 90%) as a yellow powder, mp 255 °C. (Found: C, 59.8; H, 5.1; N, 10.5. $C_{13}H_{14}N_2O_4$ requires C, 59.5; H, 5.4; N, 10.7%). λ_{max}: 258 nm (ε 11,000 cm^{-1} M^{-1}), 279 (8200), 346 (7400). ν_{max}: 3448, 3152, 1691, 1612, 1594, 1274, 1224, 1104, 850 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.79 and 3.88 (2s, 6H, OMe), 4.02 (s, 3H, NMe), 6.47 (d, $J=2.1$ Hz, 1H, H6), 7.36 (d, $J=2.0$ Hz, 1H, H4), 7.69 and 8.01 (2bs, 2H, NH_2), 8.50 (s, 1H, H2). ^{13}C NMR spectrum (DMSO-d_6): δ 37.1 (NMe), 55.3 and 55.8 (OMe), 95.1 and 96.1 (aryl CH), 110.9, 121.2 and 129.3 (aryl C), 141.6 (aryl CH), 148.0 and 157.2 (aryl C), 166.0 ($CONH_2$), 182.2 (CO). Mass spectrum: *m*/*z* 262 (M, 29%), 218 (100).

4.5.5. *N*,*N*-Dimethyl-2-(1-benzyl-5,7-dimethoxyindol-3-yl)glyoxylamide (46). Glyoxylamide **46** was prepared by the method used for glyoxylamide **45** using 1-benzyl-5,7-dimethoxyindole **3** (0.30 g, 1.12 mmol), oxalyl chloride (0.15 mL, 1.72 mmol), anhydrous diethyl ether (40 mL) and 40% aqueous dimethylamine solution (2×10 mL, excess). Glyoxylamide **46** (0.37 g, 89%) was obtained as pale tan plates, mp 174–175 °C (from dichloromethane and petroleum ether). (Found: C, 68.7; H, 6.0; N, 7.5. $C_{21}H_{22}N_2O_4$ requires C, 68.8; H, 6.1; N, 7.6%). λ_{max}: 273 nm (e 12,000 cm^{-1} M^{-1}), 330 (9900). ν_{max}: 1622, 1286, 1242, 1051, 981, 936, 827 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.06 and 3.07 (2s, 6H, NMe_2), 3.78 and 3.89 (2s, 6H, OMe), 5.56 (s, 2H, CH_2), 6.39 (d, $J=2.0$ Hz, 1H, H6), 7.12 (m, 2H, aryl H), 7.29 (m, 3H, aryl H), 7.46 (d, $J=2.1$ Hz, 1H, H4), 7.75 (s, 1H, H2). ^{13}C NMR spectrum ($CDCl_3$): δ 34.4 and 37.5 (NMe), 53.4 (CH_2), 55.4 and 55.7 (OMe), 95.2 and 96.9 (aryl CH), 113.6 and 121.8 (aryl C), 126.7, 2C (aryl CH), 127.6 (aryl CH), 128.6, 2C (aryl CH), 128.8 and 137.5 (aryl C), 138.9 (aryl CH), 147.7 and 157.7 (aryl C), 167.5 ($CONMe_2$), 185.6 (CO). Mass spectrum: *m/z* 366 (M, 5%), 294 (100).

4.5.6. *N*-Methyl-2-(4-formyl-5,7-dimethoxy-1-methylindol-3-yl)glyoxylamide (47). To a stirred solution of glyoxylamide **43** (0.20 g, 0.73 mmol) in dry dimethylformamide (5.0 mL) at 0 °C was added dropwise an ice-cold solution of phosphoryl chloride (0.14 mL, 1.50 mmol) in dry dimethylformamide (1.5 mL). The reaction mixture was stirred at room temperature for 24 h, then poured over ice, basified with 10% aqueous sodium hydroxide solution and allowed to stand overnight, and the solution was extracted with dichloromethane. The extract was washed with water, dried (Na_2SO_4), and concentrated to give an orange powder. Preparative thin layer chromatography (95:5 chloroform–methanol) afforded glyoxylamide **47** (40 mg, 18%) as yellow crystals, mp 219–221 °C (from dichloromethane and petroleum ether). λ_{max}: 212 nm (ε 26,000 cm^{-1} M^{-1}), 220 (24,000), 248 (18,000), 319 (14,000). ν_{max}: 3389, 1693, 1652, 1636, 1579, 1290, 1224 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 2.66 (d, $J=4.6$ Hz, 3H, NH*Me*), 3.93, 4.01 and 4.04 (3s, 9H, OMe and NMe), 6.68 (s, 1H, H6), 8.13 (s, 1H, H2), 8.49 (d, $J=5.1$ Hz, 1H, NHMe), 10.27 (s, 1H, CHO). ^{13}C NMR spectrum ($CDCl_3$): d 25.9 (NHMe), 37.7 (NMe), 55.8 and 57.1 (OMe), 91.3 (aryl CH), 111.9, 113.6, 122.2 and 126.7 (aryl C), 140.7 (aryl CH), 152.9 (aryl C), 160.8 and 163.3 (aryl C and CONH), 182.3 (CO), 188.9 (CHO). Mass spectrum: *m/z* 304 (M, 11%), 246 (100). HRMS (ES) Found: $M+Na^+$, 327.0953. $C_{15}H_{16}N_2O_5$ requires $M+Na^+$, 327.0951.

4.5.7. Methyl 4-oxamoyl-5,7-dimethoxyindole-2-carboxylate (48). A stirred solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (0.100 g, 0.425 mmol) and oxalyl chloride (0.07 mL, 0.8 mmol) in anhydrous diethyl ether (10.0 mL) was heated at reflux under nitrogen for 27 h. The mixture was then cooled in a salt-ice slurry and treated with aqueous ammonia (2.5 M, 6.0 mL). The open flask was heated for 15 min, the ether allowed to evaporate, and the resulting precipitate was filtered through a frit, washed with water, and dried to give glyoxylamide **48** (0.10 g, 79%) as a cream powder, mp 254–258 °C. (Found: C, 52.4; H, 5.1; N, 8.5. $C_{14}H_{14}N_2O_6.0.8H_2O$ requires C, 52.3; H, 4.9; N, 8.7%). λ_{max}: 242 nm (ε 20,000 cm^{-1} M^{-1}), 277 (7600, sh), 325 (15,000), 353 (11,000, sh). ν_{max}: 3404, 3306, 3171, 1698, 1618, 1573, 1259, 1216 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.84 (s, 6H, OMe), 4.04 (s, 3H, OMe), 6.73 (s, 1H, H6), 7.56 (s, 1H, H3), 7.26 and 7.68 (2s, 2H, $CONH_2$), 12.22 (s, 1H, indole NH) (the signals at 7.26, 7.68 and 12.22 ppm exchanged with D_2O). ^{13}C NMR spectrum (DMSO-d_6): δ 52.18, 56.63 and 58.18 (OMe), 94.48 (C6), 109.21 (C3), 107.97, 124.51, 126.79, 129.96, 153.25 and 160.86 (aryl C), 161.56 (CO_2Me), 170.44 ($CONH_2$), 190.88 (CO). Mass spectrum: *m/z* 306 (M, 10%), 262 (76), 230 (100), 144 (25).

4.5.8. Methyl 5,7-dimethoxy-4-(3′,5′-dimethoxyphenyloxamoyl)indole-2-carboxylate (49). A stirred solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (0.10 g, 0.43 mmol) and oxalyl chloride (0.07 mL, 0.8 mmol) in anhydrous diethyl ether (10 mL) was heated at reflux under nitrogen for 27 h. The mixture was then cooled in a salt-ice slurry and treated with 3,5-dimethoxyaniline (50 mg, 0.50 mmol). The open flask was then heated for 15 min, the ether allowed to evaporate, and the resulting precipitate was filtered through a frit, washed with water, and dried to give glyoxylamide **49** (0.40 g, 84%) as a cream powder, mp 212–215 °C (from methanol). (Found: C, 57.5; H, 5.2; N, 5.5. $C_{22}H_{22}N_2O_8.H_2O$ requires C, 57.4; H, 5.3; N, 6.1%). λ_{max}: 242 nm (ε 32,900 cm^{-1} M^{-1}), 280 (10,800, sh), 311 (20,000), 326 (18,000, sh). ν_{max}: 3310, 3229, 1696, 1614, 1579, 1259, 1213, 1171 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.81 (s, 6H, OMe), 3.89, 3.94 and 4.04 (3s, 9H, OMe), 6.29 (d, $J=1.9$ Hz, 1H, H4′), 6.43 (s, 1H, H6), 6.91 (d, $J=1.9$ Hz, 2H, H2′ and H6′), 7.57 (d, $J=2.3$ Hz, 1H, H3), 8.02 (s, 1H, indole NH), 9.12 (s, 1H, CONH) (the signals at 8.02 and 9.12 ppm exchanged with D_2O). ^{13}C NMR spectrum ($CDCl_3$): δ 52.59, 55.86, 56.38 and 58.49 (OMe), 93.46 (C6), 97.38 (C4′), 98.63 (C2′ and C6′), 109.46 (C3), 109.06, 124.21, 127.90, 129.58, 139.64, 152.63, 160.76 and 161.54 (aryl C), 162.39 and 163.87 (CONH and CO_2Me), 190.48 (CO). Mass spectrum: *m/z* 442 (M, 2%), 279 (100), 262 (40), 248 (20), 247 (95), 246 (34), 230 (34), 218 (83), 204 (26), 160 (22). HRMS Found: M^+, 442.1375. $C_{22}H_{22}N_2O_8$ requires M^+, 442,1376.

4.6. Mannich reaction

4.6.1. Methyl 5,7-dimethoxy-4-*N*,*N*-dimethylaminomethylindole-2-carboxylate (50). A solution of 40% aqueous dimethylamine (1.0 mL) and 38% aqueous formaldehyde (0.10 mL) in glacial acetic acid (1.0 mL) was added dropwise over 7 min to a stirred solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (0.25 g, 1.06 mmol) in glacial acetic acid (4.0 mL). The resulting solution was stirred for 2 days at ambient temperature before it was diluted with water, cooled in an iced water bath, and basified to high pH with 5 M sodium hydroxide. The resulting precipitate was filtered, washed with water, and dried to give compound **50** (0.24 g, 78%) as a fine white powder, mp 148–151 °C. (Found: C, 59.8; H, 6.8; N, 9.0. $C_{15}H_{20}N_2O_4\cdot0.5H_2O$ requires C, 60.0; H, 7.0; N, 9.3%). λ_{max}: 241 nm (ε 29,700 cm^{-1} M^{-1}), 296 (19,600), 331 (4750). ν_{max}: 3325, 1698, 1324, 1282, 1252, 1216 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 2.26 (s, 6H, NMe_2), 3.64 (s, 2H, CH_2), 3.86, 3.92 and 3.96 (3s, 9H, OMe), 6.52 (s, 1H, H6), 7.29 (s, 1H, H3), 8.94 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 45.37 (NMe_2), 54.31 (CH_2), 51.73, 55.44 and 58.05 (OMe), 94.67 (C6), 107.91 (C3), 110.98, 123.73, 127.22, 129.52, 145.95 and 152.88 (aryl C), 162.09 (CO_2Me). Mass spectrum: *m/z* 292 (M, 22%), 248 (82), 216 (100).

4.7. Bromination

4.7.1. Methyl 4-bromo-5,7-dimethoxyindole-2-carboxylate (51). Bromine (0.02 mL, 0.4 mmol) was added all at once to a vigorously stirred solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (91 mg, 0.39 mmol) in glacial acetic acid (5.0 mL) at room temperature. Stirring was continued for 30 s before water was added and the resulting emulsion extracted with ethyl acetate. The organic extract was washed twice with a saturated sodium hydrogen carbonate solution, then once with brine, dried ($MgSO_4$), and concentrated to give the bromo compound **51** (122 mg, 100%) as a white powder, mp 183–185 °C. (Found: C, 46.0; H, 4.0; N, 4.3. $C_{12}H_{12}BrNO_4$ requires C, 45.9; H, 3.9; N, 4.5%). λ_{max}: 241 nm (ε 33,800 $cm^{-1}\,M^{-1}$), 296 (20,600), 329 (5160). ν_{max}: 3312, 1704, 1316, 1249, 1211 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.93, 3.94 and 3.97 (3s, 9H, OMe), 6.55 (s, 1H, H6), 7.18 (d, $J=2.6$ Hz, 1H, H3), 9.04 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 51.98, 55.69 and 58.53 (OMe), 95.76 (C6), 108.70 (C3), 94.64, 123.79, 127.60, 129.11, 146.13 and 151.03 (aryl C), 161.76 (CO_2Me). Mass spectrum: *m/z* 315 (M, ^{81}Br, 61%), 313 (M, ^{79}Br, 60), 283 (85), 282 (25), 281 (100), 268 (26), 266 (24), 254 (34), 252 (36), 240 (37), 238 (34), 144 (35).

4.8. Nitration

4.8.1. Methyl 5,7-dimethoxy-4-nitroindole-2-carboxylate (52). A solution of methyl 5,7-dimethoxyindole-2-carboxylate **6** (0.20 g, 0.85 mmol) in dichloromethane (10.0 mL) was stirred at room temperature in the presence of concentrated nitric acid adsorbed on silica gel, for 35 min. The resulting dark mixture was diluted with dichloromethane and then immediately purified via flash column chromatography (dichloromethane) to give the nitro compound **52** (96 mg, 40%) as a yellow powder, mp 236–237 °C. (Found: C, 51.2; H, 4.3; N, 10.3. $C_{12}H_{12}N_2O_6$ requires C, 51.4; H, 4.3; N, 10.0%). λ_{max}: 237 nm (ε 19,000 $cm^{-1}\,M^{-1}$), 250 (15,000, sh), 273 (7400, sh), 333 (11,000), 370 (10,000). ν_{max}: 3304, 1704, 1286, 1249, 1214 cm^{-1}. ^1H NMR spectrum (DMSO-d_6): δ 3.85, 4.00 and 4.07 (3s, 9H, OMe), 6.77 (s, 1H, H6), 7.35 (d, $J=2.3$ Hz, 1H, H3), 12.59 (s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6): δ 52.36, 57.00 and 58.07 (OMe), 94.01 (C6), 108.06 (C3), 122.87, 123.41, 123.80, 130.41, 152.68 and 154.15 (aryl C), 161.16 (CO_2Me). Mass spectrum: *m/z* 280 (M, 58%), 249 (20), 248 (100), 219 (22), 218 (35), 216 (84), 172 (20), 145 (20), 144 (60).

4.8.2. Methyl 4-amino-5,7-dimethoxyindole-2-carboxylate (53). A suspension of methyl 5,7-dimethoxy-4-nitroindole-2-carboxylate **52** (66 mg, 0.24 mmol) and 10% palladium/charcoal (9 mg) in absolute ethanol (15.0 mL) was heated at reflux during the dropwise addition of a solution of hydrazine hydrate (0.2 mL) in absolute ethanol (1.5 mL) over 2 min. Heating was continued for 2 h before the hot mixture was filtered and concentrated. The remaining syrup was dissolved in dichloromethane and washed three times with brine, dried ($MgSO_4$), and concentrated to give a dark solid that was purified via flash column chromatography (dichloromethane) to give the amine **53** (35 mg, 59%) as a yellow powder, mp 141–144 °C. (Found: C, 57.4; H, 5.8; N, 11.4. $C_{12}H_{14}N_2O_4$ requires C, 57.6; H, 5.6; N, 11.2%). λ_{max}: 254 nm (ε 28,500 $cm^{-1}\,M^{-1}$), 299 (13,300), 367 (3880). ν_{max}: 3403, 3323, 1687, 1539, 1519, 1444, 1360, 1330, 1270 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): δ 3.86, 3.91 and 3.92 (3s, 9H, OMe), 6.56 (s, 1H, H6), 7.14 (d, $J=1.9$ Hz, 1H, H3), 8.91 (s, 1H, NH). ^{13}C NMR spectrum ($CDCl_3$): δ 52.33, 56.50 and 59.08 (OMe), 97.84 (C6), 106.17 (C3), 120.09, 123.18, 125.09, 126.86, 128.11 and 139.30 (aryl C), 162.46 (CO_2Me). Mass spectrum: *m/z* 250 (M, 45%), 235 (26), 218 (27), 203 (100), 202 (10), 109 (27).

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References and notes

1. Black, D. St.C.; Bowyer, M. C.; Bowyer, P. K.; Ivory, A. J.; Kim, M.; Kumar, N.; McConnell, D. B.; Popiolek, M. *Aust. J. Chem.* **1994**, *47*, 1741–1750.
2. Black, D. St.C.; Bowyer, M. C.; Catalano, M. M.; Ivory, A. J.; Keller, P. A.; Kumar, N.; Nugent, S. J. *Tetrahedron* **1994**, *50*, 10497–10508.
3. Black, D. St.C.; Craig, D. C.; Kumar, N. *Aust. J. Chem.* **1996**, *49*, 311–318.
4. Black, D. St.C.; Kumar, N.; McConnell, D. B. *Tetrahedron* **1996**, *52*, 8925–8936.
5. Süs, V. O.; Glos, M.; Müller, K.; Eberhardt, H. D. *Justus Liebigs Ann. Chem.* **1953**, *583*, 150–160.
6. Mulligan, P. J.; LaBerge, S. *J. Med. Chem.* **1970**, *13*, 1248–1249.
7. Crohare, R.; Merkuza, V. M.; Gonzalez, H. A.; Ruveda, E. A. *J. Heterocycl. Chem.* **1970**, *7*, 729–732.
8. Hemetsberger, H.; Knittel, D.; Weidmann, H. *Monatsh. Chem.* **1970**, *101*, 161–165.
9. Moody, C. J. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 7, pp 21–38.
10. Forster, M. O.; Fierz, H. E. *J. Chem. Soc.* **1908**, *93*, 72–85.
11. Knouzi, N.; Vaultier, M.; Carrié, R. *Bull. Soc. Chim. Fr.* **1985**, *5*, 815–819.
12. Allen, M. S.; Hamaker, L. K.; La Loggia, A. J.; Cook, J. M. *Synth. Commun.* **1992**, *22*, 2077–2102.
13. Lloyd, H. A.; Kielar, E. A.; Highet, R. J.; Uyeo, S.; Fales, H. M.; Wildman, W. C. *J. Org. Chem.* **1962**, *27*, 373–377.
14. Ezquerra, J.; Pedregal, C.; Lamas, C.; Barluenga, J.; Pérez, M.; García-Martín, M. A.; González, J. M. *J. Org. Chem.* **1996**, *61*, 5804–5812.
15. Hiremath, S. P.; Purohit, M. G. *Indian J. Chem.* **1974**, *12*, 493–495.
16. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H.; Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961–8971.
17. Muratake, H.; Okabe, K.; Takahashi, M.; Tonegawa, M.; Natsume, M. *Chem. Pharm. Bull.* **1997**, *45*, 799–806.

18. Giua, M. *Gazz. Chim. Ital.* **1924**, *54*, 593–597.
19. Black, D. St.C.; Kumar, N.; McConnell, D. B. *Tetrahedron* **1996**, *52*, 8925–8936.
20. Kruse, L. I.; Meyer, M. D. *J. Org. Chem.* **1984**, *49*, 4761–4768.
21. Tani, M.; Ikegami, H.; Tashiro, M.; Hiura, T.; Tsukioka, H.; Kaneko, C.; Notoya, T.; Shimizu, M.; Uchida, M.; Aida, Y.; Yokoyama, Y.; Murakami, Y. *Heterocycles* **1992**, *34*, 2349–2362.
22. Jones, A. W.; Purwono, B.; Bowyer, P. K.; Mitchell, P. S. R.; Kumar, N.; Nugent, S. J.; Jolliffe, K. A.; Black, D. St.C. *Tetrahedron* **2004**, *60*, 10779–10786.

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Hetero Diels–Alder reactions (HDAR) of α,α′-dioxothiones on solid support

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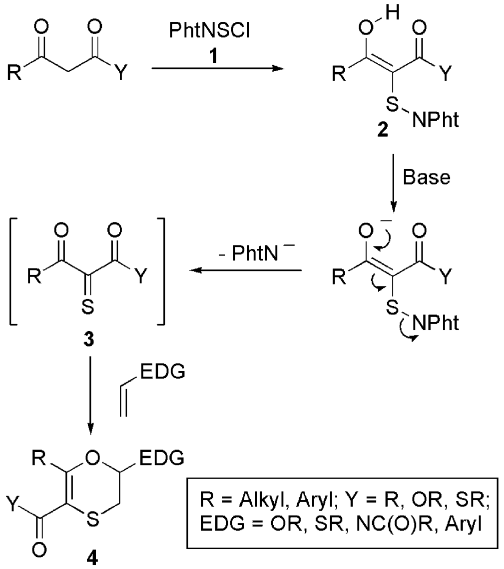
Abstract—Solid-supported α,α′-dioxothiones are easily obtained starting from β-ketoester modified Wang and hydroxymethylated polystyrene resins. The hetero Diels–Alder reactions (HDAR) of these species, used either as electron-poor dienes or dienophiles, followed by a simple cleavage of the products from the resin by trans-esterification with sodium methoxide, allowed the isolation of the desired cycloadducts in overall yields up to 90%.

1. Introduction

Hetero Diels–Alder reactions represent one of the more efficient and valuable tool for the construction of six-membered heterocycles which, in turn, probably are the most common cyclic moiety encountered in bio-organic and medicinal chemistry.[1] The development of [4+2] cycloadditions on solid-phase represents an appreciated target either for a possible simplification of the synthetic procedure or for their potential application in combinatorial chemistry.

We have developed a practical procedure for the formation of α,α′-dioxothiones and studied their behaviour as electron-poor heterodienes in inverse electron demand hetero Diels–Alder reactions with a plethora of electron-rich alkenes (Scheme 1). The reaction of β-dicarbonyls with the phthalimidesulfenyl chloride **1** (PhtNSCl, Pht = Phthaloyl) affords the corresponding α,α′-dioxothiophthalimides **2** which, in the presence of weak bases like Pyridine or Et_3N, undergo a 1,4-elimination at sulfur of the phthalimide anion causing the formation of the transient thiones **3** (Scheme 1). These species react smoothly with vinyl ethers, vinyl sulfides, vinyl amides, and styrenes to give 1,4-oxathiin heterocyclic systems **4** with a total control of chemo- and regiochemistry.[2–4]

Due to the simplicity, mildness, generality and usefulness of this approach,[5] we decided to study the possibility of its extension to solid support. In this paper, we report the results, scope and limitations of this methodology when applied to solid-supported α,α′-dioxothiones.[6]



Scheme 1. General procedure for the generation and trapping of α,α′-dioxothiones in solution-phase.

2. Results and discussion

Following the general protocol optimized for this chemistry

Keywords: Phthalimidesulfenyl chloride; Dioxothiones; Hetero Diels–Alder reactions; Solid-phase synthesis; Sulfur heterocycles.

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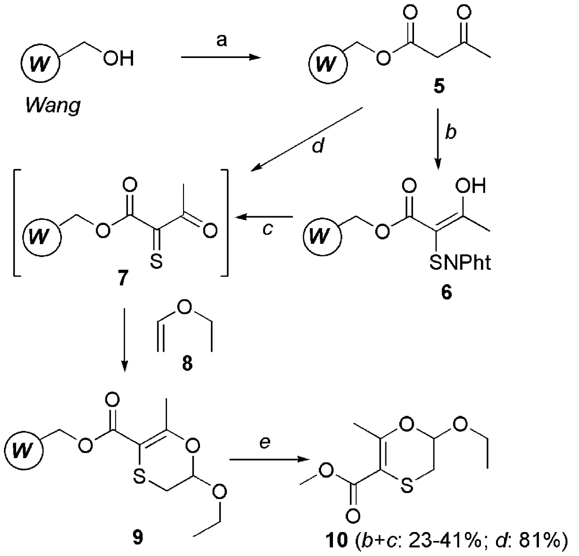
in solution-phase, we tried to transform a resin-linked β-ketoester into the corresponding acylthione. Using the Wang resin as solid support, the β-ketoester function was attached after considering several possible methods,[7] by direct trans-esterification with *tert*-butylacetoacetate[8] in toluene at 110 °C, to obtain immobilised ketoester **5** (Scheme 2). The reaction of a DCM pre-swelled suspension of resin **5** with 1.4 equiv of phthalimidesulfenyl chloride (**1**) at room temperature, allowed the transformation into the α,α′-dioxothiophthalimide modified resin **6** (Scheme 2). Eventually, the reaction of **6** with 1 equiv of Pyridine in $CHCl_3$ at room temperature afforded the solid-supported α,α′-dioxothione **7** that, in the presence of 5 equiv of ethyl vinyl ether (**8**), underwent an inverse electron demand HDAR to give the resin-linked oxathiin **9** as reported in Scheme 2.



Scheme 2. General procedure for the generation and trapping of an α,α′-dioxothione on solid-phase. Reagents: (a) *tert*-butylacetoacetate, toluene, 110 °C, 24 h; (b) **1**, DCM, rt, 5 h; (c) Py or Et_3N, ethyl vinyl ether (**8**), $CHCl_3$, rt −60 °C, 18–22 h; (d) **1**, Et_3N, **8**, DCM, 60 °C, 19 h; (e) MeONa/MeOH, THF, rt, 5 h.

After each step, the resin was repeatedly washed with DCM, MeOH, Et_2O, DCM and dried under vacuum over KOH. The progress of the reactions and the formation of intermediates **5**, **6** and **9** was monitored by FTIR and ^1H NMR.[9]

The use of Wang resin would have allowed the cleavage of the cycloadduct from the solid-phase by reaction with TFA in DCM, however, we found that oxathiin cycloadducts of type **4** (or **9**) are unstable under these conditions. Alternatively, a clean cleavage was achieved by trans-esterification with a freshly prepared methanolic sodium methoxide solution in dry THF; the isolation of cycloadduct **10** was thus possible in 23% yield (Scheme 2). Despite the low yield, these preliminary results were quite encouraging due to the simplicity of the entire procedure and since **10** was obtained as a pure compound (purity >95% by ^1H NMR) directly after evaporation of the solvents used to wash the resin (see Section 4). Identification of **10** was easily obtained by comparison with an authentic sample obtained in solution-phase reactions from methylacetoacetate[3] (R = Me, Y = OMe, EDG = OEt) as described in Scheme 1.

Several examples of Diels–Alder reactions on solid phase have been reported[10] but, at the best of our knowledge, this is the first example of hetero cycloaddition employing a solid-supported acylthione. We studied how to improve the yield of sequence reported in Scheme 2. A first advance was easily obtained using a stronger base to generate the thione **7** and performing the cycloaddition at higher temperature. As a matter of fact repeating the procedure as depicted in Scheme 2 but using 2 equiv of Et_3N on step c and carrying on the cycloaddition of **7** with **8** at 60 °C for 18 h, after cleavage with MeONa, derivative **10** was isolated in a 41% yield. Other simple modifications to the procedure, among those often suggested to optimize a reaction on solid-phase, such as duplication of trans-esterification and/or sulfenylation steps (a and/or b steps in Scheme 2) did not allow, in our case, any satisfactory improvement.

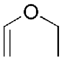
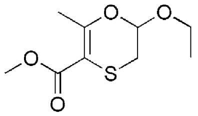
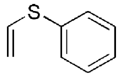
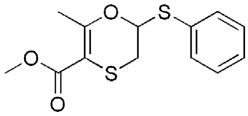
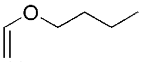
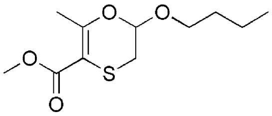
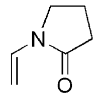
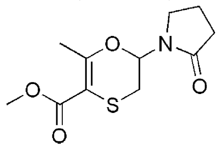
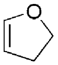
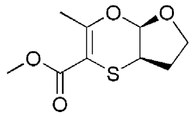
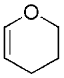
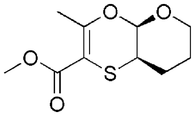
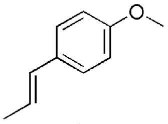
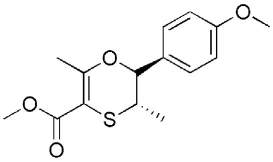
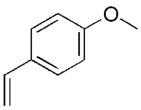
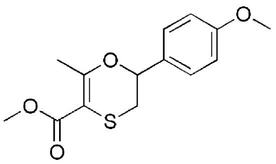
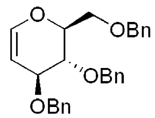
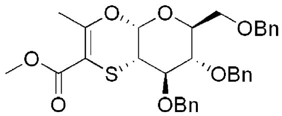
A crucial enhance was eventually obtained using a simplified procedure that allowed to run the cycloaddition without the isolation of the intermediate dioxothiophthalimide derivative, and we recently verified to be valuable for the cycloaddition of thiones obtained from acid sensitive sugar-containing β-ketoesters.[11] In particular 2 equiv of **1** and 2.5 equiv of Et_3N were added in sequence to resin **5** at room temperature in DCM, followed, after 20 min, by the addition of vinyl ether **8** (Scheme 2, step d). The reaction mixture was heated to 60 °C for 19 h; the obtained resin-linked oxathiin **9** was then reacted with MeONa to give **10** in a 81% yield. This satisfactory result indicated that each of the five steps carried out on solid-phase to isolate **10** (i.e., introduction of the β-ketoester, sulfenylation, formation of the thione, cycloaddition and cleavage) must occur with almost quantitative yield. The nearly simultaneous addition of **1** and Et_3N to **5**, which avoids the formation of a high concentration of HCl, the obvious by-product of the sulfenylation step, and consequently the acid decomposition of the Wang resin, can likely explain this successful result. However, it must be mentioned that the analysis of the solvents used to wash the resin **6** after sulfenylation (i.e., step b in Scheme 2) did not show any compound deriving from a possible deterioration of the resin **5**.

We decided to verify the generality of this solid-supported HDAR repeating the cycloaddition with the electron-rich alkenes **11–18** as dienophiles as reported in Table 1.

In any case, the supported thione **7** was generated following the simplified procedure without isolation of the sulfenylated resin **6** and the final yields refer to the cycloadducts **19–26** isolated as pure compounds (>95% by ^1H NMR) after cleavage with MeONa (see Section 4). Apart from the reaction with enol ether **8**, all the other results are for oxathiin cycloadducts obtained in a single run without optimization. Identification of compounds **19–26** was achieved by comparison with authentic samples previously prepared in solution-phase.[3]

Data reported in Table 1 are in good agreement with the informations, we have accumulated during the last years for these cycloaddition reactions. In fact when the dienophile

Table 1. Oxathiins **10**, **19**–**26** obtained by cycloaddition of solid-supported thione **7**

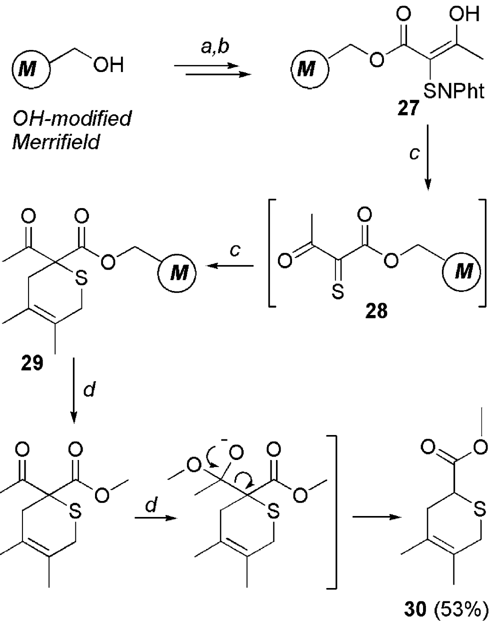
Dienophil	Cycloadduct (reac. time, yield)	Dienophile	Cycloadduct (reac. time, yield)
 **8**	 **10** (19 h 81%)	 **15**	 **23** (40 h, 13%)
 **11**	 **19** (12 h, 50%)	 **16**	 **24** (23 h, 5%)
 **12**	 **20** (18 h, 53%)		
 **13**	 **21** (18 h, 40%)	 **17**	 **25** (48 h, trace)
 **14**	 **22** (32 h, 41%)	 **18**	 **26** (48 h, trace)

chosen presents either a poor reactive HOMO orbital, that is an high gap between the LUMO of the thione and the HOMO of the alkene,[4] or a remarkable steric demand[2–4,12] the reaction time becomes very long and the yields decrease. In such circumstances the cycloaddition on solid-phase, which requires, as expected, harsher reaction conditions, becomes poorly efficient for dienophiles like vinyl pyrrolidone **16**, anethole **17** or tri-*O*-benzylglucal **18** that allowed the isolation of only trace amounts of the corresponding oxathiins **24**, **25** or **26**.

On the other hand, the methodology depicted in Scheme 2 (path d) is valuable and more convenient, in terms of simplicity and overall yields, than the procedure in solution-phase for reactive steric undemanding dienophiles.

A further development of this methodology we studied was, the possibility of using different solid-supporting resins for the generation of α,α′-dioxothiones. Thus, a β-ketoester group was inserted on a hydroxymethyl polystyrene (OH-modified Merrifield) resin following the same procedure reported in Scheme 2. In this case, due to the insensitivity of this resin to acid hydrolysis, the sulfenylation reaction was carried out using an excess of **1** and repeated twice since to have an exhaustive transformation to the resin-linked dioxothiophathalimide **27** (Scheme 3). Indeed, we verified that generating the supported thione **28** from **27** and Et_3N, in the presence of ethyl vinyl ether (**8**), after 18 h at 60 °C and cleavage with MeONa, the oxathiin **10** was isolated in 77% yield.

With completely sulfenylated resin **27** in hand we could also carry out cycloaddition reactions in solvents like DMF, that are not suitable for the one-pot [(i) sulfenylation (ii) thione generation] procedure. With minute amounts[13] of DMF as



Scheme 3. Formation of the modified Merrifield supported-thione **28**, and its trapping as electron-poor dienophile. Reagents: (a) *tert*-butylacetoacetate, toluene, 110 °C, 24 h; (b) **1**, DCM, rt, 5 h; (c) Et_3N, 2,3-dimethyl-1,3-butadiene, $CHCl_3$, 60 °C, 20 h; (d) MeONa/MeOH, THF, rt, 5 h.

solvent and prolonging the reaction times we observed a little, but promisingly, further improvement on the total yield of some cycloadditions, including those that had given poor results with resin **5**. Thus, under these conditions derivatives **10**, **23**, **24** and **25** were isolated in 92, 28, 12 and 13% yield, respectively.

Another noticeable reaction of α,α′-dioxothiones[14] is their participation as electron-poor dienophiles in direct electron demand Diels–Alder reactions with 1,3-dienes.[2] We tried to trap solid-supported α,α′-dioxothione **28** as dienophile using 2,3-dimethyl-1,3-butadiene. In this case, cycloaddition afforded the dihydrothiopyran OH-modified Merrifield resin **29**, that, when reacted with MeONa in dry THF, allowed the isolation of derivative **30** in 53% yield (Scheme 3).

The modification of the heterocyclic ring structure with loss of the acetyl group was expected since we have already observed[15] that basic hydrolysis, or trans-esterification with alkoxides, in α-keto-α′-carboxythiopyrans is associated with a very easy and unavoidable retro-Claisen rearrangement that causes the formation of a 2-carboxythiopyran of type **30** (Scheme 3). However, the isolation of derivative **30**, proving the formation of thiopyran **29**, indicates that α,α′-dioxothiones retain their double nature of efficient electron-poor dienes and dienophiles in HDAR on solid-support as well.

As already mentioned, α,α′-dioxothiones **3** are transient species that cannot be isolated or detected. When α-keto-α′-carboxy-*N*-thiophthalimides of type **2** (R = Me, Y = OMe, OEt) are reacted with a base in the absence of a suitable trapping agent, the produced thiones **3** undergo a base catalysed reversible self-condensation process with formation of a dimeric species which, in turn, can be isolated and identified.[2] It has been recently reported[16] that the formation of a quinone methide on a solid support allowed its spectroscopic characterization since the resin acts as an appropriate ‘isolator’ for such highly reactive species.

We speculated whether this possibility exists also for α,α′-dioxothione **28**. Operatively, resin **27** was reacted with Et_3N in dry DCM for 2 h at room temperature in the dark. After being thoroughly washed and dried, the resin **28** formally containing the free thione moiety was reacted with **8** in dry DCM at 60 °C for 18 h. Satisfactorily, after the usual cleavage, oxathiin **10** was isolated in 16% yield indicating that, under this condition, a monomeric α,α′-dioxothione moiety is, at least in part, stable on the resin **28**.[17]

3. Conclusion

In this paper, we reported the successful extension on solid-phase of the *N*-thiophthalimide mediated generation of α,α′-dioxothiones. Using the easily available Wang or OH-modified Merrifield resins as solid-supports and avoiding the introduction of any linker, dioxothiones were obtained under very mild reaction conditions and reacted in HDAR either as electron-poor dienes or dienophiles. Using highly reactive small dienophiles the isolation from the solid phase of the oxathiin cycloadducts was more simple and convenient than the parallel solution-phase procedure. Further developments and applications of this new aspect of dioxothiones chemistry are under study.

4. Experimental

4.1. General

DCM, DMF, $CHCl_3$, THF, CH_3OH, toluene, Pyridine and Et_3N were dried using standard procedures. All commercial reagents were used without further purification. Solution phase ^1H NMR spectra were recorded in $CDCl_3$ at 200 or 400 MHz, using residual $CHCl_3$ at δ_H 7.26 as reference. Phthalimidesulfenyl chloride **3** was prepared as published elsewhere.[18] Oxathiins **19–26** were identified by comparison of the ^1H NMR spectra with authentic samples previously prepared in solution.[3]

4.2. Solid-phase synthesis

Wang and hydroxymethylated Merrifield resins were purchased from Novabiochem. They are all based on 1% cross-linked divinylbenzene-styrene copolymer and are 100–200 mesh with a loading of 1.2 and 0.98 mmol/g. Solid-phase reactions were carried in sure sealed vials and the resin suspensions transferred by plastic-pipettes. Solid-phase work-up was carried out by means of the plastic-siring technique. Flat-bottom PE syringes were equipped with sintered Teflon filters, teflon tubing and valves which allow suction to be applied to the syringe from below. Functionalized resins were analysed with FTIR and/or MAS solid-phase NMR. MAS solid-phase NMR were acquired on a 400 MHz Varian MercuryPlus spectrometer using a PFG-ID-Varian Nanoprobe (Pulsed Field Gradient, Indirect Detection), at 25 °C using $CDCl_3$ as solvent with a CPMG modified sequence to minimise the resin signals. In the following ^1H NMR spectra of the intermediated resins **5**, **6**, **9** the signals due to the resin’s protons are omitted apart from the terminal benzilic CH_2. Unless otherwise stated reactions were worked-up as it follows: the resin was transferred from the vial to the syringe, filtered and washed with DCM (four times), MeOH (four times), Et_2O (four times) and DCM (four times), and dried under vacuum over KOH for 2–24 h before to run the following step.

4.2.1. Immobilised β-ketoester 5.[8] Wang resin (1 g, 1.2 mmol/g) was swelled at room temperature in dry toluene (5 mL) for 2 h. *tert*-Butylacectoacetate (1.9 g, 12 mmol) was added and the mixture heated at 110 °C for 24 h. The general work-up afforded 1.064 g of resin **5**.

IR (KBr) 1742 (s, C=O ester), 1717 (s, C=O ketone) cm^{-1}.[7,8] ^1H NMR (400 MHz, $CDCl_3$) δ: 2.32 (s, $C{=}COCH_3$, 3H), 3.55 (s, $OCOCH_2CO$, 2H), 5.23 (s, resin-$ArCH_2OCO$, 2H).

4.2.2. Sulfenylated resin 6. β-Ketoester functionalised resin **5** (1.070 g, 1.2 mmol) was swelled at room temperature in dry DCM (6.5 mL) for 1 h. Sulfenyl chloride **1** (0.356 g, 1.7 mmol) was added and the mixture kept at room temperature for 5 h. The general work-up afforded 1.081 g of resin **6**.

IR (KBr) 1785+1740+1718 (N–C=O Pth+C=O ester) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.73 (s, C=$COCH_3$, 3H), 5.25 (s, resin-ArCH_2OCO, 2H); 7.70–7.90 (m, Phthaloyl, 4H), 13.93 (s, C=COH, 1H).

4.2.3. Immobilised oxathiin 9 from sulfenylated resin 6. Sulfenylated resin **6** (180 mg, 0.15 mmol) was swelled in dry $CHCl_3$ (4 mL) at room temperature for 2 h, then ethyl vinyl ether (**8**) (56 mg, 0.78 mmol) and Et_3N (33 mg, 0.32 mmol) were added in sequence and the mixture heated to 60 °C for 18 h. The general work-up afforded 164 mg of resin **9**.

IR (KBr) 1702 (s, C=O ester conjugated), 1600 (s, C=C conjugated) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (bs, OCH_2CH_3, 3H), 2.40 (s, C=$COCH_3$, 3H), 2.95–3.05 (m, SCH_2, 2H), 3.87–3.92 (m, OCH_2CH_3, 1H), 4.01–4.06 (m, OCH_2CH_3, 1H), 5.25 (bs, OCHO, 1H), 5.40 (s, resin-ArCH_2OCO, 2H).

4.2.4. Immobilised oxathiin 9 from resin 5: simplified procedure. To a pre-swelled suspension of resin **5** (153 mg, 0.17 mmol) in dry DCM (2.5 mL) at room temperature for 1 h, PhtNSCl **1** (75 mg, 0.35 mmol) in dry DCM (1.5 mL) and dry Et_3N (44 mg, 0.43 mmol) were added in succession. After 20 min at room temperature vinyl ether **8** (62 mg, 0.86 mol) was introduced and the mixture heated to 60 °C for 19 h. The general work-up afforded 170 mg of resin **9**.

4.2.5. Resin 9 cleavage with MeONa: oxathiin 10. The resin **9** (170 mg, 0.16 mmol) was swelled in dry THF (6 mL) at room temperature for 1 h; a solution of freshly prepared MeONa 1.6 M in MeOH (0.5 mL, 0.8 mmol) was added by syringe and the mixture kept at room temperature for 5 h. The resin was washed with Et_2O (four times) and DCM (four times), the organic solvents recollected, washed with saturated aqueous NH_4Cl and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded cycloadduct **10** (28 mg, 81%) as a pale yellow oil identical to an authentic sample.[3]

^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.25 (t, J=7.5 Hz, 3H), 2.32 (s, 3H), 2.76–2.94 (AB part of an ABX system, J_{AB}= 13.5 Hz, 2H), 3.64–3.76 (m, 1H), 3.73 (s, 3H), 3.84–3.96 (m, 1H); 5.24 (X part of an ABX system, J=4.0, 2.0 Hz, 1H).

All the above reported procedures can be considered as representative also for the functionalization of the hydroxymethyl polystyrene (OH-modified Merrifield) resin, and for all the cycloadditions described in Table 1.

4.2.6. Cycloadduct 23 from resin 27 in DMF. To a pre-swelled sulfenylated resin **27** (260 mg, 0.20 mmol) in dry DMF (1 mL) at room temperature for 2 h, phenyl vinyl sulfide (**15**) (136 mg, 1.00 mmol) and Et_3N (21 mg, 0.21 mmol) were added in sequence and the mixture heated to 60 °C for 52 h. The general work-up afforded 239 mg an oxathiin-immobilised resin, which was dried and reacted with MeONa in dry THF as described above. Evaporation of the solvent gave cycloadduct **23** (16 mg, 28%) as a pale yellow oil with spectroscopic data identical of those reported in the literature.[3]

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.37 (s, 3H), 3.01 (AB part of an ABX system, J_{AB}=13.2 Hz, 2H), 3.77 (s, 3H), 5.68 (X part of an ABX system, J=5.4, 2.7 Hz, 1H), 7.30–7.60 (m, 5H).

4.2.7. Thiopyran 30. To a pre-swelled sulfenylated resin **27** (160 mg, 0.12 mmol) in dry $CHCl_3$ (4 mL) at room temperature for 2 h, 2,3-dimethyl-1,3-butadiene (54 mg, 0.66 mmol) and Et_3N (14 mg, 0.14 mmol) were added in sequence and the mixture heated to 60 °C for 20 h. The general work-up afforded 154 mg of immobilised thiopyran resin **29** which was dried and reacted with MeONa in dry THF as described above. Evaporation of the solvent gave thiopyran **30** (13 mg, 53%) as a colourless oil with spectroscopic data identical of those reported in the literature.[19]

^{1}H NMR:[17] (200 MHz, $CDCl_3$) δ: 1.69 (bs, 3H), 1.71 (bs, 3H), 2.45 (AB system, 2H, J=6.2 Hz), 3.08 (AB system, 2H, J=16.2 Hz), 3.6 (t, 1H, J=6.2 Hz), 3.73 (s, 3H).

4.2.8. Immobilised monomeric thione 28. In a flat-bottom PE syringe equipped with two sintered Teflon filters, resin **27** (212 mg, 0.16 mmol) was swelled in dry DCM (2 mL) at room temperature for 1 h, then the syringe was covered with aluminium foil and Et_3N (49 mg, 0.50 mmol) was added in the dark. After 2 h at room temperature the deep pink resin obtained was washed with dry DCM, until no trace of Et_3N was detected in the washing, transferred into a vials with dry DCM (2 mL) and vinyl ether **8** (36 mg, 0.50 mmol) and heated to 60 °C for 18 h. The usual work-up and cleavage with MeONa gave cycloadduct **10** in 16% isolated yield.

Acknowledgements

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References and notes

1. Nicolaou, K. C.; Snyder, S. A.; Montagnon, T.; Vassilikogiannakis, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1668–1698 and reference cited therein.
2. Capozzi, G.; Nativi, C.; Menichetti, S.; Rosi, A.; Valle, G. *Tetrahedron* **1992**, *48*, 9023–9032.
3. Capozzi, G.; Franck, R. W.; Mattioli, M.; Menichetti, S.; Nativi, C.; Valle, G. *J. Org. Chem.* **1995**, *60*, 6416–6426.
4. Capozzi, G.; Dios, A.; Franck, R. W.; Geer, A.; Marzabadi, C.; Menichetti, S.; Nativi, C.; Tamarez, M. *Angew. Chem., Int. Ed.* **1996**, *35*, 777–779.
5. Menichetti, S.; Nativi, C. Hetero Diels–Alder approach to oxathiins. In *Targets in Heterocyclic Systems—Chemistry and Properties*, 2003; Vol. 7, pp 108–139.
6. A preliminary report of this work has been presented at 21st

International Symposium on the Organic Chemistry of Sulfur, ISOCS-XXI, Madrid, Spain, July 4–9, 2004.

7. (a) Tietze, L. F.; Hippe, T.; Stainmetz, A. *Synlett* **1996**, 1043–1044. (b) Witzeman, J. S. *Tetrahedron Lett.* **1990**, *31*, 1401–1404. (c) Tietze, L. F.; Evers, H.; Hippe, T.; Steinmetz, A.; Töpken, E. *Eur. J. Org. Chem.* **2001**, 1631–1634.
8. Strohmeier, G. A.; Kappe, O. C. *J. Comb. Chem.* **2002**, *4*, 154–161.
9. FTIR and ^{1}H NMR spectra registered on the resin immobilised compounds **5**, **6**, **9** were compared with those of the corresponding *O*-benzylester derivatives previously prepared in solution-phase (i.e. in Scheme 1: R = Me, Y = OBn, EDG = OEt).
10. (a) Lorsbach, B. A.; Kurth, M. J. *Chem. Rev.* **1999**, *99*, 1549–1581. (b) Yli-Kauhaluoma, J. *Tetrahedron* **2001**, *57*, 7053–7071 and reference cited therein.
11. Bartolozzi, A.; Pacciani, S.; Benvenuti, C.; Cacciarini, M.; Liguori, F.; Menichetti, S.; Nativi, C. *J. Org. Chem.* **2003**, *68*, 8529–8533.
12. Leconte, S.; Dujardin, G.; Brown, E. *Eur. J. Org. Chem.* **2000**, 639–643.
13. Alexandros Kiriazis, A.; Leikoski, T.; Mutikainen, I.; Yli-Kauhaluoma, J. *J. Comb. Chem.* **2004**, *6*, 283–285.
14. We demonstrated that α,α'-dioxothiones are also involved in ene-reactions: Capozzi, G.; Fragai, M.; Menichetti, S.; Nativi, C. *Eur. J. Org. Chem.* **2000**, 3653–3657.
15. A similar Retro-Claisen rearrangement was observed on α-keto-α'-carboxythiopyrans obtained by cycloaddition of thiones **3** (R = Me, Y = OMe, OEt) with anthracene: (a) Capozzi, G.; Menichetti, S.; Nativi, C.; Vergamini, C. *Synthesis* **1998**, 915–918. (b) Capozzi, G.; Giacomelli, S.; Menichetti, S.; Nativi, C. *Synthesis* **2001**, 409–412.
16. Zanaletti, R.; Freccero, M. *Chem. Commun.* **2002**, 1908–1909.
17. Preliminary attempts to identify the dioxothione moiety on resin **28** either by FTIR or ^{13}C NMR were unsatisfactory.
18. Boccardo, G.; Capozzi, G.; Giuntini, M.; Menichetti, S.; Nativi, C. *Tetrahedron* **1997**, *53*, 17383–17394.
19. Shimizu, H.; Kudo, N.; Kataoka, T.; Hori, M. *J. Chem. Soc., Perkin Trans 1* **2001**, 2269–2276.

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Tetrahedron

Base catalyzed intramolecular transamidation of 2-aminoquinazoline derivatives on solid phase☆

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Abstract—A novel intramolecular cyclo-elimination via transamidation on the Rink Amide AM resin under mild basic conditions is presented. The methodology led to the synthesis of an important class of cardiotonic agents: imdiazo- and pyrimido-quinazolines from the corresponding 2-aminoquinazoline hydrobromide salt under mild basic conditions. NMR based titration studies revealed the role of hydrobromide as a molecular switch, which on removal triggers the cyclisation of aminoquinazoline to tricyclic structures. The main advantage of transamidation under basic conditions over the TFA cleavage is the recyclability of the resin obtained after cyclo-elimination. This has been demonstrated by successive synthesis of four structurally diverse imidazoquianzolin-2-ones using the same batch of resin without any cross contamination.

1. Introduction

Solid-phase cyclative cleavage[1] strategy through intramolecular nucleophilic substitution is an attractive route for the preparation of a variety of medicinally important five-, six- and seven-membered heterocyclic compounds[2] in high purity. As part of our continuing program to develop novel strategies for solid-phase synthesis of heterocyclic compounds[3] of therapeutic interest, we recently reported formation of imidazoquinazolin-2-ones[4] from immobilized amino acid derived 2-aminoquinazolines. The tricyclic structures were obtained either by acid/base-induced cyclo-elimination from Wang resin bound aminoquinazolines or by acid induced cyclo-elimination from RAM resin bound aminoquinazolines. However, during the course of our studies we observed that (i) 2-aminoquinazolines derived from glycine failed to produce imidazoquinazolin-2-ones under acidic conditions both on Wang and RAM resin, and (ii) the base mediated cleavage of Wang resin linked 2-aminoquinazoline derived from proteogenic amino acids gave the desired product, together with the diisopropylammonium bromide salt as a byproduct and requiring further purification.

In order to address these issues and circumvent the above problems, we studied the mechanistic aspects of cyclization of aminoquinazolines to imidazoquinazolines both under acidic and basic conditions, which in turn led to the synthesis of imidazoquinazolinones via a novel intramolecular transamidation strategy under mild basic conditions on solid-phase. Interestingly, by this route the desired compounds derived from proteogenic amino acids were found to be devoid of hydrobromide salt. Most of the transamidations on solid phase have been described strictly under acidic conditions and there is no report dealing with intramolecular amide exchange under basic conditions. Herein, we report a convenient method for the synthesis of imidazoquinazolin-2-one and one of its analogous chemotypes quinazolinoquinazolin-5-one from the Rink amide AM bound aminoquinazolines via transamidation under mild basic conditions. Further the resin recovered after the final transamidation step was found to be reusable for solid-phase synthesis. To the best of our knowledge this is the first report dealing with the base catalyzed intramolecular amide exchange reaction (transamidation) on solid-phase.

2. Results and discussion

In the first instance, it was thought worthwhile to ascertain

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Keywords: Transamidation; Solid-phase synthesis; Imidazoquinazolinone; Pyrimidoquinazolinone.

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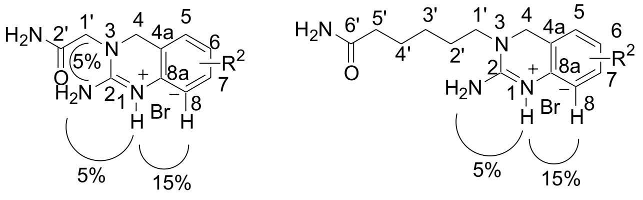
the site of protonation in the 2-aminoquinazoline **1** derived from glycine.[4] The NOE difference experiments revealed quaternisation at N-1 as expected[5] (Fig. 1) which was found to be variant with the earlier report by Venuti et al.,[6] wherein $–NH_2$ was reported as the site for protonation. Further, NOE between $–NH_2$ at 7.89 ppm and H-1′ at 4.51 ppm suggested the existence of a fixed conformation along the amino acid substructure. In order to substantiate our finding, aminoqinazolines **2** derived from γ-aminobutyric acid[4] was investigated by NMR. The absence of NOE between $–NH_2$ and H-1′ in **2** (Fig. 1) indicated hydrogen bonding as the probable reason for the existence of an extra NOE in **1**. To verify the existence of the hydrogen bond in **1**, ^{1}H VT NMR studies were carried out. A linear correlation ($r^2=0.998$) was observed between the shielding of guanidine $–NH_2$ signal (7.95 ppm) at 25 °C and the temperature. The $d\delta/dt$ value was calculated to be 2.4 ppb $°C^{-1}$ in DMSO-d_6 confirming the involvement of guanidine-NH_2 proton in the intramolecular H-bonding with the carbonyl group.



1 (Ref 4) **2 (Ref 4)**

Figure 1. NOE correlations.

Next, the unexpected disparity observed between the resin bound glycine and other C-α substituted amino acid derived 2-aminoquinazolines in the cyclative cleavage step to form 2-aminoquinazolines (**1**) and imidazoquinazolin-2-ones (**4**), respectively, on solid-phase under acidic conditions was examined (Fig. 2). Here, it is appropriate to mention that the general notion about the precise role of substitution at C-α position in amino acids is to have an appropriate reactive conformer population rather than decrease in the effective angle (Thorpe–Ingold effect)[7] in order to facilitate the formation of five-membered ring. The real challenge still confronted us as to how under basic conditions glycine derived aminoquinazoline cyclized on Wang resin.

Elucidation of the mechanistic principles for the intramolecular attack of amine on the carbonyl under both acidic and basic conditions was thus warranted to provide the evidence for the anomaly observed. In order to understand the working mechanistic aspects, titration studies of the methyl ester of glycine derived 2-aminoquinazoline **5** in solution was attempted against TFA and DIPA (diisopropylamine) (Fig. 3). The aim of this study was to provide some insight of cyclative cleavage in acidic and basic conditions on solid phase. The progress of the reaction was monitored using NMR spectroscopy. Addition of TFA to compound **5** produced no change in the NMR spectrum except for the change in the chemical shifts of NH and NH_2 protons (Supporting information). Based on these observations, a mechanism for the acid catalyzed cycloelimination on solid-phase was thus conceived as depicted in Figure 2. Lack of intramolecular cyclization in TFA to form imidazo ring in the resin bound glycine based

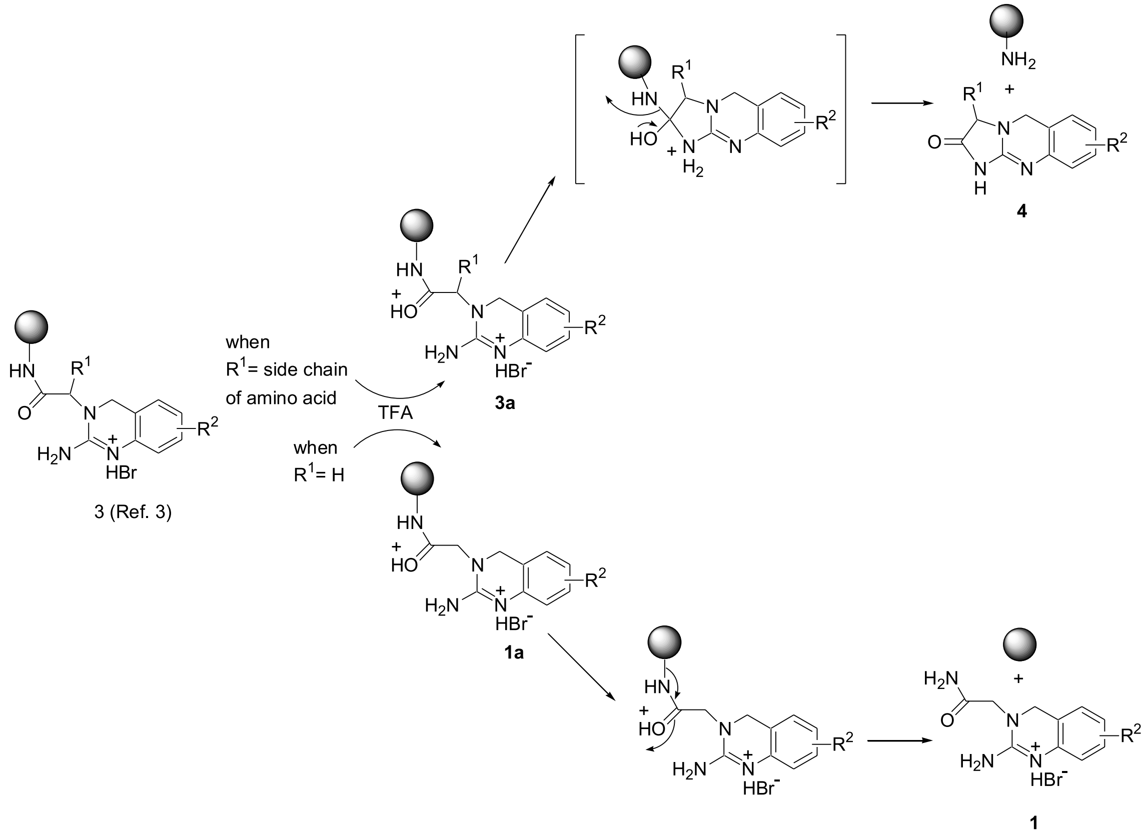


Figure 2. Proposed mechanism for the acid catalyzed cyclative cleavage.

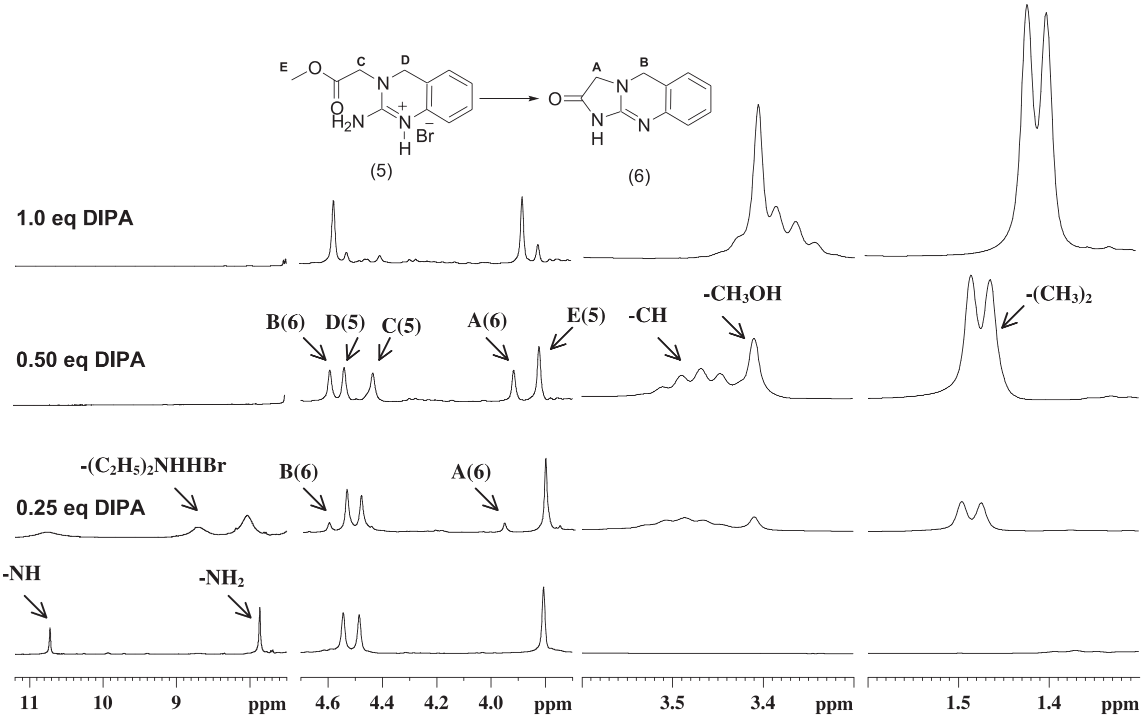
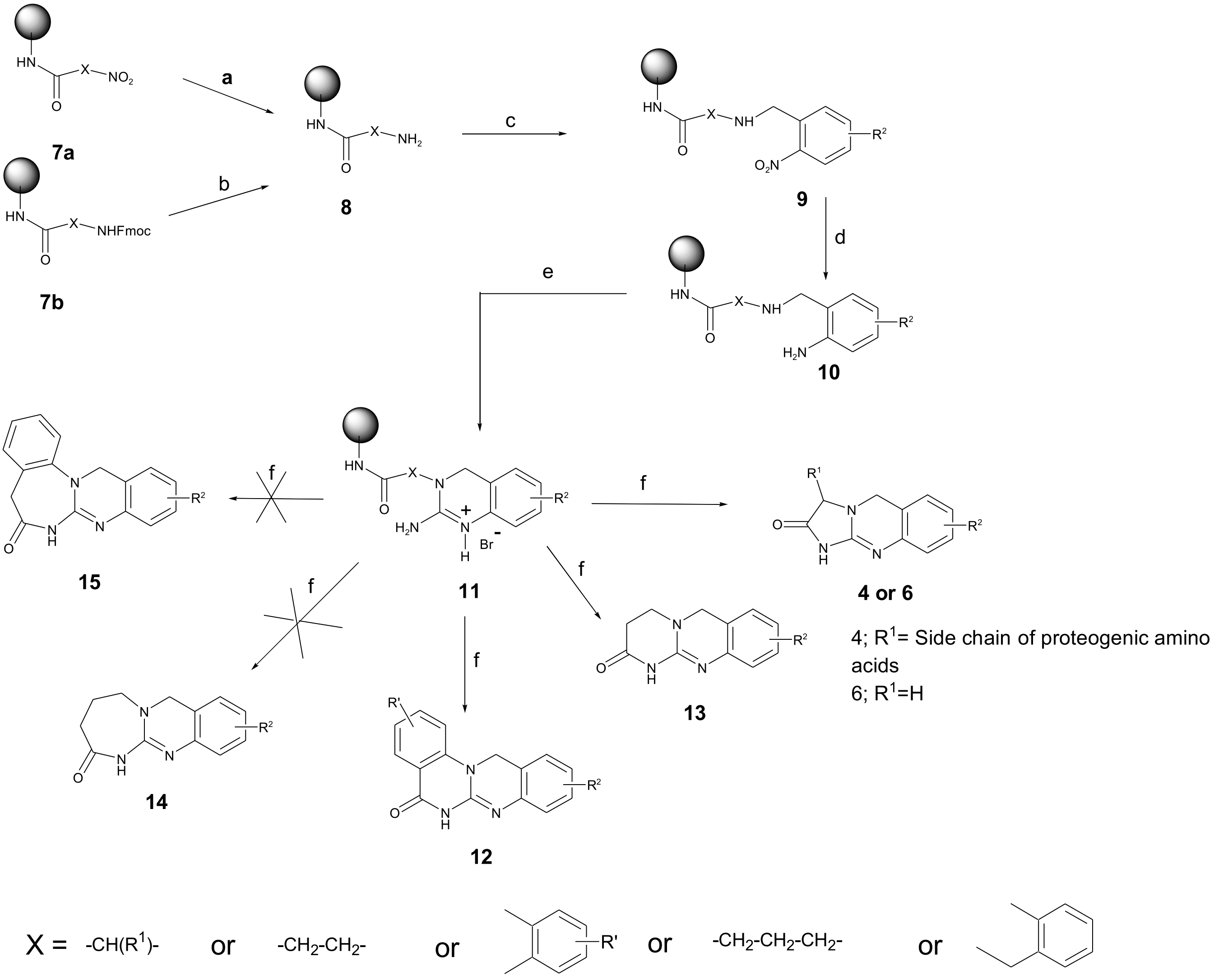


Figure 3. Titration studies of 2-aminoquinazoline 5 with diisopropyl amine leading to the formation of **6**.

compound (**1a**) may be attributed to the pronounced delocalisation of the hydrobromide salt **1** in comparison to its unprotonated counterpart. As a consequence, conformational restriction is induced along the C–NH_2 bond thereby hampering the formation of the favorable transition state that leads to the intramolecular cyclization. However, in 2-aminoquinazolines derived from amino acids (**3a**) other than glycine, intramolecular cyclization under acidic condition proceeds with protonation (activation) of the carbonyl group[8] followed by attack of the NH_2 group which releases HBr due to the poor availability of the lone pair via resonance at N-1 (Fig. 2). These studies clearly singled out the crucial role of HBr being played in the cyclo-elimination process on solid-phase. Subsequently, cyclization of **5** under basic conditions was studied using DIPA as base. The stock solution of DIPA in CD_2Cl_2 was added in four aliquots (0.25 equiv each) at an interval of 2 min to compound **5** dissolved in deuterated dichloromethane in an NMR tube. The respective spectra were recorded according to the procedure described in the experimental section. As shown in Figure 3, spectrum acquired immediately after the addition of the first aliquot of DIPA (0.25 equiv) resulted in the broadening of NH_2 and NH signals, indicated fast exchange and loss of hydrogen bonding.

Emergence of a new set of signals at 8.2 (broad), 4.60, 3.95 and 3.40 ppm corresponds to $[(CH_3–CH_2)_2CH]_2NHBr$, H_2-B(**6**), H_2-A(**6**) and CH_3OH, respectively. Subsequent addition of the second aliquot of DIPA (0.50 equiv) caused the complete disappearance of the –NH, –NH_2 signals coupled with the increase in the intensity of H_2-B(**6**), H_2-A(**6**) and CH_3OH signals representing the progress of the reaction. Addition of the last aliquot of DIPA (1.0 equiv) led to the complete conversion in the tricyclic imidazoquinazolin-2-one (**6**; Fig. 3).

Based on the instant and facile intramolecular cyclization observed by NMR, we envisaged that it might be possible to carry out cyclo-elimination reaction on Rink amide (RAM) resin with an amide linker via transamidation under mild basic conditions. In order to prove our hypothesis, the Rink Amide AM resin bound 2-aminoquinazoline derived from Gly (3; $R^1 = H$) was treated with 25% DIPA in DCM for 12 h. The filtrate after evaporation afforded imidazoquinazolin-2-one **6** in high yield and purity. The compound was characterized by NMR and as expected it was found to be a mixture of imidazoquinazolin-2-one and diisopropylammonium bromide salt. To avoid the presence of diisopropylammonium bromide salt as contaminant, an alternative strategy was devised. The Rink Amide AM resin bound 2-aminoquinazoline derived from Gly was first treated with 25% aqueous solution of NH_4OH in dioxane for 2 min with the view to generate free base. The ammonical solution was removed by filtration and the resin was washed twice each with 20%water in dioxane, dioxane, ether and DCM. The resin beads were then immediately suspended in DCM and were shaken for 12 h at rt. The filtrate after evaporation furnished product in high yields and generally did not require further purification as determined by ^1H NMR. This methodology was then extended to other proteogenic amino acid derived 2-aminoquinazoline which resulted in imidazoquinazolinones **5** (Scheme 1). Based on the above results coupled with NMR titration studies, a mechanism is proposed for transamidation on solid phase under basic condition as depicted in Figure 4. The resin recovered after the final cyclo-elimination step (Fig. 4) was subjected to ninhydrin test, which showed the presence of –NH_2 group on the bead. Further, the SBFTIR spectrum of the recovered resin also matched with the spectrum of the fresh Rink Amide AM resin. This led us to examine the possibility to reusing the recovered resin with the view to make



Scheme 1. Reagents and conditions: (a) $SnCl_2$(2 M in DMF) 5 h, rt; (b) 30% piperidine in DMF, 30 min; (c) aldehydes, $NaCNBH_3$,1% AcOH/TMOf, 3 h, rt; (d) $SnCl_2$(2 M in DMF) 5 h, rt; (e) BrCN, DMF–ethanol (2:1); (f) 25% NH_4OH in dioxane, 20% H_2O in dioxane, dioxane, ether, DCM 12 h.

solid-phase synthesis a commercially viable technique for this class of molecules. We succeeded in reusing the same batch of Rink Amide AM resin for the successive synthesis of up to four structurally diverse imidazoquinazolin-2-ones **4**(**a–d**) in high yields and purities (Table 1) and as expected the resin was still found to be reusable.

Finally, to examine the role of the linker in cyclo-elimination, it was considered appropriate to examine the cyclo-elimination on other widely used amide resins such as Sieber Amide and Rink Amide. The products from these resins were obtained in high yields and purities. This can be attributed to the better nucleophilicity of the guanidino-NH_2 in comparison to the linker –NH_2 that facilitates the transamidation to form the five-membered ring.[9]

In light of these observations, we next extended the general applicability of our strategy for the synthesis of six- and seven-membered rings fused to aminoquinazolines such as pyrimido and diazepino quinazolinones. In the first instance, we selected β-alanine for the synthesis of pyrimidoquinazolin-2-ones whereas GABA and 2-nitrophenylacetic acid for diazepanquinazolin-2-ones (Scheme 1). It was observed that six-membered ring **13** (Scheme 1) derived from β-alanine was formed in low yield (30%), whereas the formation of seven-membered ring **14** and **15** (Scheme 1) from GABA and 2-nitrophenylacetic acid, respectively, was not observed at all. This may be attributed to the increase in the distance between the two reaction centers which in turn reduces the feasibility of transamidation thereby suggesting that the cyclative cleavage via transamidation appears to be more favored in the formation of five-membered ring than for six-membered ring. Next, it was decided to synthesize quinazolinoquinazolin-5-ones **12**, another six-membered ring fused to aminoquinazolines. The synthesis was carried out on Rink Amide AM resin starting from *o*-nitrobenzoic acid instead of β-alanine. We envisaged that *o*-nitrobenzoic acid may provide a rigid geometry that may favor the final cyclo-elimination via transamidation.

Our strategy for **12** commenced with the synthesis of resin **7a** and **7b** by the coupling of substituted 2-nitro benzoic acid and Fmoc anthranilic acid to the Rink Amide AM resin using DIC/HOBt method. The completion of loading was

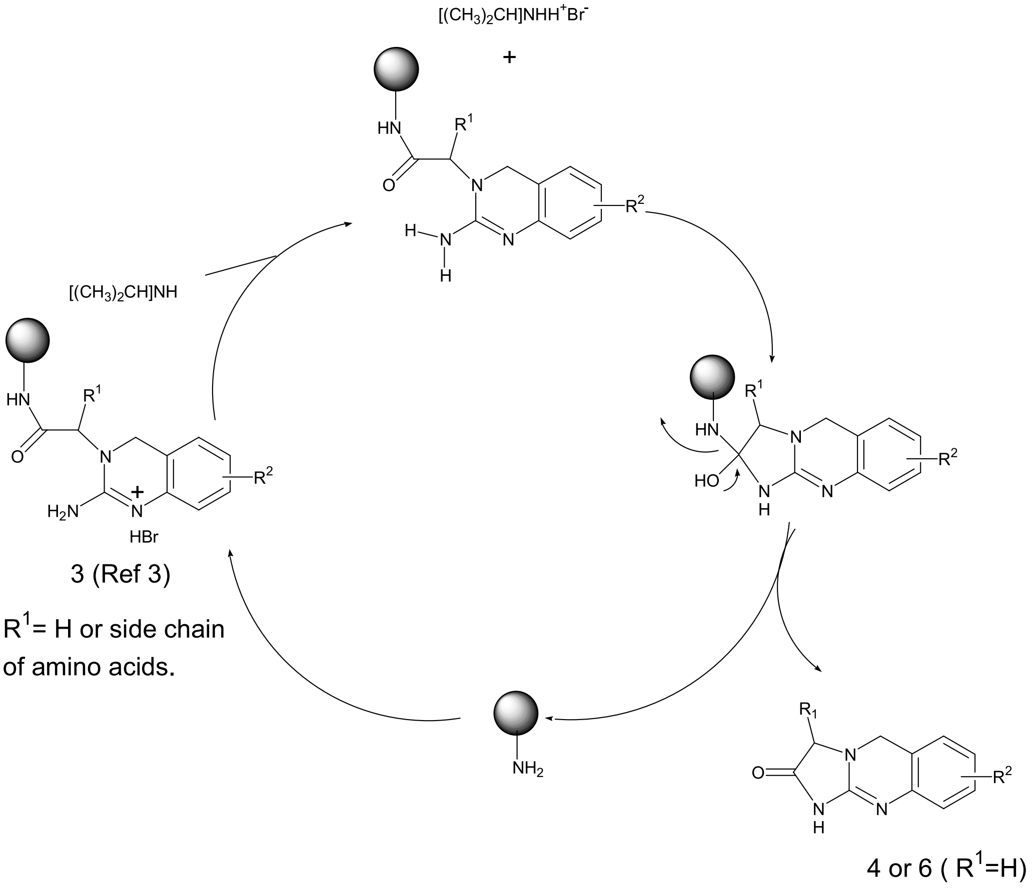


Figure 4. Proposed mechanism for the base-catalyzed cyclative cleavage.

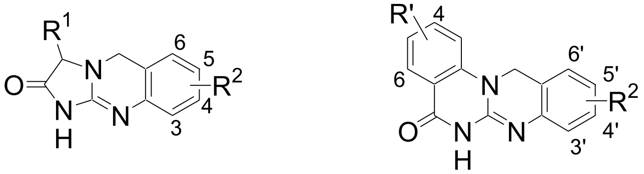
Table 1. Purity and ESMS of compounds based on prototype **4**

Product	R^1	R^2	ESMS$(M+H)^+$	t_R (min)	Yield[a] crude/ isolated (%)	Purity[a] (%)
4a	$–CH[CH(CH_3)_2]–$	3,5-OMe	290.39	11.23	90/78	95
4b	$–CH[CH(CH_3)_2]–$	–H	230.37	10.77	86/75	93
4c	$–CH(CH_2C_6H_5)–$	3,5-OMe	337.83	11.52	88/77	94
4d	$–CH(CH_2C_6H_5)–$	–H	278.53	10.89	93/79	96

Crude yields based on weight of cleaved compounds.

[a] Analysis of crude products was carried out on Agilent liquid chromatograph using a 5 μm, 4.8×150 mm C_{18} reverse phase column with a linear gradient 10–100% ACN in water (v/v) over 25 min. The flow rate was 1.0 mL/min, and UV detection at 220/254 nm.

monitored by a negative Kaiser test. This was followed by reduction of the *o*-nitro group with $SnCl_2 \cdot 2H_2O$ to give an amine **8**. The amine **8** was also obtained from resin **7b** by the deprotection of Fmoc anthranilic acid, which was treated with *o*-nitro benzaldehyde by reductive alkylation to give **9** followed by $SnCl_2 \cdot 2H_2O$ reduction to give amine **10**. Subsequently, its cyclization was carried out in the presence of cyanogen bromide to give immobilized 2-aminoquinazolines **11**. Finally, its cleavage using ammonium hydroxide resulted in a crude product **12**, which after purification using reverse phase HPLC was eventually characterized by NMR and ESMS. Using the synthetic method outlined above, a library containing eighteen single compounds based on structure **12**(**a**–**p**) were synthesized (Fig. 5) using substituted nitrobenzoic acid/Fmoc anthranilic acid and *o*-nitrobenzaldehydes (Table 2). The library was generated using an Advanced Chemtech multiple organic synthesizer MOS 496Ω. Structural diversity in nitrobenzoic acid/ anthranilic acid had no effect on the final yield except 4-chloro substituted compounds **12**(**l**) and **12**(**n**), which resulted in poor yield. Additionally, *o*-nitrobenzaldehydes having 4-chloro substituents did not furnish the final product. Rest of the compounds otherwise were obtained in moderate to good isolated yields (30–60%). Interestingly the purity of the crude products remained in the range of 60–100%. The reason for high order of purity



4 **12**

Figure 5. Numbering of representative compound based on prototypes **4** and **12**.

can be attributed to the release of only the desired product formed by transamidation under basic conditions.

3. Conclusion

In conclusion, this constitutes the first report of intramolecular transamidation on RAM AM, RAM and Sieber amide resin under mild basic conditions for the formation of imidiazoquinazolin-2-ones from resin bound 2-aminoquinazolines in quantitative yields and excellent purity. Attempts to extend this strategy for the synthesis of six-membered ring via transamidation, furnished the desired compounds in moderate to good yields and with excellent purity. The main advantage of this process is the recyclability of the resin, which has been demonstrated by synthesizing four different imidazoquinazolin-2-ones using same batch of resin without any cross contamination. Recycling of the resin, purity and ease of synthesis makes it a method of choice for the synthesis of imidazoquinazolin-2-ones class of molecules. We strongly believe that the above results are worthy of further investigation for the synthesis of other class of five-membered heterocyclic compounds with the advantage of recycling of the resin. The strategy is amenable to automation and can be successfully used for the generation of large libraries of biologically active imidazoquinazolin-2-ones and pyrimidoquinazolin-2-ones.

4. Experimental

4.1. General

Rink amide AM resin (1% divinylbenzene, 100–200 mesh, 0.63 mmol/g substitution), Wang resin (1% divinylbenzene, 100–200 mesh, 0.63 mmol/g substitution) and amino acids were purchased from Novabiochem, Switzerland. *N*-Hydroxybenzotrizaole (HOBt) was purchased from Janseen Chemica, Belgium. *O*-nitrobenzaldehyde, *N*,*N*′-diisopropylcarbodiimide, piperidine and trifluoroacetic acid were purchased from Aldrich. Anhydrous solvents were used for reactions. All other reagents were obtained from commercial sources and were used without further purification. The reactions were carried out in polypropylene syringes of 5 mL capacity, which were shaken on an orbital shaker (IKA-VIBRAX-VXR). The ^{1}H and ^{13}C spectra were obtained on 300 MHz spectrometer, and chemical shifts are reported in ppm (δ) relative to TMS. Because of solubility properties, the solvents used are mixture of $CDCl_3$ and CD_3OD. RP-HPLC analysis of crude products was carried out on Agilent liquid chromatograph using a 5 μm, 4.8× 150 mm^2 C-18 reverse phase column with a linear gradient 0–100% (Tables 1 and 2) ACN in water (v/v) over 25 min. The flow rate was 1.0 mL/min. and UV detection was observed at 220/254 nm. Mass spectra were recorded using electron spray ionization (ESI) technique.

4.2. General procedure for preparation of compound 12 on Rink Amide AM resin

The Fmoc groups of Rink Amide AM resin (0.63 mM/g; 100 mg) were removed by treating with 25% piperidine in DMF (1 mL) twice for 5 and 25 min. The resin was filtered and washed with DMF (9×5 mL). The resin so obtained was coupled with Fmoc protected proteogenic amino acids (3 equiv)/ Fmoc protected anthranilic acids (3 equiv) or 2-nitro benzoic acid (3 equiv) by using HOBt (3 equiv), DIC (3 equiv) and DMF (1 mL) as solvent for 16 h at rt. The resin was filtered and washed successively with DMF (3× 2 mL), MeOH (3×2 mL), DCM (3×2 mL) and Ether (3× 2 mL) and finally dried in vacuo. Completion of the reaction was confirmed by a negative Kaiser test. The nitro group of resin **7a** was reduced to amine with 2 M $SnCl_2 \cdot 2H_2O$ in DMF (1 mL) for 5 h at rt and in resin **7b** Fmoc was removed by treating with 25% piperidine in DMF (1 mL) twice for 5

Table 2. Purity and ESMS of compounds based on prototype **12**

Product no.	R′	R^2	ESMS $(M+H)^+$	t_R (min)	Yield[a] crude/ isolated (%)	Purity[a] (%)
12a	–H	–H	250.39	14.23	86/55	81
12b	–H	3′-OMe	280.10	13.11	78/53	85
12c	–H	3′,5′-OMe	310.43	12.52	88/50	82
12d	5-OMe	–H	280.14	13.35	76/49	79
12e	5-OMe	3′-OMe	310.53	12.71	79/56	84
12f	5-OMe	3′,5′-OMe	340.93	12.34	90/58	76
12g	5-OMe	6′-Cl	314.31	13.33	73/48	73
12h	5-OMe	6′-OH	296.14	12.74	72/49	81
12i	3-OMe	–H	280.48	13.87	74/53	89
12j	3-OMe	3′-OMe	310.41	12.34	85/57	88
12k	3-OMe	3′,5′-OMe	340.83	12.14	73/47	80
12l	4-Cl	–H	284.53	13.89	72/41	72
12m	4-Cl	3′-OMe	314.73	12.99	75/40	75
12n	4-Cl	3′,5′-OMe	344.58	12.14	65/42	68
12o	5-Me	–H	264.17	14.11	82/50	73
12p	5-Me	3′-OMe	294.47	13.23	81/48	74
12q	5-Me	3′,5′-OMe	324.49	13.14	73/43	90
12r	3,5-di-Me	–H	278.27	14.03	75/47	90
12s	3,5-di-Me	3′-OMe	308.41	12.77	74/41	83
12t	3,5-di-Me	3′,5′-OMe	338.43	12.93	81/48	87

Crude yields based on weight of cleaved compounds.

[a] Analysis of crude products was carried out on Agilent liquid chromatograph using a 5 μm, 4.8×150 mm C_{18} reverse phase column with a linear gradient 10–100% ACN in water (v/v) over 25 min. The flow rate was 1.0 mL/min, and UV detection at 220/254 nm.

and 25 min. The resin was filtered and washed with DMF (9×5 mL). The resin so obtained was treated with *o*-nitro benzaldehydes (5 equiv) in TMOF for 3 h at rt. The resin was then filtered and treated with 1% AcOH solution of TMOF and $NaCNBH_3$ (10 equiv) for 2 h at rt. The resin was filtered and washed successively with MeOH (3×2 mL), DMF (3×2 mL), DCM (3×2 mL) and ether (3×2 mL) and finally dried in vacuo to give **9**. The nitro group of resin **9** was reduced to amine with 2 M $SnCl_2 \cdot 2H_2O$ in DMF (1 mL) for 5 h at rt. There upon the resin was washed successively with DMF (3×2 mL), MeOH (3×2 mL), DCM (3×2 mL) and Ether (3×2 mL) and finally dried in vacuo to give **10**. Next the resin **10** was treated with cyanogen bromide (10 equiv) in DMF–EtOH (2:1) mixture at rt. for 16 h The resin was successively washed with DMF (3×2 mL), EtOH (3×2 mL), MeOH (3×2 mL), DCM (3×2 mL) and ether (3×2 mL) and dried in vacuo to give **11**. The resulting resin **11** was then subjected to basic cleavage first with 25% NH_4OH in dioxane for 2 min followed by sequential washing with 20% water in dioxane (2×2 mL), dioxane (2×2 mL) and ether (4×2 mL). The resin so obtained was finally prewashed once with DCM and then suspended in DCM (1 mL) for 12 h at r.t. The resulting mixture was filtered and the filtrate was evaporated to dryness in vacuum. The residue was freeze dried after dissolving in ^{t}BuOH/water (4:1) to give the desired compounds based on prototype **12**.

4.3. NMR titration studies

To the solution of compound **1** (8.0 mg) 0.036 mmol in CD_2Cl_2 (500 μL, Aldrich 99.9%D) in NMR tube, 50 μL each aliquots of DIPA stock solution (5 μL DIPA in 200 μL of CD_2Cl_2) 0.12 mmol were successively added. Recording of the ^{1}H NMR was recorded just after addition at 25 °C. The chemical shifts are referenced at 0.0 ppm to TMS.

4.3.1. Isopropyl-7,8-dimethoxy-1,5-dihydro-imidazo[2,1-*b*]quinazolin-2-one 4(a) (Table 1). Pale yellow solid, HPLC purity 95% t_R 11.23 min. ^{1}H NMR (300 MHz, DMSO-d_6): 0.91 (d, 3H, J=6.6 Hz, CH_3), 0.99 (d, 3H, J=6.6 Hz, CH_3), 2.20 (m, 1H,$CHCH_3$), 3.72 (s, 6H, 2×OCH_3), 3.79 (brs, 1H, CO*CH*N), 4.40 (d, 1H, J=13.5 Hz, CH_2Ph), 4.50 (d, 1H, J=13.5 Hz, CH_2Ph), 6.64 (s, 1H, ArH), 6.82 (s, 1H, ArH). ESMS: *m*/*z* 290.39 $[M+H]^+$.

4.3.2. 3-Isopropyl-1,5-dihydro-imidazo[2,1-*b*]quinazolin-2-one 4(b) (Table 1). White solid, HPLC purity 93% t_R 10.77 min. ^{1}H NMR (300 MHz, DMSO-d_6): 0.91 (d, 3H, J=6.6 Hz, CH_3), 0.99 (d, 3H, J=6.6 Hz, CH_3), 2.21 (m, 1H, $CHCH_3$), 3.77 (brs, 1H, CO*CH*N), 4.48 (d, 1H, J=16.0 Hz, CH_2Ph), 4.58 (d, 1H, J=16.0 Hz, CH_2Ph), 6.99 (d, 1H, J=7.8 Hz, ArH), 7.04 (t, 1H, J=7.5 Hz, ArH), 7.19 (d, 1H, J=7.5 Hz, ArH), 7.24 (t, 1H, J=7.8 Hz, ArH). ESMS: *m*/*z* 230.37 $[M+H]^+$.

4.3.3. 3-Benzyl-7,8-dimethoxy-1,5-dihydro-imidazo-[2,1-*b*]quinazolin-2-one 4(c) (Table 1). Pale yellow solid, HPLC purity 94% t_R 11.52 min. ^{1}H NMR (300 MHz, DMSO-d_6): 2.99 (dd, 1H, J=14.9, 4.0 Hz, $CHCH_{2a}Ph$), 3.14 (dd, 1H, J=14.9, 4.0 Hz, $CHCH_{2b}Ph$), 3.70 (s, 6H, 2×OCH_3), 4.14 (t, 1H, J=4.0 Hz, COCHN), 4.30 (d, 1H, J=16.2 Hz, $CH_{2a}Ph$), 4.36 (d, 1H, J=16.2Hz, $CH_{2b}Ph$), 6.56 (s, 1H, ArH), 6.72 (s, 1H, ArH), 7.20 (m, 5H, Ph). ESMS: *m*/*z* 337.83 $[M+H]^+$.

4.3.4. 1,3,4,6-Tetrahydro-2*H*-pyrimido[2,1-*b*]quinazolin-2-one 13. White solid, HPLC purity 81% t_R 11.77 min. ^{1}H NMR (300 MHz, DMSO-d_6): 2.63 (t, 2H, J=6.9 Hz, CH_2), 3.51 (t, 2H, J=7.0 Hz, CH_2), 4.59 (s, 2H, N*CH*$_2$Ph), 6.98 (d, 1H, J=7.5 Hz, ArH), 7.04 (d, 1H, J=7.2 Hz, ArH), 7.12 (d, 1H, J=7.2 Hz, ArH), 7.21 (t, 1H, J=7.5 Hz, ArH). ESMS: *m*/*z* 202.43 $[M+H]^+$.

4.3.5. 3-Methoxy-6,12-dihydro-5*H*-quinazolino[3,2-*a*]-quinazolin-5-one 12(d) (Table 2). White solid, HPLC purity 79% t_R 13.35 min. ^{1}H NMR (300 MHz, $CDCl_3$: CD_3OD (4:1)): 3.92 (s, 3H, OCH_3), 5.49 (s, 2H, CH_2), 7.05 (d, 1H, J=7.6 Hz, ArH), 7.17 (brt, 2H, ArH), 7.29 (d, 1H, J=7.9 Hz, ArH), 7.33 (t(o), 1H, J=7.6 Hz, ArH), 7.42 (d, 1H, J=7.9 Hz, ArH), 7.48 (dd, 1H, J=7.6, 2.6 Hz, ArH), 7.68 (s, 1H, ArH). ESMS: *m*/*z* 280.14 $[M+H]^+$.

4.3.6. 3,8-Dimethoxy-6,12-dihydro-5*H*-quinazolino-[3,2-*a*]quinazolin-5-one 12(e) (Table 2). Pale yellow solid, HPLC purity 84% t_R 12.71 min. ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD (4:1)): 3.91 (s, 3H, OCH_3), 3.96 (s, 2H, OCH_3), 5.38 (s, 2H, N*CH*$_2$Ph), 6.94 (d(o), 1H, J=7.7 Hz, ArH), 6.98 (d(o), 1H, J=7.7 Hz, ArH), 7.11 (t(o), 1H, J=7.7 Hz, ArH), 7.15 (d, 1H, J=7.9 Hz, ArH), 7.43 (dd,1H, J=7.9, 2.6 Hz, ArH), 7.64 (s, 1H, ArH). ESMS: *m*/*z* 310.53 $[M+H]^+$.

4.3.7. 3,9,10-Trimethoxy-6,12-dihydro-5*H*-quinazolino[3,2-*a*]quinazolin-5-one 12(f) (Table 2). White solid, HPLC purity 76% t_R 12.34 min. ^{1}H NMR (300 MHz, DMSO-d_6): 3.68 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.68 (s, 2H, N*CH*$_2$Ph), 6.64 (d, 1H, J=8.2 Hz, ArH), 6.91 (d, 1H, J=8.2 Hz, ArH), 7.15 (s, 1H, ArH), 7.66 (s, 1H, ArH), 7.95 (s, 1H, ArH). ESMS: *m*/*z* 340.93 $[M+H]^+$.

4.3.8. 1-Methoxy-6,12-dihydro-5*H*-quinazolino[3,2-*a*]-quinazolin-5-one 12(i) (Table 2). White solid, HPLC purity 89% t_R 13.87 min. ^{1}H NMR (300 MHz, DMSO-d_6): 3.66 (s, 3H, OCH_3), 4.72 (s, 2H, CH_2), 6.81 (t, 1H, J=7.9 Hz, ArH), 7.01 (d, 1H, J=7.9 Hz, ArH), 7.18 (d, 1H, J=7.9 Hz, ArH), 7.51 (d(o), 1H, J=7.3 Hz, ArH), 7.51 (t(o), 1H, J=7.3 Hz, ArH), 7.62 (t, 1H, J=7.3 Hz, ArH), 7.98 (d, 1H, J=7.3 Hz, ArH). ESMS: *m*/*z* 280.48 $[M+H]^+$.

4.3.9. 9,10-Dimethoxy-3-methyl-6,12-dihydro-quinazolino[3,2-*a*]quinazolin-5-one 12(q) (Table 2). Pale yellow solid, HPLC purity 90% t_R 13.14 min. ^{1}H NMR (300 MHz, DMSO-d_6): 2.15 (s, H, CH_3), 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2), 6.53 (d, 1H, J=8.1 Hz, ArH), 7.01 (d, 1H, J=8.1 Hz, ArH), 7.16 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.67 (s, 1H, ArH). ESMS: *m*/*z* 324.49 $[M+H]^+$.

Supplementary data

1D NOE spectrum of compound **1** and **2**, ^{1}H NMR stack plot of the titration studies of compound **5** with TFA-d.

Titration of compound **5** with diisopropyl ethylamine and triethylamine. ^{1}H NMR spectrum of **4a–4d**, **13**, **12d**, **12e**, **12f**, **12i**, **12q**.

Supplementary data associated with this article can be found in the online version, at 10.1016/j.tet.2005.03.047

References and notes

1. Krchnak, V.; Holladay, M. W. *Chem. Rev.* **2002**, *102*, 61–91.
2. Blaney, P.; Grigg, R.; Sridharan, V. *Chem. Rev.* **2002**, *102*, 2607–2624.
3. (a) Kesarwani, A. P.; Grover, R. K.; Roy, R.; Kundu, B. *Tetrahedron* **2005**, *61*, 629–635. (b) Roy, A. D.; Sharma, S.; Grover, R. K.; Kundu, B.; Roy, R. *Org. Lett.* **2004**, *6*, 4763–4766. (c) Grover, R. K.; Mishra, R. C.; Kundu, B.; Tripathi, R. P.; Roy, R. *Tetrahedron Lett.* **2004**, *45*, 7331–7334. (d) Saha, B.; Srivastava, G. K.; Kundu, B. *Synlett* **2004**, 2242–2244. (e) Singh, S. K.; Gupta, P.; Duggineni, S.; Kundu, B. *Synlett* **2003**, 2147–2150. (f) Kesarwani, A. P.; Srivastava, G. K.; Rastogi, S. K.; Kundu, B. *Tetrahedron Lett.* **2002**, *43*, 5579–5581. (g) Rastogi, S. K.; Srivastava, G. K.; Singh, S. K.; Grover, R.; Roy, R.; Kundu, B. *Tetrahedron Lett.* **2002**, *43*, 8327–8330.
4. Srivastava, G. K.; Kesarwani, A. P.; Grover, R. K.; Roy, R.; Srinivasan, T.; Kundu, B. *J. Comb. Chem.* **2003**, *5*, 769–774.
5. Kowalski, J.; lipton, M. A. *Tetrahedron Lett.* **1996**, *37*, 5839–5840.
6. Venuti, M. C.; Stephenson, R. A.; Alvarez, R.; Bruno, J. J.; Strosberg, A. M. *J. Med. Chem.* **1988**, *31*, 2136–2145.
7. Jung, M. E.; Gervay, J. *Tetrahedron Lett.* **1988**, *29*, 2429–2432.
8. Kirk, K. L.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 8142–8147.
9. Lasri, J.; Gonalez-Rosende, M. E.; Sepulveda-Arques, J. *Org. Lett.* **2003**, *5*, 3851–3853.

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Tetrahedron

Electron deficiency of aldehydes controls the pyrrolidine catalyzed direct cross-aldol reaction of aromatic/heterocyclic aldehydes and ketones in water

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Abstract—A synthetically useful pyrrolidine catalyzed direct cross-aldol reaction of aromatic/heterocyclic aldehydes with ketones in water affords the aldol addition product in up to 93% yield. Electrophilicity of the aldehydes controls the course of the reaction.

1. Introduction

The aldol reaction constitutes a fundamental synthetic methodology for forming carbon–carbon bonds and nature itself seems to prefer this reaction in its biosynthetic processes, for example, in the prebiotic formation of saccharides.[1] The reaction is typically catalyzed by aldolases (Class I and Class II) for direct aldolization of two unmodified carbonyl compounds. Synthetic methodologies involving both aldolase[2] and catalytic antibodies[3] have been extensively developed for accomplishing aldol reactions with high efficiency and selectivity.

Small organic molecules, especially proline have been shown to catalyze aldol reactions both in organic[4] and aqueous[5] media mimicking the type I aldolase mechanism. Recently, Janda[5a] and Barbas[5b] have reported the accomplishment of cross-aldol reactions in buffered aqueous medium using nornicotine and other pyrrolidine based catalysts. We have been interested in developing synthetic methodologies based on the principles of green chemistry and the use of water as a solvent in organic reactions. The use of small organic molecules in aqueous systems is a developing area of synthetic organic chemistry where small organic molecules act as enzyme mimics in water, which is the natural environment of biological reactions catalyzed by enzymes. The recent reports[5] in buffered aqueous solutions have initiated us to report our results, since these methods are low to moderate in yield and require long reaction times. An entry into such carbon–carbon bond formation from prochiral precursors when further refined has the potential of involving asymmetric induction in an environmentally friendly manner. Further, the sodium hydroxide catalyzed aldol reaction in aqueous medium mainly provides condensation products.[6]

We herein report that heteroaromatic and aromatic aldehydes undergo fast direct cross-aldol reactions with ketones in water in the presence of a catalytic amount of pyrrolidine. To the best of our knowledge this is the first report on the use of a water–pyrrolidine combination for the aldol reaction.[7] Further, knowledge of the catalytic efficiency of pyrrolidine in water will be useful in understanding the importance of pyrrolidine based natural products in biochemical processes. Synthetically, the cross-aldol addition products can serve as a useful starting material[20] for the synthesis of nikkomycin[21] and potentially bioactive non-natural and pseudonatural products.

2. Results and discussion

Initially, we studied the reactions of acetone with pyridine-2-carboxaldehyde (**1**) using pyrrolidine (30 mol%) as catalyst. To our surprise, the reaction was complete within 5 min. Work-up of the reaction and subsequent column chromatography gave aldol addition product 4-hydroxy-4-(2-pyridyl)-butan-2-one (**2a**) in 93% yield with no apparent formation of the dehydration product. In the absence of catalyst the aldol product was not formed. To determine if the observed catalysis was due to the unique property of pyrrolidine or if other cyclic secondary amines could also

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Keywords: Pyrrolidine catalysis; Heterocyclic aldol; Cross-aldol; Aqueous medium; β-Hydroxy ketones.

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Table 1. Direct catalytic aldol reaction of acetone with pyridine-2-carboxaldehyde in water

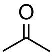
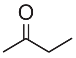
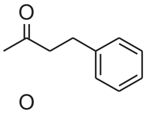
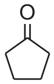
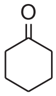
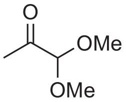
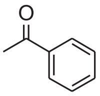
Entry	Catalysts	Time	Yield of **2a**[a]
1.	Pyrrolidine	5 min	93%
2.	None	48 h	—
3.	Proline	24 h	47%
4.	Piperidine	50 min	83%
5.	Morpholine	> 24 h	Traces
6.	$(C_6H_{11})_2NH$	> 24 h	—

[a] Isolated yield after column chromatography.

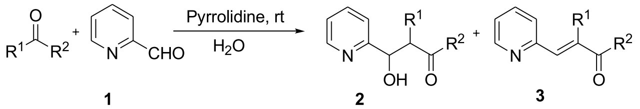
catalyze the aldol reaction in water, the reactions of acetone and pyridine-2-carboxaldehyde were performed in the presence of piperidine, morpholine, dicyclohexylamine and proline (Table 1). The results revealed that pyrrolidine was the most efficient catalyst, giving the aldol addition product in 5 min. The reactivity order of the tested amines was: pyrrolidine > piperidine > proline > morpholine > dicyclohexylamine. This observation is supported by examination of the structure of the compounds reported to be efficient organocatalysts, where all of these compounds possess a five-membered amine containing heterocycle.[8]

To determine the scope and limitation of this reaction we performed the reaction of pyridine-2-carboxaldehyde (**1**) with different donor ketones using pyrrolidine as the catalyst (Table 2, Scheme 1). The reaction of 2-butanone and 4-phenyl-2-butanone with **1** affords aldol adducts **2** in good yield and regioselectivity but no diastereoselectivity (entries 2 and 3). The observed regioselectivity in favour of linear aldol adducts (**2b** and **2d**) possibly originates from the greater reactivity of the less substituted enamine intermediate (kinetically stable) vis-à-vis more substituted enamine intermediate (thermodynamically stable). Thus we propose that regioselective formation of the enamine intermediate is a kinetic phenomenon. This observed regioselection is contrary to the reported aldol addition in the presence of alkali metal bicarbonates.[22] Cyclic donors, cyclopentanone and cyclohexanone gave products **2f** and **2g**, respectively, with good to moderate diastereoselectivity. Pyruvaldehyde-1,1-dimethylacetal reacts with **1** to afford **2h**—a potential precursor for the synthesis of biologically relevant molecules.[21] Acetophenone is unreactive under the reaction conditions (Scheme 1).

Table 2. Pyrrolidine catalyzed aldol reaction of various ketones with pyridine-2-carboxaldehyde (**1**) in water

Entry	Ketone	Time (min)	Product(s) (yield)[a]	dr[b] *anti/syn*
1.		5	**2a** (93%)	—
2.		10	**2b** (72%) **2c** (12%) **2b**:**2c**; 89:11[b]	— 1:1
3.		15	**2d** (76%) **2e** (11%) **2d**:**2e**; 87:13[b]	1:1 —
4.		15	**2f** (62%)	1:4
5.		15	**2g** (86%)	2:1
6.		5	**2h** (87%)	—
7.		6 h	—	—

[a] Isolated yield after column chromatography.
[b] Determined by ^1H NMR spectroscopy.



a; R^1 = H, R^2 = CH_3 **b**; R^1 = H, R^2 = CH_2CH_3
c; R^1 = CH_3, R^2 = CH_3 **d**; R^1 = H, R^2 = $-CH_2CH_2Ph$
e; R^1 = CH_2Ph, R^2 = $-CH_3$ **f**; R^1R^2 = $-(CH_2)_3-$
g; R^1R^2 = $-(CH_2)_4-$ **h**; R^1 = H, R^2 = $-CH(OMe)_2$

Scheme 1.

The organocatalytic cross-aldol reactions in buffered aqueous media employing phosphate buffer at pH 8 and 7.4 have been shown to involve an enamine mechanism ruling out the involvement of a general base mechanism.[5] This has also been suggested in the literature involving amine catalysis of the self aldol condensation reaction.[9]

In order to propose a mechanism for the pyrrolidine catalyzed cross-aldolization in water we argued that if the enamine mechanism was feasible in aqueous buffer[5] then it should also be possible in water. The general base catalysis involving an enolate mechanism was also a possibility. A comparison of the pK_a of acetone (19.16, H_2O) and acetophenone (18.24, H_2O) suggested that acetophenone should be more reactive than acetone,[10] if the enolate mechanism was involved. However, we observed that acetone reacted in 5 min, whereas acetophenone did not react, even after 6 h (Table 2, entry 7), suggesting an enamine mechanism.

The comparison of the reactivity of pyrrolidine and piperidine indicated that the catalytic activity of the pyrrolidine was higher than piperidine, which is consistent with the rate of enamine formation observed with these amines in non-aqueous conditions.[11] This observation supports the involvement of the enamine mechanism. Further, we determined the pH of the 30 mol% of pyrrolidine and piperidine in water (Table 3, entries 4 and 5). Although the difference in pH of both is small but they show substantial difference in reactivity and this difference in reactivity again suggested the involvement of the enamine mechanism as observed under non-aqueous conditions. Also, by using sodium hydroxide as base, the reaction performed at pH 11.75 (Table 3, entry 6) required a longer reaction time than the pyrrolidine-catalyzed reaction, which again supports the enamine mechanism. We also observed the effect of the amount of pyrrolidine on reaction rate; using pyrrolidine at lower than 5 mol% loading the reaction is slower. Although we cannot completely rule out

Table 3. Correlation of catalysts loading with pH of reaction mixture

Entry	Catalyst	Mol% of catalyst	pH of the reaction mixture	Reaction time	Yield of **2a**[a]
1	Pyrrolidine	1 mol%	9.40	7 h	73%
2	Pyrrolidine	2 mol%	10.48	2 h	89%
3	Pyrrolidine	5 mol%	10.78	5 min	90%
4	Pyrrolidine	30 mol%	11.75	5 min	93%
5	Piperidine	30 mol%	11.59	50 min	83%
6	NaOH	—	11.75[b]	30 min	87%

[a] Isolated yield after column chromatography.

[b] The pH of the solution was adjusted by drop wise addition of 1 N NaOH solution using pH meter.

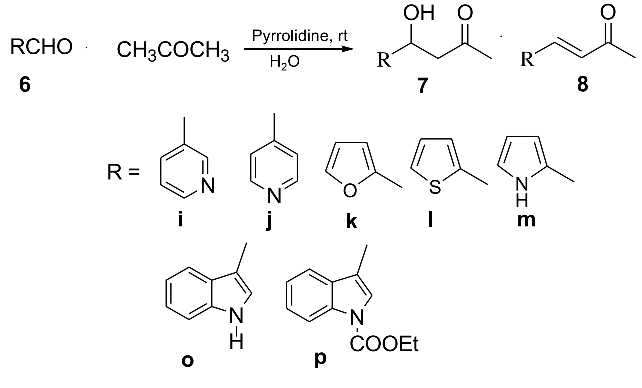
the involvement of a general base mechanism, the high rate of reaction seems to be due to the involvement of an enamine mechanism.

To elaborate the synthetic utility further, other heterocyclic aldehydes were used (Table 4). Pyridine-3-carboxaldehyde (**6i**) and pyridine-4-carboxaldehyde (**6j**) with acetone gave the aldol product in 71 and 68% yield, respectively, whereas the electron rich heterocyclic aldehyde, pyrrole-2-carboxaldehyde (**6m**) gave only aldol condensation product. Furan-2-carboxaldehyde (**6k**) and thiophene-2-carboxaldehyde (**6l**) gave both the aldol addition (**7**) and aldol condensation (**8**) products (Scheme 2).

Table 4. Pyrrolidine catalyzed aldol reaction of various aldehydes with acetone as ketone donor in water

Entry	Aldehyde	Time (min)	Product yield (%)[a]	
			7	**8**
1	**6i**	5	71	Nil
2	**6j**	5	68	Nil
3	**6k**	5	44	52
4	**6l**	5	30	65
5	**6m**	5	Nil	62
6	**6o**	60	Nil	Nil
7	**6p**	60	Nil	Nil

[a] Isolated yield after column chromatography.



Scheme 2.

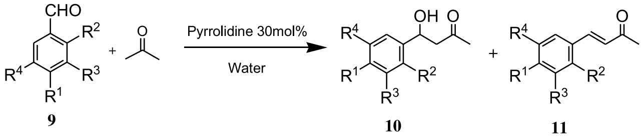
There is a clear distinction of product formation based on the electron rich/deficient nature of the heterocyclic aldehydes. Electron deficient aldehydes favour aldol addition products (**7**) whereas electron rich prefer aldol condensation products (**8**). Indole aldehydes and *N*-protected indole aldehydes failed to react in water possibly due to the insolubility of the aldehydes. Indole aldehydes were also found to be unreactive when the reaction was performed in water–DMSO mixture probably due to the reduced electrophilic nature of the carbonyl group of indole aldehydes.[19] Thus, the nature of heterocycle controls the product formation.

Similar effects of the electron rich/deficient nature of aldehydes can be observed in the direct aldol reaction of acetone with substituted aromatic aldehydes. The electrophilic nature of the aromatic aldehydes can be varied by appropriate substitution on the aromatic ring (Table 5, Scheme 3).

Table 5. Pyrrolidine catalyzed reaction of acetone (40 mmol) with aromatic aldehydes (2 mmol) with a reaction time of 5 min

Entry	Aldehyde	Product yield (%)[a]		
		10	**11**	Recovered **9**
1	**9q**	91	2	—
2	**9r**	92	2	—
3	**9s**	90	—	traces
4	**9t**	92	—	4
5	**9u**	93	6	—
6	**9v**	66	—	32
7	**9w**	83	—	13
8	**9x**		No reaction	
9	**9y**		No reaction	
10	**9z**		No reaction	

[a] Isolated yield after column chromatography.



q: $R^1 = NO_2$; $R^2 = R^3 = R^4 = H$ **r**: $R^1 = H$; $R^2 = NO_2$; $R^3 = R^4 = H$
s: $R^1 = Cl$; $R^2 = R^3 = R^4 = H$ **t**: $R^1 = H$; $R^2 = Cl$; $R^3 = R^4 = H$
u: $R^1 = R^2 = R^3 = R^4 = H$ **v**: $R^1 = OCH_3$; $R^2 = R^3 = R^4 = H$
w: $R^1 = OCH_3$; $R^2 = H$; $R^3 = R^4 = OCH_3$ **x**: $R^1 = N(CH_3)_2$; $R^2 = R^3 = R^4 = H$
y: $R^1 = OH$; $R^2 = R^3 = R^4 = H$ **z**: $R^1 = OH$; $R^3 = NO_2$; $R^2 = R^4 = H$

Scheme 3.

The results show that aromatic aldehydes substituted with nitro-/chloro substituents (**9q–t**) gave the direct cross-aldol product **10q–t** in 90–92%. Astonishingly, benzaldehyde—which does not have any substituent also affords **10u** in 93% yield along with a minor amount of **11u**. The aldol reaction of aromatic aldehydes substituted with a methoxy group was also successful but slow as evident from the recovered aldehyde (entries 6 and 7). The aromatic aldehydes substituted with –OH and –$N(CH_3)_2$ were unreactive under the reaction conditions. Thus, the feasibility of the pyrrolidine catalyzed direct cross-aldol reaction of acetone with aromatic aldehydes in water, is dependent on the electrophlicity of the respective aldehydes.

3. Conclusion

In conclusion, we have demonstrated that pyrrolidine catalyzes a fast, direct cross-aldol reaction of acetone with a variety of heterocyclic and aromatic aldehydes in water. The electron deficiency of the heterocyclic and aromatic aldehydes is an important requirement for the success of the reaction. The process provides an environmentally friendly, high yielding synthetic methodology for procuring β-hydroxy ketones in 5 min.

4. Experimental

4.1. General

^{1}H NMR spectra were measured on Jeol AL-300 (300 MHz) or Bruker AC 200E (200 MHz) spectrometer at ambient temperature. Data were recorded as follows: chemical shift in ppm from internal tetramethylsilane on the δ scale, multiplicity (b = broad, s = singlet, d = doublet, dd = double doublet, dt = double triplet, t = triplet, and m = multiplet), coupling constant (Hz) and integration. ^{13}C NMR spectra were recorded on Jeol AL 300 (75.45 MHz) spectrometer at ambient temperature. Chemical shifts are recorded in ppm from the solvent resonance employed as the internal standard (deuterated chloroform at 77.07 ppm). IR spectra were obtained with Nicolet Avatar 320 FTIR and are reported in wave numbers (cm^{-1}). Mass spectra were recorded on GCMS-QP-2000 and Jeol JMS-D 300 mass spectrometer. All aldol reactions were carried out under an atmosphere of air in a closed system. For thin-layer chromatography (TLC) analysis throughout this work, glass plates (7.5×2.5 cm) coated with Acme's silica gel GF 254 containing 13% calcium sulfate as binder using ethyl acetate and hexane as the eluents. Visualization of the spots was accomplished by exposing to UV light or iodine vapors. The crude products were purified by preparative column chromatography on silica gel with 60–120 mesh. Organic substrates, 2-, 3-, 4-pyridine carboxaldehydes were procured from Merck, 2-pyrrolecarboxaldehyde, 2-thiophenecarboxaldehyde were obtained from Aldrich and 2-furfural was procured from SISCO, India and used as received. Acetone extra pure grade was purchased from SISCO, India. Pyrrolidine was procured from Spectrochem and used directly.

4.2. General procedure for the aldol reaction of heterocyclic aldehydes with acetone using pyrrolidine as catalyst

To a stirring mixture of respective heterocyclic aldehyde (1 mmol), acetone (0.37 mL, 5 mmol), water (5 mL) and amine (30 mol%) were added. The reaction was monitored by TLC and on completion was extracted with 100 mL of CH_2Cl_2 (3×30 mL). The organic layer was washed with water (2×50 mL) and dried over anhydrous Na_2SO_4 and evaporated to obtain crude product. Column chromatography of the crude on silica gel (60–120) using mixture of ethyl acetate and hexane in varying proportions as eluent, gave pure product.

4.2.1. 4-Hydroxy-4-(2′-pyridyl)butan-2-one (2a). Yield, 93% (153 mg); colourless crystalline solid, mp: 74–76 °C (lit.[12] 75.5–76 °C); MS (*m/z*) CI: 166 $(M+1)^+$. Anal. Calcd for $C_9H_{10}NO_2$: C, 65.45; H, 6.66; N, 8.48. Found: C, 65.54; H, 6.56; N, 8.01; IR (KBr): 3133, 1718, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.21 (s, 3H, CH_3), 2.90 (dd, 1H, $J=17.1$, 8.2 Hz, CH of CH_2), 3.06 (dd, 1H, $J=17.1$, 3.9 Hz, CH of CH_2), 5.19 (dd, 1H, $J=8.2$, 3.9 Hz, CH), 7.16–7.23 (m, 1H, PyH), 7.45 (d, 1H, $J=7.9$ Hz, PyH), 7.71 (dt, 1H, $J=7.9$, 1.7 Hz, PyH), 8.50–8.53 (m, 1H, PyH); ^{13}C DEPT NMR ($CDCl_3$): δ 30.2 (+ve), 50.4 (−ve), 69.4 (+ve), 120.0 (+ve), 121.9 (+ve), 136.6 (+ve), 147.9 (+ve), 161.5, 207.8.

4.2.2. 4-Hydroxy-4-(3′-pyridyl)butan-2-one (7i). Yield, 71% (117 mg); pale yellow liquid; HRMS (EI): exact mass calcd for $C_9H_{11}NO_2$: 165.0790. Found: 165.0785; IR (neat): 3200, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 2.81–2.87 (m, 2H, CH_2), 5.18 (dd, 1H, $J=8.4$, 4.0 Hz, CH), 7.26–7.30 (m, 1H, PyH), 7.71–7.75 (m, 1H, PyH), 8.45–8.50 (m, 1H, PyH); ^{13}C DEPT NMR ($CDCl_3$): δ 30.7 (+ve), 51.7 (−ve), 67.3 (+ve), 123.2 (+ve), 133.7 (+ve), 138.3, 146.9 (+ve), 148.2 (+ve), 208.0.

4.2.3. 4-Hydroxy-4-(4′-pyridyl)butan-2-one.[13] (7j) Yield, 68% (112 mg); yellow liquid; MS (*m/z*) EI: 165 (M^+); IR (neat): 3250, 1714, 1604 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 2.69 (br s, 1H, OH), 2.82 (d, 2H, $J=6.1$ Hz, CH_2), 5.15 (t, 1H, $J=6.1$ Hz, CH), 7.28 (d, 2H, $J=5.8$ Hz, PyH), 8.54 (d, 2H, $J=6.0$ Hz, PyH); ^{13}C DEPT NMR ($CDCl_3$): δ 30.7 (+ve), 51.6 (−ve), 67.9 (+ve), 120.3 (+ve), 149.7 (+ve), 153.5, 207.5.

4.2.4. 4-Hydroxy-4-(2′-furyl)butan-2-one (7k). Yield, 44% (68 mg); yellow liquid; HRMS (EI): exact mass calcd for $C_8H_{10}O_3$: 154.0630. Found: 154.0629; IR (neat): 3416, 1712 cm^{-1}; ^{1}H NMR[14a] ($CDCl_3$): δ 2.21 (s, 3H, CH_3), 2.95 (dd, 1H, $J=17.7$, 3.6 Hz, CH_2), 3.07 (dd, 1H, $J=17.7$, 8.6 Hz, CH_2), 3.24 (br s, 1H, OH), 5.19 (dd, 1H, $J=8.6$, 3.6 Hz, CH), 6.26–6.27 (m, 1H, ArH), 6.32–6.34 (m, 1H, ArH), 7.37 (d, 1H, $J=1.5$ Hz, ArH); ^{13}C DEPT NMR ($CDCl_3$): δ 30.7 (+ve), 48.2 (−ve), 63.7 (+ve), 106.2 (+ve), 110.3 (+ve), 142.1 (+ve), 155.1, 207.5.

4.2.5. 4-(2′-Furyl)but-3-en-2-one.[15] (8k) Yield, 52% (70 mg); dark brown liquid. Spectroscopic data consistent with literature.[14c]

4.2.6. 4-Hydroxy-4-(2′-thiophen)butan-2-one (7l). Yield, 30% (51 mg); dark brown liquid; HRMS (EI): exact mass calcd for $C_8H_{10}SO_2$: 170.0402. Found: 170.0391; IR (neat): 3419, 1710 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.21 (s, 3H, CH_3), 2.89–3.07 (m, 2H, CH_2), 5.19 (dd, 1H, $J=8.1$, 3.9 Hz, CH), 6.94–6.97 (m, 2H, ArH), 7.23–7.26 (m, 1H, ArH); ^{13}C DEPT NMR ($CDCl_3$): δ 30.7 (+ve), 51.7 (−ve), 66.1 (+ve), 123.5 (+ve), 124.7 (+ve), 126.7 (+ve), 146.5 and 207.5.

4.2.7. 4-(2′-Thiophenyl)but-3-en-2-one.[16] (8l) Yield, 65% (99 mg); dark brown liquid; MS (*m/z*) EI: 152 (M^+); IR (neat): 1664, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 6.53 (d, 1H, $J=16.0$ Hz, CH), 7.05–7.11 (m, 1H, ArH), 7.23–7.30 (m, 1H, ArH), 7.40 (d, 1H, $J=5.1$ Hz, ArH), 7.63 (d, 1H, $J=16.0$ Hz, CH,); ^{13}C DEPT NMR ($CDCl_3$): δ 27.7 (+ve), 125.7 (+ve), 128.2 (+ve), 128.8 (Ar-C), 131.4 (+ve), 135.6 (Ar-C), 139.7, 197.6.

4.2.8. 4-(Pyrrol-2-yl)but-3-en-2-one (8m). Yield, 62% (84 mg); colourless crystalline solid, mp: 115–117 °C (lit.[17] mp: 117–120 °C); MS (*m/z*) EI: 135 (M^+). Spectroscopic data consistent with literature.[14b,d]

4.3. General procedure of aldol reaction of 1 with different donor ketones

To a stirring mixture of **1** (107 mg, 1 mmol), methyl ketone (5 mmol) and water (5 mL), pyrrolidine (30 mol%) was added as catalyst. The monitoring of the reaction with TLC showed that reaction was completed in 5 min. The reaction was worked up by extraction with CH_2Cl_2 and washing the CH_2Cl_2 layer with water. The organic layer was dried over anhydrous Na_2SO_4 and distilled to obtain crude product. Column chromatography of the crude on silica gel (60–120) using mixture of ethyl acetate and hexane in varying proportions as eluent, gave pure product.

4.3.1. 1-Hydroxy-1-(2′-pyridyl)pentan-3-one (2b). Yield, 72% (129 mg); pale yellow liquid; HRMS (CI): exact mass calcd for $C_{10}H_{13}NO_2$: 179.0946. Found: 180.1024 (M+1); IR (neat): 3389, 1711, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.07 (t, 3H, $J=7.3$ Hz, CH_3,), 2.49 (q, 2H, $J=7.3$ Hz, CH_2), 2.84 (dd, 1H, $J=16.9$, 8.2 Hz, CH of CH_2), 3.04 (dd, 1H, $J=16.9$, 3.8 Hz, CH of CH_2), 4.36 (br s, 1H, OH), 5.16 (dd, 1H, $J=8.2$, 3.8 Hz, CH), 7.16–7.22 (m, 1H, PyH), 7.48 (d, 1H, $J=7.8$ Hz, PyH), 7.70 (dt, 1H, $J=7.8$, 1.6 Hz, PyH) and 8.50 (d, 1H, $J=4.7$ Hz, PyH,); ^{13}C DEPT NMR ($CDCl_3$): δ 7.7 (+ve), 36.5 (−ve), 49.3 (−ve), 69.8 (+ve), 120.2 (+ve), 122.2 (+ve), 136.5 (+ve), 148.5 (+ve), 161.42 and 208.0.

4.3.2. 1-Hydroxy-2-methyl-1-(2′-pyridyl)butan-3-one (2c). Yield, 11% (20 mg); yellow thick liquid, as inseparable mixture of *syn* and *anti* diastereomers in 1:1; MS (*m/z*) ESI: 202 $(M+Na)^+$; IR (neat): 3384, 1708, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.99 (d, 3H, $J=7.0$ Hz, CH_3) and 1.10 (d, 3H, $J=7.0$ Hz, CH_3), 2.18 (s, 3H, CH_3) and 2.24 (s, 3H, CH_3), 3.05–3.19 (m, 1H, CH of two diastereomers), 4.18 (d, 1H, $J=6.3$ Hz, CH *anti/threo*), 5.17 (d, 1H, $J=3.3$ Hz, CH *syn/erythro*), 7.19–7.23 (m, 1H, PyH of two diastereomers), 7.34–7.42 (m, 1H, PyH of two diastereomers), 7.66–7.72 (m, 1H, PyH of two diastereomers) and 8.52 (d, 1H, $J=3.9$ Hz, PyH,). ^{13}C DEPT NMR: ($CDCl_3$) δ 9.7 (+ve), 13.5 (+ve), 29.6 (+ve), 75.7 (+ve), 121.0 (+ve), 122.7 (+ve), 136.9 (+ve), 148.6 (+ve), 160.5 and 213.5.

4.3.3. 5-Hydroxy-1-phenyl-5-(2′-pyridyl)pentan-3-one (2d). Yield, 76% (194 mg); dark yellow liquid; HRMS (EI): exact mass calcd for $C_{16}H_{17}NO_2$: 255.1259. Found: 255.1249; IR (neat): 3395, 1711, 1593 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.76–3.02 (m, 6H, CH_2), 5.14 (dd, 1H, $J=7.8$, 3.8 Hz, CH), 7.10–7.21 (m, 6H, ArH), 7.40 (d, 1H, $J=7.8$ Hz, PyH), 7.65 (dt, 1H, $J=7.6$, 1.25 Hz, PyH), 8.46 (d, 1H, $J=4.7$ Hz, PyH); ^{13}C DEPT NMR ($CDCl_3$): δ 29.3 (−ve), 45.1 (−ve), 49.9 (−ve), 120.5 (+ve), 122.6 (+ve), 126.1 (+ve), 128.2 (+ve), 128.44 (+ve), 128.7 (+ve), 128.9 (+ve), 137.1 (+ve), 140.74, 148.2 (+ve), 161.1, 200.1.

4.3.4. 2-[Hydroxy(2′-pyridyl)methyl]cyclopentanone (2f). Yield, 62% (119 mg); colourless crystalline solid, mp 118–120 °C, as inseparable mixture of *syn* and *anti* diastereomers in 4:1; MS (*m/z*) 214 $(M+Na)^+$. Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.10; H, 6.80; N, 7.32. Found: C, 69.20; H, 6.64; N, 7.49; IR (neat): 3221, 1708, 1592 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.57–2.30 (m, 6H, CH_2), 2.54–2.59 (m, 1H, CH), 4.98 (d, 1H, $J=6.3$ Hz, CH *anti/threo*), 5.29 (d, 1H, $J=2.5$ Hz, CH *syn/erythro*), 7.16–7.23 (m, 1H, PyH), 7.32 (d, 1H, $J=7.7$ Hz, PyH), 7.66 (dt, 1H, $J=7.7$, 1.8 Hz, PyH) and 8.52 (d, 1H, $J=4.4$ Hz, PyH). ^{13}C DEPT NMR: ($CDCl_3$) δ 20.5 (−ve), 22.1 (−ve), 38.9 (−ve), 54.8 (+ve), 70.7 (+ve), 120.1 (+ve), 122.2 (+ve), 136.8 (+ve), 147.9 (+ve), 160.43, 219.53.

4.3.5. 2-[Hydroxy(2′-pyridyl)methyl]cyclohexanone (2g). Yield, 86% (176 mg); colourless crystalline solid, mp: 120–122 °C, as inseparable mixture of *syn* and *anti* diastereomers[18] in of 1:2; MS: (*m/z*) 228 $(M+Na)^+$. Anal. Calcd for $C_{12}H_{13}NO_2$: C, 70.24; H, 7.31; N, 6.62. Found: C, 70.40; H, 7.09; N, 7.42; IR (neat): 3215, 1707, 1592 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.52–1.93 (m, 4H, CH_2), 2.01–2.19 (m, 2H, CH_2), 2.25–2.41 (m, 2H, CH_2), 3.02–3.11 (m, 1H, CH), 4.88 (d, 1H, $J=5.5$ Hz, CH *anti/threo*), 5.36 (d, 1H, $J=2.45$ Hz, CH *syn/erythro*), 7.15–7.20 (m, 1H, PyH), 7.48 (d, 1H, $J=7.9$ Hz, PyH), 7.68 (dt, 1H, $J=7.9$, 1.5 Hz, PyH), 8.51 (d, 1H, $J=4.4$ Hz, PyH); ^{13}C DEPT NMR ($CDCl_3$): δ 24.6 (−ve), 26.2 (−ve), 27.6 (−ve), 42.4 (−ve), 55.9 (+ve), 70.9 (+ve), 120.8 (+ve), 121.9 (+ve), 136.5 (+ve), 148.3 (+ve), 160.9, 213.8.

4.3.6. 4-Hydroxy-1,1-dimethoxy-4-(2′-pyridyl)butan-2-one (2h). Yield, 87% (196 mg); yellow thick liquid; HRMS (CI): exact mass calcd for $C_{11}H_{15}NO_4$: 225.1001. Found: 226.1074 (M+1); IR (neat): 3442, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.07–3.15 (m, 2H, CH_2), 3.40 (s, 3H, OCH_3), 3.41 (s, 3H, OCH_3), 4.54 (s, 1H, CH), 5.23–5.27 (m, 1H, CH), 7.18–7.27 (m, 1H, Py-H), 7.43 (d, 1H, $J=8.1$ Hz, Py-H), 7.70 (dt, 1H, $J=7.8$, 1.8 Hz, Py-H), 8.51–8.54 (m, 1H, Py-H). ^{13}C DEPT NMR ($CDCl_3$): 45.5 (−ve), 54.5 (+ve), 69.2 (+ve), 103.6 (+ve), 120.3 (+ve), 122.3 (+ve), 136.8 (+ve), 148.2 (+ve), 161.1, and 209.1.

4.4. Typical procedure for the aldol reaction of acetone and pyridine-2-carboxaldehyde using NaOH as base

To a stirring mixture of acetone (0.37 ml, 5 mmol) and water (5 mL) in a round bottom flask prefitted with a pH meter, a solution of 1 N NaOH was added dropwise to make the pH of the solution 11.75. At this constant pH of 11.75 pyridine-2-carboxaldehyde **1** (107 mg, 1 mmol) was added. The reaction mixture was stirred for another 30 min, diluted with 50 mL water and extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4 and distilled to get crude 4-hydroxy-4-(2′-pyridyl)butan-2-one (**2a**). Column chromatography of the crude on silica gel (60–120) using mixture of ethyl acetate and hexane in varying proportions as eluent, gave 4-hydroxy-4-(2′-pyridyl) butan-2-one (**2a**) as a crystalline solid (128 mg, 83%).

4.5. General procedure for the aldol reaction of aromatic aldehydes with acetone using pyrrolidine as catalyst

To a stirring mixture of respective aromatic aldehydes (2 mmol), acetone (40 mmol) and water (3 mL), amine (30 mol%) was added as catalyst. The reaction was monitored by tlc and after 5 min the reaction was stopped and extracted with CH_2Cl_2 (2×25 mL). The organic layer was washed with water (2×50 mL), dried over anhydrous Na_2SO_4 and evaporated to obtain crude product. Column chromatography of the crude on silica gel (60–120) using mixture of ethyl acetate and hexane in varying proportions as eluent, gave pure product.

4.5.1. 4-Hydroxy-4-(4′-nitrophenyl)butan-2-one (10q). Yield, 91% (380 mg); yellow crystalline solid, mp: 59–61 °C (lit.[25] mp: 59–61 °C). Spectroscopic data consistent with literature.[25]

4.5.2. 4-Hydroxy-4-(2′-nitrophenyl)butan-2-one (10r). Yield, 92% (384 mg); crystalline solid, mp: 52–55 °C (lit.[13] mp: 65–68 °C); MS (*m/z*) EI: 192 (M^+-H_2O); IR ($CHCl_3$): 3432, 1712 cm^{-1}; ^1H NMR[24] ($CDCl_3$): δ 2.16 (s, 3H, CH_3), 2.68 (dd, 1H, $J=17.7$, 9.4 Hz, CH_2), 3.11 (dd, 1H, $J=17.7$, 2.0 Hz, CH_2), 5.65 (dd, 1H, $J=9.4$, 2.0 Hz, CH), 7.40 (t, 1H, $J=8.4$ Hz, ArH), 7.65 (t, 1H, $J=8.4$ Hz, ArH), 7.93–7.98 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 30.2, 51.1, 65.4, 124.3, 128.0, 128.1, 133.7, 138.5, 146.9, 208.6.

4.5.3. 4-Hydroxy-4-(4′-chlorophenyl)butan-2-one (10s). Yield, 90% (356 mg); crystalline solid, mp: 47–50°C (lit.[23] mp: 59–61 °C). Spectroscopic data consistent with literature.[23]

4.5.4. 4-Hydroxy-4-(2′-chlorophenyl)butan-2-one (10t). Yield, 92% (364 mg); colourless thick liquid; MS (*m/z*) EI: 180 (M^+-H_2O); IR (neat): 3432, 1710 cm^{-1}; ^1H NMR[24] ($CDCl_3$): δ 2.19 (s, 3H, CH_3), 2.67 (dd, 1H, $J=17.5$, 9.5 Hz, CH_2), 2.93 (dd, 1H, $J=17.5$, 2.3 Hz, CH_2), 5.49 (dd, 1H, $J=9.5$, 2.3 Hz, CH), 7.14–7.31 (m, 3H, ArH), 7.60 (d, 1H, $J=7.8$ Hz, ArH); ^{13}C NMR ($CDCl_3$): δ 30.4, 50.0, 66.4, 127.0, 128.4, 129.1, 130.9, 140.1, 208.9.

4.5.5. 4-Hydroxy-4-phenylbutan-2-one (10u). Yield, 93% (305 mg); colourless thick liquid. Spectroscopic data consistent with literature.[14a]

4.5.6. 4-Hydroxy-4-(4′-methoxyphenyl)butan-2-one (10v). Yield, 66% (255 mg); low melting solid (Lit.[23] mp: 36–38 °C); MS (*m/z*) EI: 193 (M^+); IR (neat): 3439, 1709 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.18 (s, 3H, CH_3), 2.77 (dd, $J=17.3$, 3.3 Hz, 1H of CH_2), 2.89 (dd, $J=17.3$, 9.0 Hz, 1H of CH_2), 5.10 (dd, $J=9.0$, 3.3 Hz, 1H, CH), 6.87 (d, $J=8.4$ Hz, 2H, ArH), 7.27 (d, $J=8.4$ Hz, 2H, ArH); ^{13}C NMR ($CDCl_3$): δ 30.6, 51.9, 55.1, 69.4, 113.7, 126.8, 134.9, 158.9, 209.0.

4.5.7. 4-Hydroxy-4-(3′,4′,5′-trimethoxyphenyl)-butan-2-one (10w). Yield, 83% (421 mg); crystalline solid, mp: 60–63 °C; MS (*m/z*) EI: 254 (M^+); IR (neat): 3405, 1706 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.22 (s, 3H, CH_3), 2.83–2.88 (m, 2H of CH_2), 3.29 (d, $J=3.0$ Hz, 1H, OH), 3.83 (s, 3H, OCH_3), 3.87 (s, 6H, 2×OCH_3), 5.08–5.11 (m, 1H, CH), 6.59 (s, 1H, ArH), 7.26 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$): δ 30.5, 51.9, 55.8, 60.5, 69.7, 102.3, 136.9, 138.6, 153.0, 208.7.

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References and notes

1. (a) Decker, P.; Schweer, H. *Origins of Life* **1984**, *14*, 335. (b) Degraaf, R. M.; Visscher, J.; Xu, Y.; Arrhenius, G.; Schwartz, W. *J. Mol. Evol.* **1998**, *47*, 501–507.
2. (a) Machajewski, T. D.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2000**, *39*, 1352–1374 and references therein. (b) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443–473. (c) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994.
3. (a) Zhong, G.; Lerner, R. A.; Barbas, C. F., III *Angew. Chem., Int. Ed.* **1999**, *38*, 3738–3741. (b) Hoffmann, T.; Zhong, G.; List, B.; Shabat, D.; Anderson, J.; Gramatikova, S.; Lerner, R. A.; Barbas, C. F., III *J. Am. Chem. Soc.* **1998**, *120*, 2768–2779.
4. (a) List, B.; Lerner, R. A.; Barbas, C. F., III *J. Am. Chem. Soc.* **2000**, *122*, 2395–2396. (b) Notz, W.; List, B. *J. Am. Chem. Soc.* **2000**, *122*, 7386–7387. (c) List, B.; Pojarliev, P.; Castello, C. *Org. Lett.* **2001**, 573–575. (d) Sakthivel, K.; Notz, W.; Bui, T.; Barbas, C. F., III *J. Am. Chem. Soc.* **2001**, *123*, 5260–5267. (e) Córdova, A.; Notz, W.; Barbas, C. F., III *J. Org. Chem.* **2002**, *67*, 301–303. (f) Hajos, Z. G.; Parrish, D. R. *J. Org. Chem.* **1974**, *39*, 1615–1621. (g) Eder, U.; Sauer, G.; Wiechert, R. *Angew. Chem., Int. Ed.* **1971**, *10*, 496–497. (h) Agami, C.; Platzer, N.; Sevestre, H. *Bull. Soc. Chim. Fr.* **1987**, *2*, 358–360. (i) Northrup, A. B.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 6798–6799. (j) Bogevig, A.; Kumaragurubaran, N.; Jorgensen, K. A. *Chem. Commun.* **2002**, 620–621.
5. (a) Dickerson, T. J.; Janda, K. D. *J. Am. Chem. Soc.* **2002**, *124*, 3220–3221. (b) Cordova, A.; Notz, W.; Barbas, C. F., III *Chem. Commun.* **2002**, 3024–3025. (c) Reymond, J.-L.; Chen, Y. *J. Org. Chem.* **1995**, *60*, 6970–6979. (d) Dickerson, T. J.; Lovell, T.; Meijler, M. M.; Noodleman, L.; Janda, K. D. *J. Org. Chem.* **2004**, *69*, 6603–6609.
6. Heathcock, C. H. In Heathcock, C. H., Ed.; Comprehensive Organic Synthesis: Additions to C-X π-Bonds Part-2; Pergamon: Oxford, 1991; Vol. 2, pp 133–179.
7. In PBS buffer hydroxyacetone reacts with p-nitrobenzaldehyde in the presence of catalytic pyrrolidine to afford aldol addition product (65%). Ref. 5(b).
8. Cavill, J. L.; Peters, J.-E.; Tomkinson, N. C. O. *Chem. Commun.* **2003**, 728–729.
9. (a) Fisher, F. G.; Marschall, A. *Ber.* **1931**, *64*, 2825. (b) Gutsche, C. D.; Redmore, D.; Buriks, R. S.; Nowotny,

K.; Grassner, H.; Armbruster, C. W. *J. Am. Chem. Soc.* **1967**, *89*, 1235–1245.

10. Guthrie, J. P. *J. Am. Chem. Soc.* **1991**, *113*, 7249–7255.
11. Haynes, L. W. In *Enamimes: Synthesis, Structure and Reactions*; Cook, A. G., Ed.; Dekker, M: London, 1969; p 63.
12. Marvel, C. S.; Stille, J. K. *J. Org. Chem.* **1957**, *22*, 1451–1457.
13. Grayson, D. H.; Tuite, M. R. J. *J. Chem. Soc., Perkin Trans. 1* **1986**, *I*, 2137–2142.
14. (a) Bernardi, A.; Gennari, C.; Goodman, J. M.; Leue, V.; Paterson, I. *Tetrahedron* **1995**, *51*, 4853–4866. (b) Hayakawa, K.; Yodo, M.; Ohsuki, S.; Kanematsu, K. *J. Am. Chem. Soc.* **1984**, *106*, 6735–6740. (c) Alvarez-Ibarra, C.; Perez, M. S. A.; Fernandez, M. J.; Serrano, D.; Sinisterra, V. *J. Chem. Res. (S)* **1992**, *326–327*(M), 2674–2686. (d) Gregory, B.; Hinz, W.; Jones, R. A.; Arques, J. S. *J. Chem. Res. (S)* **1984**, *311*(M), 2801–2821.
15. Furniss, S. B.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; ELBS/Longman: London, 1989; p 1034.
16. (a) Keskin, H.; Miller, R. E.; Nord, F. F. *J. Org. Chem.* **1951**, *16*, 199–206. (b) Huang, Y.; Chen, C.; Shen, Y. *Synth. Commun.* **1989**, *19*, 501–509. (c) Kasahara, A.; Izumi, T.; Ogihara, T. *J. Heterocycl. Chem.* **1989**, *26*, 597–599.
17. Herz, W.; Brasch, J. *J. Org. Chem.* **1958**, *23*, 1513–1516.
18. Loh, T.-P.; Pei, J.; Koh, K. S.-V.; Cao, G.-Q.; Li, X.-R. *Tetrahedron Lett.* **1997**, *38*, 3465–3468.
19. Kumar, S.; Kumar, V.; Chimni, S. S. *Tetrahedron Lett.* **2002**, *43*, 8029–8031.
20. (a) Trost, B. M., Fleming, I., Heathcock, C. H., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 2. (b) Mukaiyama, T. *Tetrahedron* **1999**, *55*, 8609–8670. (c) Nicolaou, K. C.; Vourloumis, D.; Winssinger, N.; Baran, P. S. *Angew. Chem., Int. Ed.* **2000**, *39*, 44–122.
21. Henderson, D. P.; Shelton, M. C.; Cotterill, I. C.; Toone, E. J. *J. Org. Chem.* **1997**, *62*, 7910–7911.
22. Wu, Y.-S.; Shao, W.-Y.; Zheng, C.-Q.; Huang, Z.-L.; Cai, J.; Deng, Q.-Y. *Helv. Chim. Acta* **2004**, *87*, 1377–1384.
23. Joly, S.; Nair, M. S. *J. Mol. Catal. B: Enzymatic* **2003**, *22*, 151–160.
24. Tang, Z.; Jiang, F.; Cui, X.; Gong, L.-G.; Mi, A.-Q.; Jiang, Y.-Z.; Wu, Y.-D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5755–5760.
25. Shokat, K.; Uno, T.; Schultz, P. G. *J. Am. Chem. Soc.* **1994**, *116*, 2261–2270.

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Tetrahedron

Can a consecutive double turn conformation be considered as a peptide based molecular scaffold for supramolecular helix in the solid state?

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Abstract—Helices and sheets are ubiquitous in nature. However, there are also some examples of self-assembling molecules forming supramolecular helices and sheets in unnatural systems. Unlike supramolecular sheets there are a very few examples of peptide sub-units that can be used to construct supramolecular helical architectures using the backbone hydrogen bonding functionalities of peptides. In this report we describe the design and synthesis of two single turn/bend forming peptides (Boc-Phe-Aib-Ile-OMe **1** and Boc-Ala-Leu-Aib-OMe **2**) (Aib: α-aminoisobutyric acid) and a series of double-turn forming peptides (Boc-Phe-Aib-Ile-Aib-OMe **3**, Boc-Leu-Aib-Gly-Aib-OMe **4** and Boc-γ-Abu-Aib-Leu-Aib-OMe **5**) (γ-Abu: γ-aminobutyric acid). It has been found that, in crystals, on self-assembly, single turn/bend forming peptides form either a supramolecular sheet (peptide **1**) or a supramolecular helix (peptide **2**), unlike self-associating double turn forming peptides, which have only the option of forming supramolecular helical assemblages.

1. Introduction

The challenge in molecular self-assembly is to design molecular building blocks that are predisposed to give definite supramolecular structures using non-covalent interactions. Supramolecular helices and sheets are the most common and indispensable form of structures in biological systems. Helicity exists in numerous biological and chemical systems. In proteins, the α-helical structure is a very common motif. In DNA-double helices,[1] collagen triple helix[2] and even in the coat protein complex of Tobacco Mosaic Virus (TMV),[3] helicity is a common observable feature. Surprisingly, a considerable amount of helicity is also present in some misfolded, neurodegenerative disease-causing protein aggregates popularly known as amyloid plaques.[4] Unnatural supramolecular helical structures can be constructed using conformational restriction of macromolecules,[5] intra or intermolecular hydrogen bonds[6] or by metal ion chelation.[7] The most common and well-studied example of supramolecular single-, double- and triple-stranded helical conformations are metal chelated, self-assembled, oligonuclear coordination compounds,[7] the helicates.[8] Different approaches to construct supramolecular helices without metal ions and stabilized only by intermolecular and intramolecular hydrogen bonding have also been pursued.[6,9] Recently pyrene-4, 5-dione derivatives have been used to design supramolecular helical structures.[10] Peptide derivatives[11] and even chiral amino acids like the chiral 2,6-pyridinedicarboxamide containing the podand L-histidyl moieties and the corresponding D-derivative[12a] and ferrocene bearing the podand chiral dipeptide moieties (-L-Ala-L-Pro-OEt) and the corresponding D-derivative[12b] have been used to form supramolecular helical assemblages. Parthasarathi and his colleagues have synthesized and characterized a series of tripeptides which form extended helical structures with intervening water molecules between two consecutive peptide molecules and they demonstrated the hydrated helix pattern in crystals.[13] Our group is involved in constructing supramolecular peptide helices utilizing the backbone hydrogen bonding functionalities of the peptide molecules.[14] From our previous report, it has been observed that the short synthetic terminally blocked peptides with single turn/bend conformations can form either supramolecular sheets[15] or supramolecular helices.[14a] However, the peptide molecules with a double turn structure, self-assemble to form

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Keywords: Aib; Double turn conformation; 'S' shaped molecular structure; Supramolecular helix.

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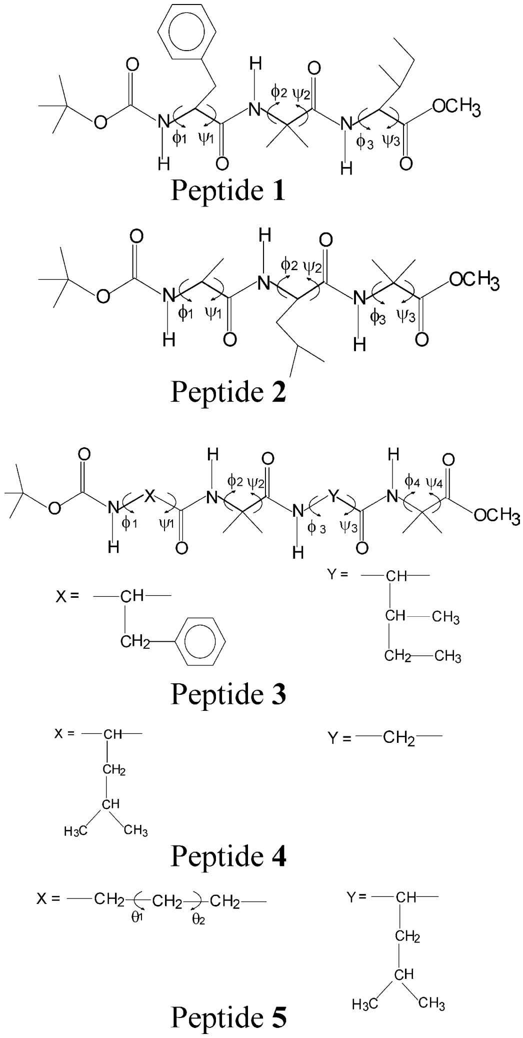


Figure 1. Schematic presentation of peptides **1**–**5**.

supramolecular helices in crystals.[14b–d] Therefore, it is necessary to define the role of a consecutive β-turn structure in the formation of supramolecular helical architecture. In this context we have designed and synthesized a series of single and double turn/ bend forming peptides (Fig. 1). All our previously reported peptides[14,15] and the peptides described in the present work are listed in Table 1. In this paper, we address the question of whether self-assembling double-turn forming peptides can only form supramolecular helical structures or whether other supramolecular architectures are possible.

2. Results and discussion

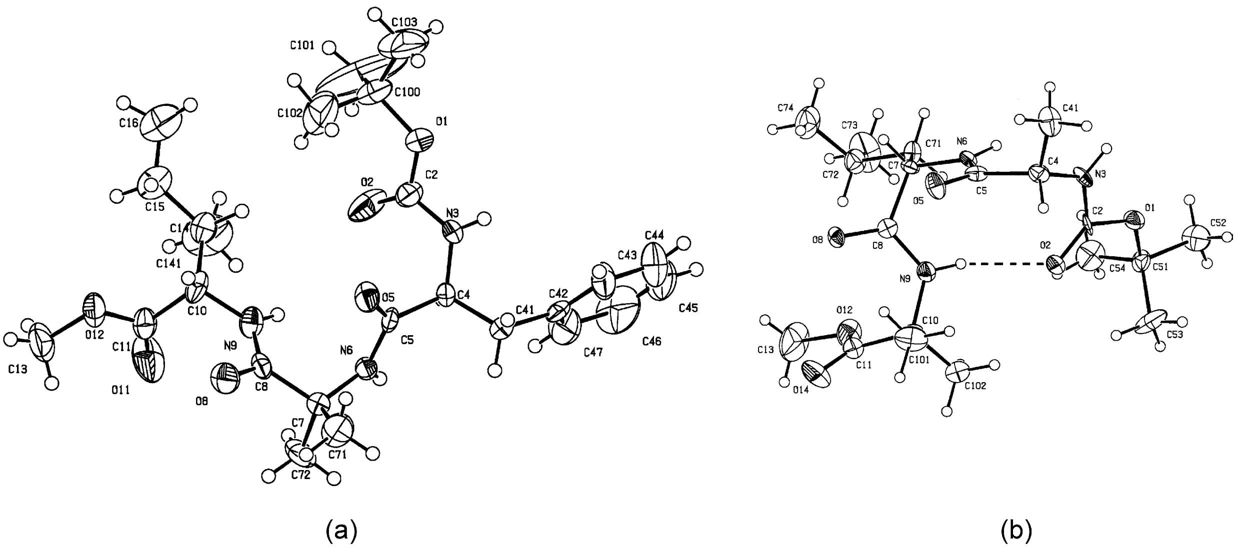
Peptides (peptides **1**, **2**, **3**, **4** and **5**) reported in this study have been synthesized with conformationally constrained, helicogenic Aib (α-aminoisobutyric acid) residue(s) in order to induce the helical nature of the individual peptide backbone.[16] The terminally protected tripeptides Boc-Phe-Aib-Ile-OMe **1** and Boc-Ala-Leu-Aib-OMe **2** (Fig. 1) have been designed and synthesized to obtain single β-turn/bend forming molecular conformations. The tetrapeptides Boc-Phe-Aib-Ile-Aib-OMe **3** and Boc-Leu-Aib-Gly-Aib-OMe **4** and Boc-γ-Abu-Aib-Leu-Aib-OMe **5**, each containing two Aib residues have been synthesized to obtain double bend conformations with two consecutive β-turns or other unusual turn structures. Previous studies from our group have established that the helix-nucleating Aib (α-aminoisobutyric acid) residue needs to be placed adjacent to the flexible N-terminally located γ-Abu (γ-aminobutyric acid residue) to obtain a turn structure.[17] All the reported peptides were studied using X-ray crystallography, NMR and mass spectrometry.

2.1. Single crystal X-ray diffraction study

Tripeptide **1** contains a helicogenic Aib residue at the central region whereas tripeptide **2** possesses the Aib residue at the C-terminus. The molecular conformations of tripeptides **1** and **2** in the crystal state are illustrated in Figure 2. Most of the ϕ and ψ values (except ψ_1 and ψ_3) of the constituent amino acid residues of tripeptide **1** fall within the helical region of the Ramachandran plot (Table 2). Hence the tripeptide **1** backbone, though it fails to form any intramolecular hydrogen bonded β turn conformation, adopts a bend (turn-like) structure (Fig. 2a), which self-assembles through two intermolecular hydrogen bonds (N6–H6···O8, 2.36 Å, 3.02 Å, 135.00° with symmetry element $-x$, $-0.5+y$, $1-z$) along the crystallographic *b* axis to form a monolayer β-sheet structure. These monolayer structures of tripeptide **1** are further self-assembled into higher order supramolecular β-sheet structure along the crystallographic *a* and *c* directions via van der

Table 1. List of single and double turn/bend forming peptides from our group

Sequence	No. of bends/turns in molecular structure	Supramolecular structure	Reference
1. Boc-Leu-Aib-Phe-OMe	One	Helix	14a
2. Boc-Leu-Aib-Phe-Aib-OMe	Two	Helix	14b
3. Boc-Ala-Aib-Leu-Aib-OMe	Two	Helix	14c
4. Boc-β-Ala-Aib-Leu-Aib-OMe	Two	Helix	14d
5. Boc-Aib-Val-Aib-β-Ala-OMe	Two	Helix	14e
6. Boc-Leu-Aib-β-Ala-OMe	One	Sheet	15a
7. Boc-Ala-Aib-Val-OMe	One	Sheet	15b
8. Boc-Ala-Aib-Ile-OMe	One	Sheet	15b
9. Boc-Ala-Gly-Val-OMe	One	Sheet	15b
10. Boc-Phe-Aib-Ile-OMe	One	Sheet	In current study
11. Boc-Ala-Leu-Aib-OMe	One	Helix	In current study
12. Boc-Phe-Aib-Ile-Aib-OMe	Two	Helix	In current study
13. Boc-Leu-Aib-Gly-Aib-OMe	Two	Helix	In current study
14. Boc-γ-Abu-Aib-Leu-Aib-OMe	Two	Helix	In current study



(a) (b)

Figure 2. (a) The structure of peptide **1** showing the atomic numbering scheme. Ellipsoids at 30% probability. (b) The ORTEP diagram of the peptide **2** showing the atomic numbering scheme. Ellipsoids at 20% probability. Intramolecular hydrogen bonds are shown as dotted line.

Table 2. Selected backbone torsional angles of peptides **1**–**5**

Compound	$\phi 1$	$\psi 1$	$\phi 2$	$\psi 2$	$\phi 3$	$\psi 3$	$\phi 4$	$\psi 4$	$\theta 1$	$\theta 2$
Peptide **1**	−59.2	154.2	62.5	30.9	−75.0	146.8	—	—	—	—
Peptide **2**	−66.6	−15.0	−53.5	−42.8	48.2	46.6	—	—	—	—
Peptide **3**	62.0	23.6	53.4	26.6	64.4	25.5	−46.4	134.6	—	—
Peptide **4**	−62.6	116.9	54.8	30.0	83.2	2.6	−47.1	−50.1	—	—
Peptide **5**										
Molecule A	−115.6	−140.8	−53.5	−37.3	−86.5	−6.0	−55.5	−47.8	57.6	64.2
Molecule B	−121.7	−134.8	−55.5	−37.3	−80.2	−14.0	42.5	−128.4	60.2	56.6

Waals interactions (Fig. 3). By comparison Figure 2b shows that there is a 4→1 hydrogen bond between Boc CO and Aib(3) N–H (N9–H···O2, 2.37, 3.04 Å, 135.00°) forming a distorted type III β-turn (10-membered hydrogen bonded ring) conformation in tripeptide **2**. The backbone torsion angles of tripeptide **2** fall within the helical region of the Ramachandran plot (Table 2). For the first two residues, that is Ala(1) and Leu(2), the ϕ, ψ values lie within the right handed helical region of the Ramachandran plot while ϕ, ψ values of the last residue (Aib(3)) fall in the left-handed helical region. Each individual sub-unit of tripeptide **2** then self-assembles through intermolecular hydrogen bonds (N3–H···O8, 2.11, 2.93 Å, 159.00° and N6–H···O5, 2.37, 3.2 Å, 164.00° with symmetry element $-0.5+x$, $-0.5-y$, $-z$) atop one another to form a supramolecular helix along the crystallographic *a* axis (Fig. 4). In the former example and other previous studies[15] it has been demonstrated that tripeptides with single β-turn conformations can form supramolecular β-sheet architectures upon self-assembly. The structure of peptide **2** reported here and other earlier work in the literature[14a] has indicated that tripeptides containing a turn/bend conformation can also form supramolecular helical structure upon self-association in the solid state. So, molecular self-assembly of single-turn forming peptides does not only lead to one particular type of supramolecular structure, but can form either supramolecular helices or supramolecular β-sheets depending upon the nature of crystal packing.

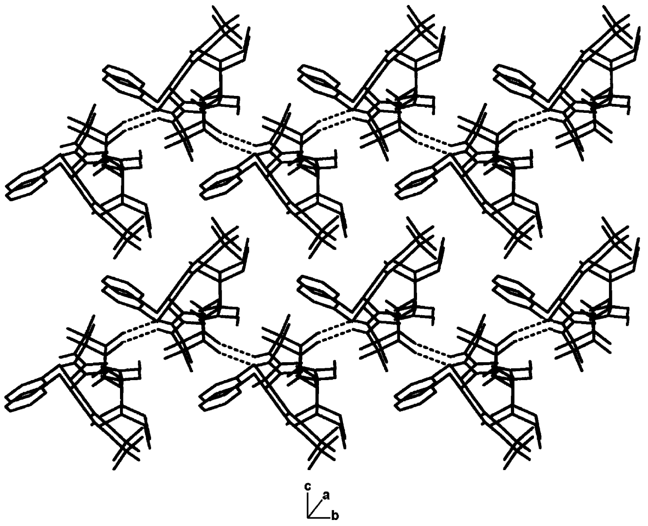


Figure 3. Packing of peptide **1** along crystallographic *b* axis showing intermolecularly hydrogen bonded sheet-like structure.

We are trying to find a peptide-based molecular scaffold, which can only form a definite type of supramolecular structure: namely the supramolecular helix. In this regard we have designed and synthesized tetrapeptides **3**, **4** and **5**. From X-ray crystallography, it is evident that the tetrapeptide **3** adopts a consecutive β-turn structure in the crystal (Fig. 5a). Incorporation of helicogenic Aib residues has driven the tetrapeptide **3** backbone to form two overlapping type III′ β-turn conformations through intramolecular

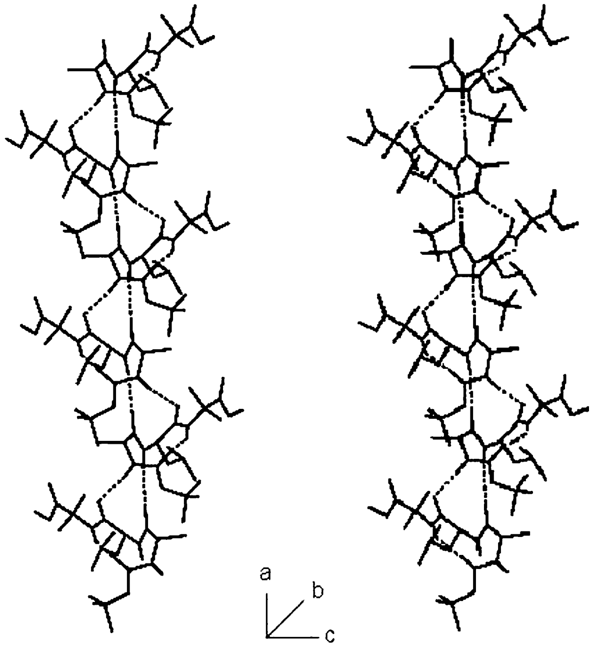


Figure 4. A cross-eye stereo representation of the higher order packing of peptide **2** along crystallographic *a* axis showing the formation of supramolecular helix via intermolecular hydrogen bonds.

hydrogen bonds (N9–H···O2, 2.31, 3.17 Å, 175.00° and N12–H···O5, 2.17 Å, 2.95 Å, 151.00°). Most of the backbone torsion angles (except $\psi 4$) of tetrapeptide **3** are in the left-handed helical region of the Ramachandran diagram (Table 2). The tetrapeptide **4** also forms two successive β-turns in the crystal (Fig. 5b). Ten-membered intramolecular hydrogen bonds are formed between the CO of the Boc group and Gly(3) NH (N13–H···O4, 2.21, 3.02 Å, 157.00°) and the CO of Leu(1) and Aib(4) NH (N17–H···O7, 2.05 Å, 2.88 Å, 162.00°), respectively, in tetrapeptide **4**. The backbone torsion angles (except ψ_1) of tetrapeptide **4** are mostly in the helical region of the Ramachandran diagram (Table 2). However, the dihedral angles of tetrapeptide **4** indicate that the first turn is a type II β-turn whereas the second turn is a type I′ β-turn. In both tetrapeptides **3** and **4**, the Aib(2) residue occupies simultaneously the $i+2$th position of first turn and the $i+1$th position of the second turn. Here the remarkable feature is that these two tetrapeptides form an ‘S’ shaped molecular scaffold as a result of the double turn structure (Fig. 5). The individual tetrapeptide **3** subunits are regularly stacked atop one another through multiple intermolecular hydrogen bonds (N6–H···O11, 2.23, 2.95 Å, 141.00° and N4–H···O8, 2.10 Å, 2.90 Å, 154.00° with symmetry element $1-x$, $0.5+y$, $0.5-z$) to form a supramolecular helix along the axis parallel to crystallographic *b* direction (Fig. 6). The hydrogen bonds between the Phe(1) and Aib(2) NH groups and the Aib(2) and Ile(3) C=O groups are responsible for connecting individual molecules to form and stabilize the supramolecular one-dimensional helical column of peptide **3**. The supramolecular building blocks of tetrapeptide **4** are further self-assembled through selective intermolecular hydrogen bonds (N5–H···O16, 2.01, 2.86 Å, 174.00° with symmetry element $2-x$, $0.5+y$, $0.5-z$) to generate a supramolecular helical architecture along the crystallographic *b* axis (Fig. 7). One interesting feature of the crystal structure of the tetrapeptide **4** is that, the individual helical columns of **4** are linked to the adjacent column exploiting the hydrogen bond functionalities of Aib(2) NH and Aib(4) CO (N8–H···O20, 2.09, 2.93 Å, 165.00° with symmetry element $1+x$, y, z).

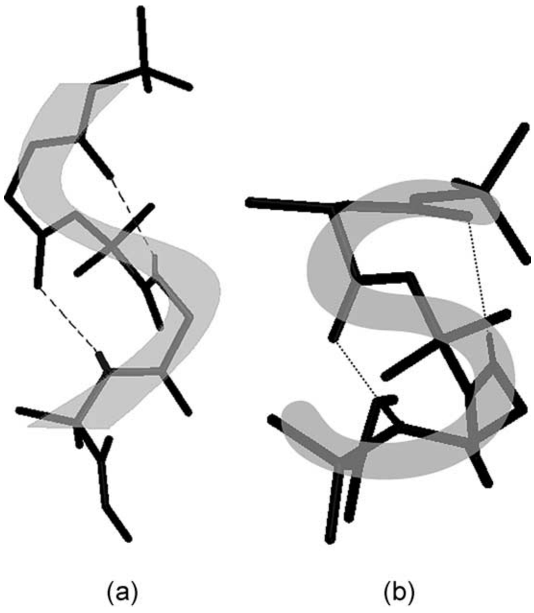

(a) (b)

Figure 5. (a) Formation of ‘S’ shaped molecular scaffold by peptide **3** and (b) Formation of ‘S’ shaped molecular scaffold by peptide **4**.

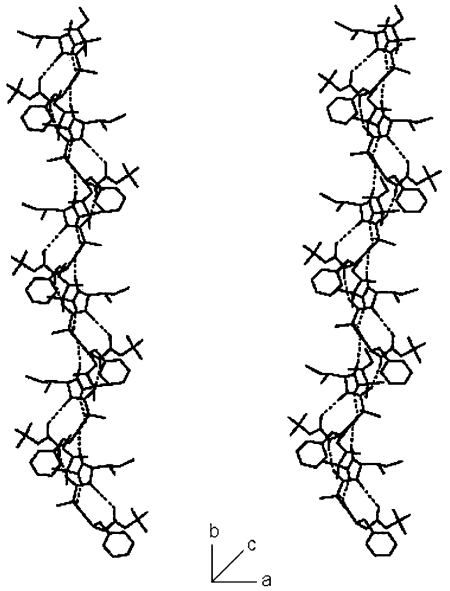


Figure 6. Cross-eye stereo representation of the packing of peptide **3** shows the intermolecular hydrogen-bonded supramolecular helix along the *b* axis. Hydrogen bonds are shown as dotted lines.

The molecular conformation of tetrapeptide **5** (Fig. 8) was also established by single crystal X-ray diffraction studies. Figure 8 illustrates that there are two molecules (namely A and B) in the asymmetric unit, which have equivalent conformations. Both molecules A and B in the asymmetric unit form a hydrogen bond to a water molecule (N3A–H···O200, 2.18, 3.00 Å, 160.00° for molecule A and N3B–H···O100, 2.22, 3.04 Å, 160.00° for molecule B with symmetry

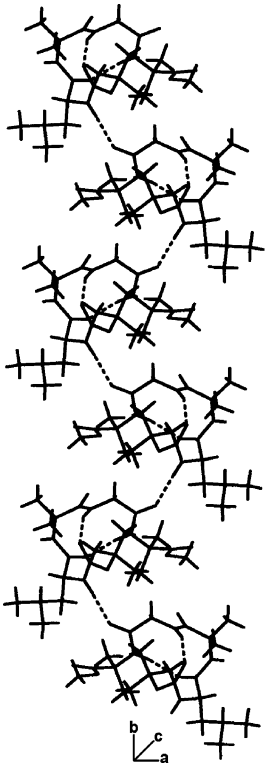


Figure 7. The packing of the peptide **4** illustrating the intermolecular hydrogen-bonded supramolecular helix along the *b* axis. Hydrogen bonds are shown as dotted lines.

equivalent *x*, −1+*y*, *z* and *x*, 1+*y*, *z*, respectively). The insertion of two CH_2 groups into the backbone (γ-Abu) causes an unusual 4→1 type hydrogen bond (N11A–H···O2A, 2.24, 3.07 Å, 163.00° for molecule A and N11B–H···O2B, 2.19, 3.03 Å, 165.00° for molecule B) between the CO of the Boc group and the NH of Leu(3) forming a 12-membered hydrogen bonded ring (Fig. 8). An additional 10-atom hydrogen bonded β-turn (N18A–H···O7A, 2.18, 3.02 Å, 165.00° for molecule A and N18B–H···O7B, 2.26, 3.05 Å, 152.00° for molecule B) between the CO of γ-Abu and the NH of Aib(4) is also formed adjacent to the 12-membered hydrogen bonded ring. Hence, tetrapeptide **5** also has a double bend 'S' shaped molecular conformation. Most of the ϕ and ψ values (except ϕ_1 and ψ_1 of molecule A and ϕ_1, ψ_1 and ψ_4 of molecule B) of the constituent amino acid residues of tetrapeptide **5** fall within the helical region of Ramachandran plot (Table 2). The torsions about the polymethylene groups of the N-terminal γ-Abu residue (namely θ_1 and θ_2) in peptide **5** are in *gauche* conformation. The peptide self-assembles through intermolecular hydrogen bonds (N8A–H···O10A, 2.15, 2.92 Å, 149.00° with symmetry equivalent 0.5−*x*, −0.5+*y*, 1−*z* for molecule A and N8B–H···O10B, 2.15, 2.92 Å, 149.00° with symmetry equivalent 0.5−*x*, 0.5+*y*, −*z* for molecule B) maintaining the proper registry of molecules A and B along the crystallographic *b* axis to form a supramolecular helical structure (Fig. 9). Crystal data for all the reported peptides are listed in Table 3.

3. Conclusion

All the reported peptides are composed of one or more conformationally constrained Aib residues and their backbone dihedral angles demonstrate that they mostly adopt helical conformations. Crystallographic studies of tripeptides **1** and **2** demonstrate that the self-association of peptides containing only one turn have two options to form a supramolecular architecture: either a supramolecular sheet or a supramolecular helix. Tetrapeptides **3** and **4** share a common structural motif, consecutive double bend molecular conformations and self-association of each peptide

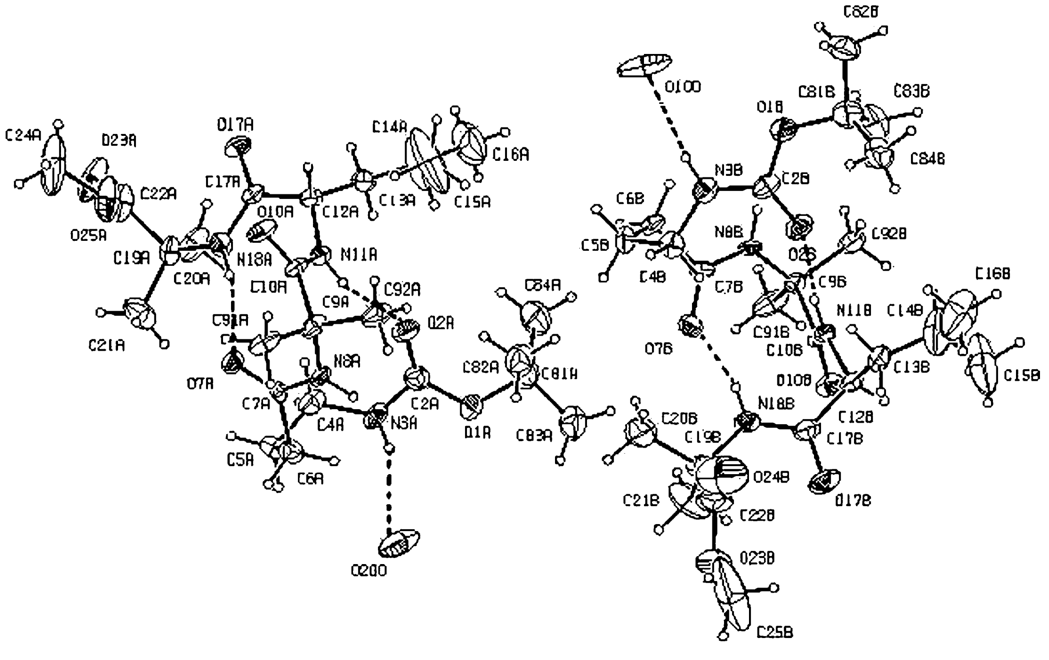


Figure 8. Molecular conformation of peptide **5** with atom numbering scheme. The asymmetric unit contains two molecules (namely A and B) each of which has a water molecule. Each molecule forms a 12 membered unusual turn adjacent with a 10 membered distorted type III β-turn. The hydrogen bonds are shown as dotted line. Thermal ellipsoids are shown at the level of 20% probability.

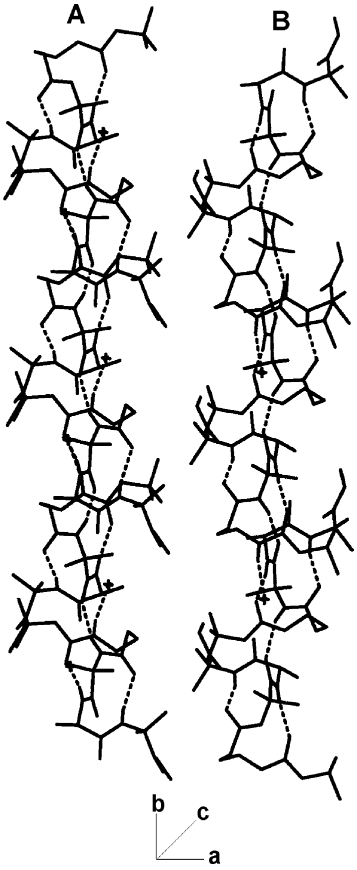


Figure 9. Packing diagram of peptide **5** along the crystallographic *b* axis showing intermolecular hydrogen-bonded supramolecular helices. The A and B molecules packed through proper registry, individual helix is formed by only a particular molecule that is either molecule A or B. Intermolecular hydrogen bonds are shown as dotted lines. The hydrogen bonded water molecule with each peptide is shown as *.

create supramolecular helices in crystals. Tetrapeptide **5**, instead of having a conformationally flexible γ-aminobutyric acid residue at the N terminus, also forms a double bend molecular conformation with two consecutive turns; one of which is an unusual turn with a 12-atom hydrogen bonded ring structure followed by a classical β-turn conformation. Thus, all these tetrapeptides, despite substantial differences in sequences adopt a double turn conformation, which in turn leads to the formation of a common S shaped molecular structure. This S shaped structure may increase the inherent propensity of individual tetrapeptide subunits to form a supramolecular helical architecture through intermolecular hydrogen bonds in the solid state.

Our work offers an elegant approach to controlling the three-dimensional structure using the stereochemical nature of a conformationally constrained amino acid (viz. Aib). This study should be useful for designing and calibrating supramolecular architectures having importance both in supramolecular chemistry and crystal engineering.

4. Experimental

4.1. Synthesis of peptide 1

4.1.1. Boc-Phe(1)-OH 6. A solution of phenylalanine (4.95 g, 30 mmol) in a mixture of dioxane (60 mL), water (30 mL) and 1 M NaOH (30 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butyldicarbonate (7.2 g, 33 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated under vacuum to about 40–60 mL, cooled in an ice water bath, covered with a layer of ethyl-acetate (about 50 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated under vacuum. The pure material as white solid was obtained.

Yield = 96% (7.63 g, 28.8 mmol). Mp 85–87 °C. Elemental Analysis Calcd for $C_{14}H_{19}NO_4$ (265): C, 63.50; N, 5.24; H, 7.20. Found: C, 63.39; N, 5.28; H, 7.17.

4.1.2. Boc-Phe(1)-Aib(2)-OMe 7. Boc-Phe-OH (7.42 g 28 mmol) was dissolved in a mixture of 30 mL dichloromethane (DCM) in an ice-water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (8.6 g, 56 mmol) by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was

Table 3. Crystal and data collection parameters of peptides **1–5**

	Peptide **1**	Peptide **2**	Peptide **3**	Peptide **4**	Peptide **5**
Empirical formula	$C_{25}H_{39}N_3O_6$	$C_{19}H_{35}N_3O_6$	$C_{29}H_{46}N_4O_7$	$C_{22}H_{40}N_4O_7$	$C_{24}H_{44}N_4O_7$, H_2O
Crystallizing solvent	Methanol–water	Methanol–water	Methanol–water	Methanol–water	Methanol–water
Crystal system	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$C2$
a (Å)	10.224(11)	10.613(12)	10.179(12)	10.421(17)	29.557(3)
b (Å)	11.378(12)	10.476(12)	15.606(17)	13.361(2)	12.237(14)
c (Å)	12.578(14)	21.132(2)	20.727(23)	20.171(3)	20.703(2)
β (°)	105.28(10)	(90)	(90)	(90)	121.31(1)
U (Å^3)	1411.5	2349.50	3292.55	2808(7)	6397.21
Z	2	4	4	4	8
Mol. Wt.	477.59	401.50	562.7	472.58	516.63
Density (Calcd, mg/mm^3)	1.124	1.135	1.135	1.117	1.073
Unique data	5066	3249	5268	4296	9986
Observed reflns. ($I > 2\sigma(I)$)	3291	1757	1994	2701	4607
R	0.0881	0.1157	0.1015	0.0839	0.1178
$wR2$	0.1331	0.2915	0.2014	0.2394	0.2280
Largest diff. peak and hole (e/Å^3)	0.259, −0.180	0.376, −0.321	0.269–0.260	0.204, −0.205	0.370, −0.251

concentrated to 10 mL. This was added to the reaction mixture, followed immediately by di-cyclohexylcarbodiimide (DCC) (5.77 g, 28 mmol). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, and the residue was taken up in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3× 50 mL), brine, then 1 M sodium carbonate (3×50 mL) and brine (2×50 mL) and dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 8.74 g (24 mmol, 86%) of **7** as white solid.

Yield = 86% (8.74 g, 24 mmol). Mp 119–121 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.22–7.35 (m, 5H); 6.3 (s, 1H); 5.1 (d, $J=9$ Hz, 1H); 4.28 (m, 1H); 3.71 (s, 3H); 2.96–3.07 (m, 2H); 1.45 (s, 6H); 1.42 (s, 9H). Elemental Analysis Calcd for $C_{19}H_{28}N_2O_5$ (364): C, 62.45; N, 7.72; H, 7.71. Found: C, 62.63; N, 7.69; H, 7.69.

4.1.3. Boc-Phe(1)-Aib(2)-OH 8. To **7** (8.5 g 23.35 mmol), MeOH (50 mL) and 2 M NaOH (20 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h methanol was removed under vacuum, the residue was taken up in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuum to yield a waxy solid.

Yield = 92% (7.525 g, 21.5 mmol). ^{1}H NMR ($(CD_3)_2SO$, 300 MHz, δ ppm): 12.27 (br, 1H); 8.05 (s, 1H); 7.17–7.26 (m, 5H); 6.77 (d, $J=9$ Hz, 1H); 4.13–4.21 (m, 1H); 2.85–3.02 (m, 2H); 1.34 (s, 6H); 1.29 (s, 9H). Elemental Analysis Calcd for $C_{18}H_{26}N_2O_5$ (350): C, 61.60; N, 7.96; H, 7.56. Found: C, 61.71; N, 8.00; H, 7.43.

4.1.4. Boc-Phe(1)-Aib(2)-Ile(3)-OMe 1. Boc-Phe(1)-Aib(2)-OH (7 g, 20 mmol) in DMF (15 mL) was cooled in an ice-water bath and H-Ile-OMe was isolated the corresponding methyl ester hydrochloride from (7.26 g, 40 mmol) by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 mL. It was then added to the reaction mixture, followed immediately by 4.12 g (20 mmol) DCC and HOBt (2.7 g, 20 mmol). The reaction mixture was stirred for 3 days. The residue was taken up in ethyl acetate (40 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×40 mL), brine, 1 M sodium carbonate (3×40 mL), brine (2×40 mL), dried over anhydrous sodium sulfate and evaporated in vacuum to yield 8.1 g (17 mmol) of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Colorless single crystals were grown from methanol/water solvent system (methanol/water = 80:20) by slow evaporation.

Yield = 85%, (8.1 g, 17 mmol). R_f (ethyl acetate) 0.78. Mp 104–106 °C. ν_{max} (KBr)/cm^{-1} 1655, 1697, 3321, 3395, 3427. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.22–7.35 (m, 5H); 7.01 (d, $J=9$ Hz, 1H); 6.17 (s, 1H); 5.07 (d, $J=9$ Hz, 1H); 4.54–4.50 (m, 1H); 4.28–4.20 (m, 1H); 3.72 (s, 3H); 3.08–3.03 (m, 2H); 1.93–1.88 (m, 1H); 1.42 (s, 9H); 1.39 (s, 6H); 1.25–1.13 (m, 2H); 0.89–0.94 (m, 6H). ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 173.58, 172.17, 170.65, 136.56, 129.15, 129.06, 128.58, 126.87, 80.24, 57.16, 56.56, 51.83, 37.49, 28.05, 25.40, 24.90, 24.80, 24.38, 15.33, 11.37. $[\alpha]_D^{25.7}=+5.57$ (*c* 2.07, $CHCl_3$). Mass Spectral data $(M+Na)^+=500.7$, $M_{Calcd}=477$. Elemental Analysis Calcd for $C_{25}H_{39}N_3O_6$ (477): C, 66.89; N, 8.80; H, 8.47. Found: C, 66.81; N, 8.85; H, 8.53.

4.1.5. Boc-Ala(1)-OH 9. See Ref. 15b.

4.1.6. Boc-Ala(1)-Leu(2)-OMe 10. Boc-Ala-OH (5.67 g, 30 mmol) was dissolved in a mixture of dichloromethane (DCM) (30 mL) in an ice-water bath. H-Leu-OMe was isolated from the corresponding methyl ester hydrochloride (10.89 g, 60 mmol) by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 10 mL. This was added to the reaction mixture, followed immediately by di-cyclohexylcarbodiimide (DCC) (6.18 g, 30 mmol). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, and the residue was taken up in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3× 50 mL), brine, then 1 M sodium carbonate (3×50 mL) and brine (2×50 mL) and dried over anhydrous sodium sulfate, and evaporated under vacuum to yield **10** as waxy solid.

Yield = 83.3% (7.9 g, 25 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.71 (d, $J=6$ Hz, 1H); 5.15 (d, $J=7.5$ Hz, 1H); 4.64–4.57 (m, 1H); 4.15–4.11 (m, 1H); 3.74 (s, 3H); 1.61–1.69 (m, 3H); 1.45 (s, 9H); 1.35 (m, 3H); 0.93 (m, 6H). Elemental Analysis Calcd for $C_{15}H_{28}N_2O_5$ (316): C, 56.85; N, 8.72; H, 8.90. Found: C, 56.96; N, 8.86; H, 8.86.

4.1.7. Boc-Ala(1)-Leu(2)-OH 11. To **10** (6.32 g, 20 mmol), MeOH (50 mL) and 2 M NaOH (20 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h methanol was removed under vacuum, the residue was taken up in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 4.23 g of **11** as white solid.

Yield = 70% (4.23 g, 14 mmol). Mp 124–126 °C. ^{1}H NMR ($(CD_3)_2SO$, 300 MHz, δ ppm): 12.41 (br, 1H); 7.88 (d, $J=9$ Hz, 1H); 6.85 (d, $J=6$ Hz, 1H); 4.24–4.17 (m, 1H); 3.99–3.94 (m, 1H); 1.49–1.66 (m, 3H); 1.35 (s, 9H); 1.14 (m, 3H); 0.81–0.88 (m, 6H). Elemental Analysis Calcd for $C_{14}H_{26}N_2O_5$ (302): C, 55.60; N, 9.21; H, 8.56. Found: C, 55.63; N, 9.27; H, 8.60.

4.1.8. Boc-Ala(1)-Leu(2)-Aib(3)-OMe 2. Boc-Ala(1)-Leu(2)-OH (2.74 g, 10 mmol) in DMF (15 mL) was cooled in an ice-water bath and H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (3.07 g, 20 mmol) by neutralization and subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 8 mL. It was then added to the reaction mixture, followed immediately by DCC (2.06 g, 10 mmol) and HOBt (1.35 g, 10 mmol). The reaction mixture was stirred for 3 days. The residue was taken up in ethyl acetate (40 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×40 mL), brine, 1 M sodium carbonate (3×40 mL), brine (2×40 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to yield 3.21 g (8 mmol) of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate–toluene (3:1) as eluent. Colorless single crystals were grown from methanol/water solvent system (methanol/water = 90:10) by slow evaporation.

Yield = 80% (3.21 g, 8 mmol). R_f (25% toluene/ethyl acetate) 0.68. Mp 117–119 °C. ν_{max} (KBr)/cm^{-1} 1659, 1684, 1753, 3244, 3321, 3350. ^{1}H NMR (CDCl$_3$, 300 MHz, δ ppm): 6.84 (s, 1H); 6.60 (d, $J=9$ Hz, 1H); 5.00 (d, $J=6$ Hz, 1H); 4.44–4.36 (m, 1H); 4.16–4.12 (m, 1H); 3.71 (s, 3H); 1.59–1.73 (m, 3H); 1.50 (s, 3H); 1.53 (s, 3H); 1.45 (s, 9H); 1.37 (d, $J=9$ Hz, 3H); 0.90–0.94 (m, 6H). ^{13}C NMR (CDCl$_3$, 300 MHz, δ ppm): 174.52, 172.73, 170.85, 155.44, 80.08, 56.07, 52.28, 51.44, 40.27, 28.09, 24.60, 24.56, 24.48, 24.39, 22.76, 21.76, 15.00. $[\alpha]_D^{25.7}=-57.27$ (c 2.10, CHCl$_3$). Mass Spectral data $(M+Na+H)^+=425.1$, $M_{Calcd}=401$. Elemental Analysis Calcd for $C_{19}H_{35}N_3O_6$ (401): C, 56.85; N, 10.47; H, 8.72. Found: C, 56.81; N, 10.85; H, 8.53.

4.1.9. Boc-Phe(1)-Aib(2)-Ile(3)-OH 12. To Boc-Phe(1)-Aib(2)-Ile(3)-OMe **1** (5 g, 10.48 mmol), methanol (50 mL) and of 2 M NaOH (20 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then pH of the aqueous layer was adjusted to 2 by adding 1 M HCl and it was extracted with ethyl acetate (3×40 mL). The extracts were pooled, dried over anhydrous sodium sulfate and evaporated under vacuum to yield a waxy solid.

Yield = 89% (4.32 g, 9.33 mmol). ^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz, δ ppm): 12.52 (br, 1H); 7.95 (s, 1H); 7.28–7.35 (m, 5H); 7.21 (d, $J=9$ Hz, H); 6.9 (d, $J=9$ Hz, 1H); 4.36–4.44 (m, 1H); 4.16–4.12 (m, 1H); 2.87–2.96 (m, 2H); 1.77–1.89 (m, 1H); 1.30 (s, 6H); 1.28 (s, 9H); 1.07–1.18 (m, 2H); 0.81–0.82 (m, 6H). Elemental Analysis Calcd for $C_{24}H_{37}N_3O_6$ (463): C, 62.26; N, 9.02; H, 8.02. Found: C, 62.20; N, 9.07; H, 7.99.

4.1.10. Boc-Phe(1)-Aib(2)-Ile(3)-Aib(4)-OMe 3. To **12** (4.2 g, 9.1 mmol) in DMF (10 mL) was cooled in an ice water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (2.8 g, 18.2 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration (7 mL) and this was added to the reaction mixture, followed immediately by DCC (1.87 g, 9.1 mmol) and HOBt (1.23 g, 9.1 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to yield 4.14 g (7.37 mmol, 81%) of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate–toluene (3:1) as eluent. Colorless single crystals were obtained from methanol–water (90:10) by slow evaporation.

Yield = 81% (4.14 g, 7.37 mmol). R_f (25% toluene/ethyl acetate) 0.65. Mp 168–170 °C. ν_{max} (KBr)/cm^{-1} 1647, 1730, 1747, 3308, 3551. ^{1}H NMR (CDCl$_3$, 300 MHz δ ppm): 7.20–7.34 (m, 5H); 7.13 (s, 1H); 6.64 (d, $J=9$ Hz, 1H); 6.31 (s, 1H); 5.01 (d, $J=7$ Hz, 1H); 4.44 (m, 1H); 4.11–4.14 (m, 1H); 3.70 (s, 3H); 3.07–3.11 (m, 2H); 2.22 (m, 1H); 1.56, 1.53 (s, 6H); 1.43 (s, 9H); 1.26 (s, 6H); 0.89–0.91 (m, 6H). ^{13}C NMR (CDCl$_3$, 300 MHz, δ ppm): 174.89, 173.64, 171.19, 170.37, 155.82, 129.13, 129.02, 128.87, 128.78, 128.61, 127.19, 80.91, 57.93, 56.91, 55.80, 52.03, 27.92, 25.20, 25.03, 24.42, 24.35, 23.99, 15.53, 15.47, 11.57, 11.51, 11.49. $[\alpha]_D^{25.7}=-15.35$ (c 2.10, CHCl$_3$). Mass Spectral data $(M+Na+2H)^+=587.8$, $M_{Calcd}=562.7$. Elemental Analysis Calcd for $C_{29}H_{46}N_4O_7$ (562): C, 62.02; N, 9.91; H, 8.12. Found: C, 61.90; N, 9.96; H, 8.19.

4.1.11. Boc-Leu(1)-OH 13. See Ref. 18.

4.1.12. Boc-Leu(1)-Aib(2)-OMe 14. See Ref. 18.

4.1.13. Boc-Leu(1)-Aib(2)-OH 15. See Ref. 18.

4.1.14. Boc-Leu(1)-Aib(2)-Gly(3)-OBz 16. Boc-Leu(1)-Aib(2)-OH (6.32 g, 20 mmol) in DMF (20 mL) was cooled in an ice-water bath and H-Gly-OBz was isolated from the corresponding benzyl ester *p*-toluenesulfonate (8.62 g, 40 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration (10 mL) and it was added to the reaction mixture, followed immediately by DCC (4.12 g, 20 mmol) and HOBt (2.7 g, 20 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to yield 8.11 g (17 mmol) of **16** as waxy solid.

Yield = 81% (7.87 g, 17 mmol). ^{1}H NMR (CDCl$_3$, 300 MHz, δ ppm): 7.26–7.30 (m, 5H); 7.35(t, $J=9$ Hz, 1H); 6.52 (s, 1H); 4.99 (d, $J=9$ Hz, 1H); 3.78–3.90 (m, 3H); 1.71 (s, 6H); 1.59–1.68 (m, 3H); 1.52 (s, 9H); 0.94–0.99 (m, 6H). Elemental Analysis Calcd for $C_{24}H_{37}N_3O_6$ (463): C, 62.20; H, 7.99; N, 9.07. Found: C, 62.28; H, 7.95; N, 10.17.

4.1.15. Boc-Leu(1)-Aib(2)-Gly(3)-OH 17. To Boc-Leu(1)-Aib(2)-Gly(3)-OBz **16** (4.63 g, 10 mmol), methanol (50 mL) and of 2 M NaOH (20 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then pH of the aqueous layer was adjusted to 2 by adding 1 M HCl and it was extracted with ethyl acetate (3×40 mL). The extracts were pooled, dried over anhydrous sodium sulfate and evaporated under vacuum to yield a white solid.

Yield = 70% (2.60 g, 7 mmol). Mp 140–142 °C. ^{1}H NMR ((CD$_3$)$_2$SO, 300 MHz, δ ppm): 12.38 (br, 1H); 8.02 (s, 1H); 7.72 (t, $J=5$ Hz, 1H); 7.01 (d, $J=6$ Hz, 1H); 3.83–3.91 (m, 2H); 3.53–3.61 (m, 1H); 1.52–1.60 (m, 3H); 1.44 (s, 6H); 1.37 (s, 9H); 0.84–0.89 (m, 6H). Elemental Analysis Calcd for $C_{17}H_{31}N_3O_6$ (373): C, 54.69; H, 8.31; N, 11.26. Found: C, 54.67; H, 8.16; N, 11.12.

4.1.16. Boc-Leu(1)-Aib(2)-Gly(3)-Aib(4)-OMe 4. To **17** (1.30 g, 5 mmol) in DMF (10 mL) was cooled in an ice water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (1.53 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration (7 mL) and this was added to the reaction mixture, followed immediately by DCC (1 g, 5 mmol) and HOBt (0.7 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuum to yield 2.25 g (4 mmol, 80%) of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Single crystals were obtained from methanol–water (methanol/water=90:10) by slow evaporation.

Yield=80% (1.90 g, 4 mmol). R_f (ethyl acetate) 0.68. Mp 145–147 °C. ν_{max} (KBr)/cm^{-1} 1657, 1728, 3273, 3368. ^{1}H NMR (10% $(CD_3)_2SO$ in $CDCl_3$, 300 MHz, δ ppm): 8.05 (s, 1H); 7.73 (t, $J=11$ Hz, 1H); 7.56 (s, 1H); 6.23 (d, $J=6$ Hz, 1H); 3.99–4.04 (m, 2H); 3.83–3.9 (m, 1H); 3.65 (s, 3H); 1.50–1.55 (m, 3H); 1.48 (6H, s); 1.47 (s, 6H); 1.44 (s, 9H); 0.92–0.97 (m, 6H). ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 174.98, 174.18, 173.25, 169.00, 156.61, 80.89, 56.95, 55.83, 54.47, 52.19, 43.32, 28.19, 25.63, 25.11, 24.96, 24.92, 24.66, 22.78, 21.89. $[\alpha]_D^{25.7}=-19.93$ (*c* 2.06, $CHCl_3$). Mass Spectral data $(M+Na)^+=495.5$. Elemental Analysis Calcd for $C_{22}H_{40}N_4O_7$ (472): C, 55.93; H, 8.74; N, 11.86. Found: C, 54.88; H, 8.77; N, 11.89.

4.1.17. Boc-γ-Abu(1)-OH 18. See Ref. 17.

4.1.18. Boc-γ-Abu(1)-Aib(2)-OMe 19. See Ref. 17.

4.1.19. Boc-γ-Abu(1)-Aib(2)-OH 20. See Ref. 17.

4.1.20. Boc-γ-Abu(1)-Aib(2)-Leu(3)-OMe 21. Boc-γ-Abu(1)-Aib(2)-OH (1.0 g, 3.5 mmol) in DMF (5 mL) was cooled in an ice-water bath and H-Leu-OMe was isolated from the corresponding methyl ester hydrochloride (1.27 g, 7 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration (10 mL) and it was added to the reaction mixture, followed immediately by DCC (0.721 g, 3.5 mmol) and HOBt (0.472 g, 3.5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off.The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to yield 1.23 g (2.9 mmol) of white solid.

Yield=85% (1.23 g, 2.9 mmol). Mp 134–136 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.35 (d, $J=9$ Hz, 1H); 6.49 (s, 1H); 4.76 (t, $J=9$ Hz, 1H); 4.63–4.56 (m, 1H); 3.71 (s, 3H); 3.20–3.18 (m, 2H); 2.21–2.19 (m, 2H); 1.79–1.82 (m, 2H); 1.61–1.64 (m, 3H); 1.53 (s, 3H); 1.55 (s, 3H); 1.45 (s, 9H); 0.99–0.92 (m, 6H). Elemental Analysis Calcd for $C_{20}H_{37}N_3O_6$ (415): C, 57.83; H, 8.91; N, 10.12. Found: C, 57.50; H, 8.40; N, 10.00.

4.1.21. Boc-γ-Abu(1)-Aib(2)-Leu(3)-OH 22. To Boc-γ-Abu(1)-Aib(2)-Leu(3)-OMe **21** (1.23 g, 2.9 mmol), MeOH (5 mL) and 2 M NaOH (3 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 0.9 g of Boc-γ-Abu(1)-Aib(2)-Leu(3)-OH as white solid.

Yield=77.58% (0.9 g, 2.25 mmol). Mp 203–205 °C. ^{1}H NMR ($(CD_3)_2SO$, 300 MHz, δ ppm): 12.34 (br, 1H); 7.75 (s, 1H); 7.58 (d, $J=6$ Hz, 1H); 6.81 (t, $J=5.4$ Hz, 1H); 4.23–4.15 (m, 1H); 2.95–2.89 (m, 2H); 2.01–2.15 (m, 2H); 1.61–1.54 (m, 2H); 1.54–1.50 (m, 3H); 1.31 (s, 9H); 1.37 (s, 6H); 0.87–0.79 (m, 6H). Elemental Analysis Calcd for $C_{19}H_{35}N_3O_6$ (401): C, 54.85; H, 8.72; N, 10.47. Found: C, 54.30; H, 8.40; N, 10.20.

4.1.22. Boc-γ-Abu(1)-Aib(2)-Leu(3)-Aib(4)-OMe 5. Boc-γ-Abu(1)-Aib(2)-Leu(3)-OH (0.8 g, 2 mmol) in DMF (5 mL) was cooled in an ice-water bath and Aib-OMe was isolated from the corresponding methyl ester hydrochloride (0.614 g, 4 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration (10 mL) and it was added to the reaction mixture, followed immediately by DCC (0.412 g, 2 mmol) and HOBt (0.270 g, 2 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to yield 0.75 g (1.5 mmol) of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Single crystals were obtained from methanol–water (methanol/water=90:10) by slow evaporation.

Yield=75% (0.75 g, 1.5 mmol). R_f (ethyl acetate) 0.72. Mp 104–106 °C. ν_{max} (KBr)/cm^{-1} 1651, 1692, 1746, 3296. ^{1}H NMR ($(CD_3)_2SO$, 300 MHz, δ ppm): 7.54 (d, $J=9$ Hz, 1H); 7.41 (s, 1H); 6.08 (s, 1H); 4.70 (t, $J=9.5$ Hz, 1H); 4.5.–4.42 (m, 1H); 3.70 (s, 3H); 3.28–3.23 (m, 2H); 2.32–3.28 (m, 2H); 2.17–2.14 (m, 1H); 1.94–1.88 (m, 1H); 1.57 (s, 3H); 1.54 (s, 3H); 1.48 (s, 6H); 1.46 (s, 9H); 0.91–0.86 (m, 6H). ^{13}C NMR ($CDCl_3$, 300 MHz, δ ppm): 175.16, 174.27, 173.00, 172.00, 157.04, 141.39, 104.48, 79.62, 56.90, 55.92, 52.20, 51.70, 38.94, 38.39, 32.37, 28.36, 27.00, 26.42, 25.35, 24.94, 24.49, 23.72, 23.29, 20.84. $[\alpha]_D^{25.7}=-21.61$ (*C* 2.04, $CHCl_3$). Mass Spectral data $(M+H)^+=501.4$, $M_{calcd}=500$. Elemental Analysis Calcd for $C_{22}H_{44}N_4O_7$ (500): C, 57.60; H, 8.80; N, 11.20. Found: C, 57.12; H, 8.40; N, 10.94.

4.2. Single crystal X-ray diffraction study

For peptides **1**–**5**, intensity data were collected with MoKα radiation using the MARresearch Image Plate System. For all peptides, the crystals were positioned at 70 mm from the Image Plate. Selected details of the structure solutions and refinements are given in Table 3. 100 frames were measured at 2° intervals with a counting time of 2 min. Data analyses were carried out with the XDS program.[19] The structures were solved using direct methods with the Shelx86[20] program. For peptide **4**, the *t*-butyl group was disordered, each methyl group taking up two different sites each refined with 50% occupancy. For peptide **5** some atoms had high thermal parameters but no satisfactory disordered model could be obtained. Apart from disordered atoms, all non-H atoms were refined with anisotropic thermal parameters. The hydrogen atoms bonded to nitrogen and carbon were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. Those bonded to the water molecules in **5** could not be located. The structures were refined on F^2 using Shelxl.[21] Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre reference CCDC 254598-254602 for peptides **1**–**5**.

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References and notes

1. Watson, J. D.; Crick, F. C. H. *Nature* **1953**, *171*, 737–738.
2. Ramachandran, G. N.; Kartha, G. *Nature* **1955**, *179*, 593–595.
3. Franklin, R. E. *Nature* **1955**, *175*, 379–381.
4. (a) Goldsbury, C.; Goldie, K.; Pellaud, J.; Seelig, J.; Frey, P.; Müller, S. A.; Kister, J.; Cooper, G. J. S.; Aebi, U. *J. Struct. Biol.* **2000**, *130*, 352–362. (b) Arvinte, T.; Cudd, A.; Drake, A. F. *J. Biol. Chem.* **1993**, *268*, 6415–6422. (c) Blanch, E. W.; Morozova-Roche, L. A.; Cochran, D. A. E.; Doing, A. J.; Hect, L.; Barron, L. D. *J. Mol. Biol.* **2000**, *301*, 553–563. (d) Sadqi, M.; Herna'ndez, F.; Pan, U. M.; Pe'rez, M.; Schaeberle, M. D.; Avila, J.; Munoz, V. *Biochemistry* **2002**, *41*, 7150–7155.
5. (a) Berl, V.; Huc, I.; Khoury, R. G.; Krische, M. J.; Lehn, J. M. *Nature* **2000**, *407*, 720–723. (b) Kolomiets, E.; Berl, V.; Odrizola, I.; Stadler, A.-M.; Kyritskas; Lehn, J. M. *Chem. Commun.* **2003**, 2868–2869.
6. (a) Hanessian, S.; Gomtsyan, A.; Simard, M.; Roelens, S. *J. Am. Chem. Soc.* **1994**, *116*, 4495–4496. (b) Hanessian, S.; Simard, M.; Roelens, S. *J. Am. Chem. Soc.* **1995**, *117*, 7630–7645.
7. (a) Rowan, I. E.; Notle, R. J. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 62–68. (b) Feiters, M. C.; Notle, R. J. M. *Adv. Supramol. Chem.* **2000**, *6*, 41–156. (c) Lehn, J. M. *Chem. Eur. J.* **2000**, *6*, 2097–2102. (d) Lightfoot, M. P.; Mair, F. S.; Pritchard, R. G.; Warren, J. E. *Chem. Commun.* **1999**, 1945–1946. (e) Tobellion, F. M.; Seidel, S. R.; Arif, A. M.; Stang, P. J. *J. Am. Chem. Soc.* **2001**, *123*, 7740–7741.
8. Lehn, J. M.; Rigault, A.; Siegel, J.; Harrowfield, J.; Chevrier, B.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2565–2569.
9. (a) Allen, W. E.; Fowler, C. J.; Lynch, V. M.; Sessler, J. L. *Chem. Eur. J.* **2001**, *7*, 721–729. (b) Geib, S. J.; Vicent, C.; Fan, E.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 119–121.
10. Wang, Z.; Enkelmann, V.; Negri, F.; Mullen, K. *Angew. Chem., Int. Ed.* **2004**, *43*, 1972–1975.
11. (a) Moriuchi, T.; Nomoto, A.; Yoshida, K.; Ogawa, A.; Hirao, T. *J. Am. Chem. Soc.* **2001**, *123*, 68–75. (b) Nomoto, A.; Moriuchi, T.; Yamazaki, S.; Ogawa, A.; Hirao, T. *Chem. Commun.* **1998**, 1963–1964.
12. (a) Moriuchi, T.; Nishiyama, M.; Yoshida, K.; Ishikawa, T.; Hirao, T. *Org. Lett.* **2001**, *3*, 1459–1461. (b) Moriuchi, T.; Nomoto, A.; Yoshida, K.; Ogawa, A.; Hirao, T. *J. Am. Chem. Soc.* **2001**, *123*, 68–75.
13. (a) Parthasarathy, R.; Chaturvedi, S.; Go, K. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 871–875. (b) Ramasubbu, N.; Parthasarathy, R. *Biopolymers* **1989**, *28*, 1259–1269.
14. (a) Haldar, D.; Maji, S. K.; Sheldrick, W. S.; Banerjee, A. *Tetrahedron Lett.* **2002**, *43*, 2653–2656. (b) Haldar, D.; Maji, S. K.; Drew, M. G. B.; Banerjee, A.; Banerjee, A. *Tetrahedron Lett.* **2002**, *43*, 5465–5468. (c) Maji, S. K.; Banerjee, A.; Drew, M. G. B.; Haldar, D.; Banerjee, A. *Tetrahedron Lett.* **2002**, *43*, 6759–6762. (d) Banerjee, A.; Maji, S. K.; Drew, M. G. B.; Haldar, D.; Banerjee, A. *Tetrahedron Lett.* **2003**, *44*, 699–703. (e) Haldar, D.; Banerjee, A.; Drew, M. G. B.; Das, A. K.; Banerjee, A. *Chem. Commun.* **2003**, 1406–1407.
15. (a) Banerjee, A.; Maji, S. K.; Drew, M. G. B.; Haldar, D.; Banerjee, A. *Tetrahedron Lett.* **2003**, *44*, 335–339. (b) Maji, S. K.; Haldar, D.; Drew, M. G. B.; Banerjee, A.; Das, A. K.; Banerjee, A. *Tetrahedron* **2004**, *60*, 3251–3259.
16. (a) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756. (b) Sacca, B.; Formaggio, F.; Crisma, M.; Toniolo, C.; Gennaso, R. *Gazz. Chim. Ital.* **1997**, *127*, 495–500. (c) Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. *Biopolymers (Pept. Sci.)* **2001**, *60*, 396–419.
17. Maji, S. K.; Banerjee, R.; Velmurugan, D.; Razak, A.; Fun, H. K.; Banerjee, A. *J. Org. Chem.* **2002**, *67*, 633–639.
18. Karle, I. L.; Banerjee, A.; Bhattacharjya, S.; Balaram, P. *Biopolymers* **1996**, *38*, 515–526.
19. Kabsch, W. *J. Appl. Crystallogr.* **1988**, *21*, 916.
20. Sheldrick, G. M. *Acta Crystallogr. Sect. A: Fundam. Crystallogr.* **1990**, *46*, 467.
21. Sheldrick, G. M. *Program for Crystal Structure Refinement*; University of Göttingen: Germany, 1993.

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A novel 3,4-dihydroisoquinoline annulation: expedient access to isoquinoline heterocycles

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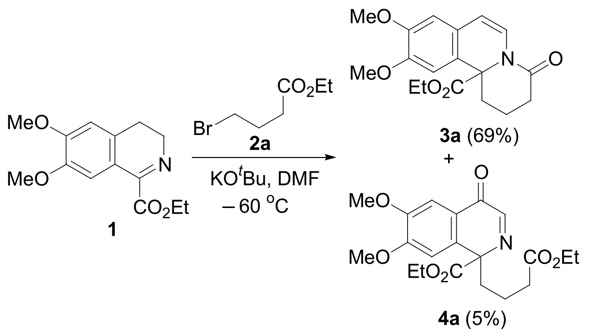
Abstract—3,4-Dihydroisoquinoline carboxylate **1** undergoes a smooth annulation with ω-bromopropionate, butyrate or *ortho*-bromomethyl benzoate **2** to afford the isoquinoline heterocycle **3** upon treatment with potassium *tert*-butoxide in DMF at −60 °C. This novel annulation constitutes a formal direct synthesis of cyclic Reissert equivalent compounds, thus offers an expedient access towards certain medicinally important isoquinoline heterocycles and relevant natural alkaloids, that is, of berberine and erythrina types.

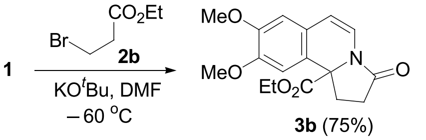
1. Introduction

In connection with our recent studies on the total synthesis of cephalotaxine,[1] we observed an unusual annulation reaction as shown in Scheme 1. An attempted Claisen condensation of 3,4-dihydroisoquinoline 1-carboxylate **1**[2] with ethyl 4-bromobutyrate (**2a**) using 1 equiv of potassium *tert*-butoxide (KO^{t}Bu) as base in DMF at −60 °C led to the formation of a majority tricyclic product in ca. 20% yield, which was identified spectroscopically as an isoquinoline annulation adduct **3a**. By employing 2 equiv of KO^{t}Bu at −60 °C in degassed DMF, the yield of the annulation product **3a** was increased to 69% on a 1 mmol scale reaction, along with a small portion (ca. 5%) of oxidized α-alkylation product characterized as **4a**.

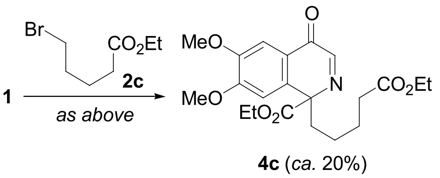


Scheme 1.

The scope of this novel annulation reaction[3] of 3,4-dihydroisoquinoline in terms of product ring-size was then examined by using ethyl 3-bromopropionate (**2b**) and ethyl 5-bromovalerate (**2c**) as the alkylating components under identical conditions (2 equiv KO^{t}Bu, degassed DMF, see Eqs. 1 and 2). It is interesting to note that, although the 5-membered ring annulation product **3b** was produced cleanly in 75% yield, the reaction of **1** with 5-bromovalerate **2c** was sluggish and the expected 7-membered annulation product **4b** was not detected. A major isolable product **4c**, a structural analog of **4a**, was obtained instead in low yield after an extended reaction time.[4] Apparently, this annulation reaction is limited to the synthesis of 5- and 6-membered annulation adducts.



(1)



(2)

Nonetheless, this unusually facile and simple annulation reaction constitutes an alternative direct synthesis of the cyclic (annulated) Reissert compound equivalents, and to a certain extent, is complementary to the conventional Reissert synthesis,[5] for the 5-membered cyclic Reissert

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Keywords: Annulation; Cephalotaxine; DMF; Isoquinoline.

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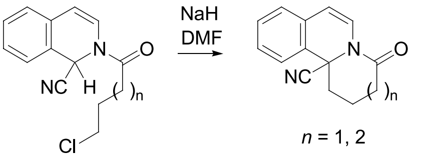
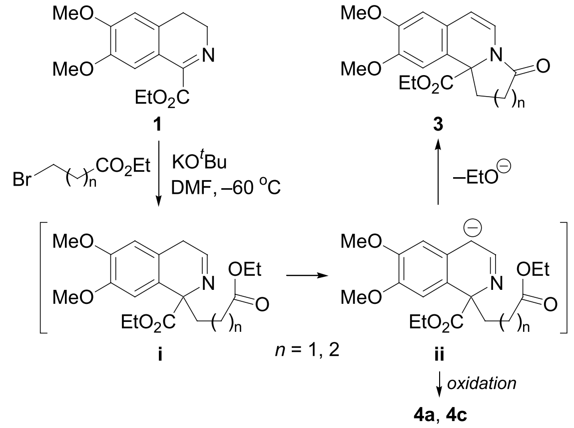
equivalent compound of type **3b** which cannot be obtained directly via the intramolecular alkylative cyclization of the corresponding Reissert precursors (Eq. 3, $n \neq 0$ and 3) as demonstrated by Popp and co-workers.[6] It is equally interesting to note that the Popp method is applicable to the synthesis of 7-membered cyclic Reissert compounds, but not to 5- and 8-membered annulation products.[7]



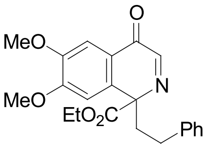
(3)

A possible reaction pathway of this base-mediated alkylative annulation is depicted in Scheme 2. The initial α-alkylation intermediate **i** may undergo intramolecular *N*-acylation through an intermediary imino benzylic anion **ii** generated by deprotonation[8] of **i**; mesomerism of **ii** and by 5- or 6-exo-trig ring-closure[9] of the corresponding enaminate leads to **3**. The oxidative products (i.e., **4a** and **4c**) might result from the imino benzylic anion **ii** by aerial oxidation.[4] The delicate increase of ring-strain may be responsible for the reluctant 7-exo-trig ring-closure to form the corresponding 7-membered annulation product from the anionic intermediate **ii** ($n=3$) after the initial α-alkylation of **1** with 5-bromovalerate.

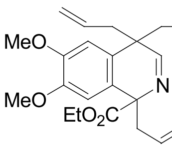


Scheme 2.

Simple alkyl bromide, phenethyl bromide (**2d**), reacted with **1** sluggishly under identical reaction conditions to give a low yield of oxidative product isoquinolinone **4d**, whilst the more reactive electrophile, allyl bromide (**2e**), led to tris-alkylated product **5** (mp 75–77 °C) in moderate yield. The use of other electrophiles, such as ethyl acrylate,[10] did not give any desired annulation product (i.e., **3b**), and the use of 1,3-dibromopropane and 1,4-dibromobutane did not give any intramolecular bis-alkylated products either. Some other reaction conditions have been examined by varying the bases and solvents[11] used, among which KO^{t}Bu in DMF was found to be the solvent system of choice. Typical phase transfer conditions involving the use of 50% aqueous KOH solution as base and *n*-tetrabutylammonium bromide (TBAB) as a phase transfer catalyst in CH_2Cl_2 or toluene also failed to promote the production of any detectable annulation product.

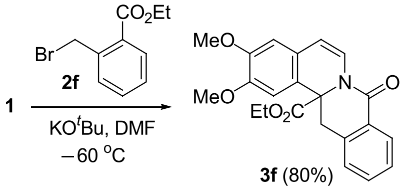


4d (ca. 20%)



5 (40%)

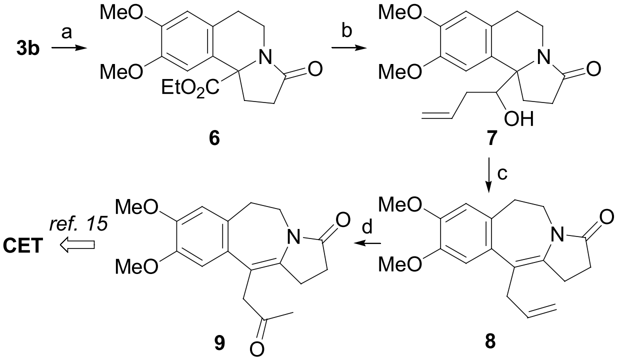
Other electrophiles, such as ethyl *ortho*-bromomethyl benzoate (**2f**), was annulated with **1** to afford an isoquinoline adduct **3f** (mp 145–147 °C) in 80% yield (Eq. 4). Analogous annulation reactions with substituted 3,4-dihydroisoquinoline and electrophiles would provide an efficient and simple method for the synthesis of berberinetype[12] and related alkaloid heterocycles (vide infra).



3f (80%)

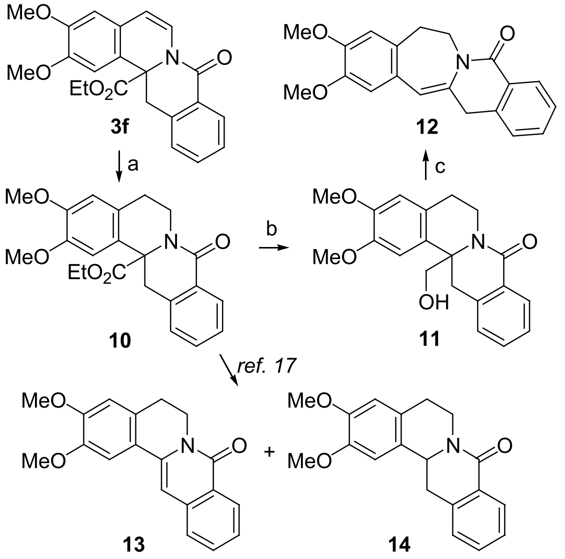
(4)

The annulated isoquinoline derivatives can be hydrogenated quantitatively to the corresponding dihydro compounds under usual catalytic hydrogenation conditions. For example, the dihydro derivative **6**, which possesses the basic pyrroloisoquinoline ring systems, appears as the major structural motif of erythrina-type alkaloids.[13] Compound **6** can be transformed into a benzazepine intermediate **9** as shown in Scheme 3, which may serves as an advanced precursor[14] in the total synthesis of cephalotaxine (CET),[15] by the following sequence: (1) carboxylate reduction by DIBAL-H to the corresponding aldehyde followed by allyl Grignard addition to give alcohol **7** (mixture of diastereomers); (2) sulfuryl chloride (SO_2Cl_2)-mediated ring-opening rearrangement[15] to give an allyl benzazepine intermediate **8**; and (3) standard Wacker oxidation to furnish the tricyclic methyl ketone **9**.



Scheme 3. (a) Conditions: (a) H_2, 10% Pd–C, MeOH, 100%. (b) (i) DIBAL-H, CH_2Cl_2, −78 °C, 72%; (ii) Allyl-MgBr, THF, −20 °C, 80%. (c) SO_2Cl_2, Et_3N, Pyr, CH_2Cl_2, −78 °C, 80%. (d) $PdCl_2$ (10 mol%), $CuCl_2$, DMF–H_2O (4:1), O_2, 40 °C, 67%.

Similarly, dihydro derivative **10** (mp 144 °C) obtained from **3f** can also be converted (Scheme 4) to a benzazepine heterocycle **12**, a structural analog of relevant natural alkaloids (i.e., lennoxamine and chilenine).[16] Further transformation of the annulation product **10** to 8-oxoprotoberberines **13** and **14**, key intermediates for the synthesis of



Scheme 4. (a) Conditions: (a) H_2, 10% Pd–C, MeOH, 100%. (b) (i) DIBAL-H, CH_2Cl_2, −78 °C, 72%; (ii) $NaBH_4$, MeOH, 0 °C, 95%. (c) SO_2Cl_2, Et_3N, Pyr, CH_2Cl_2, −78 °C, 80%.

various medicinally important berberine-type heterocycles,[17] would be feasible by the Reimann method.[18] Thus, this novel annulation method constitutes a simple and useful alternative annulation approach[3,19] for berberine alkaloid synthesis.

In conclusion, we have demonstrated here a novel base-mediated 3,4-dihydroisoquinoline annulation reaction with ω-bromo-propionate and butyrate, or substituted derivatives (i.e., **2f**). The simple annulation method would find application in the synthesis of various kinds of natural alkaloids or isoquinoline heterocycles. This annulation method is also complementary to the classical cyclic Reissert compound synthesis, to provide an efficient alternative synthesis of cyclic Reissert compound equivalents. Applications of this method to the synthesis of alkaloid and possible further extension of this method are underway in our Laboratory.

2. Experimental

For product purification by flash column chromatography, silica gel (200–300 mesh) and light petroleum ether (bp 60–90 °C) are used. All solvents were purified and dried by standard techniques, and distilled prior to use. All organic extracts were dried over $MgSO_4$, unless otherwise noted. IR spectra were recorded as a liquid film. ^1H and ^{13}C NMR spectra were recorded with TMS as an internal standard and $CDCl_3$ as solvent. Melting points were measured on a Kofler hot stage and are uncorrected.

2.1. General

2.1.1. General procedure for the annulation of 3,4-dihydroisoquinoline carboxylate 1 is as follow (preparation of compounds 3a, 3b, 3f). To a cooled (−60 °C) solution of 3,4-dihydroisoquinoline carboxylate **1** (1.0 mmol) in 4 mL of degassed anhydrous DMF was added potassium *tert*-butoxide (224 mg, 2.0 mmol) in one portion. The resulting dark green mixture was stirred for 10 min at this temperature, and a solution of ethyl ω-bromo alkanoate (1.2 mmol) in DMF (1 mL) was added dropwise over 5 min. After stirring for an additional 10 min, the reaction mixture was quenched by saturated aqueous $NaHCO_3$, extracted with ethyl acetate (3×), dried and concentrated. The crude residue was purified by column chromatography on silica gel to give the corresponding annulation products. Compound **3a** (69%), colorless gum. IR (film) ν_{max} 1732, 1666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35 (1H, d, $J=7.8$ Hz), 6.94 (1H, s), 6.56 (1H, s), 5.90 (1H, d, $J=7.8$ Hz), 4.15–4.03 (2H, m), 3.91 (3H, s), 3.87 (3H, s), 2.95 (1H, d, $J=13.0$ Hz), 2.64 (1H, dd, $J=5.9$, 16.8 Hz), 2.55–2.45 (1H, m), 2.29 (1H, dd, $J=3.4$, 13.4 Hz), 2.06–1.95 (1H, m), 1.86–1.79 (1H, m), 1.15 (3H, t, $J=7.4$ Hz) ppm; ^{13}C NMR (DEPT) (100 MHz, $CDCl_3$) δ 171.9, 149.1, 148.2, 124.6, 124.0, 66.3 (C), 124.5, 109.9, 109.4, 107.9 (CH); 62.1, 31.3, 31.1, 17.3 (CH_2), 56.3, 55.8, 14.0 (CH_3) ppm. HRMS (ESI) *m*/*z*: found for $[M+H]^+$ 332.1497, Calcd for $C_{18}H_{22}O_5N$: 332.1498. Compound **3b** (75%), colorless gum. IR (film) ν_{max} 1730, 1695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (1H, d, $J=7.5$ Hz), 6.82 (1H, s), 6.61 (1H, s), 5.93 (1H, d, $J=7.5$ Hz), 4.16–4.05 (2H, m), 3.93 (3H, s), 3.87 (3H, s), 2.96–2.92 (1H, m), 2.65–2.50 (3H, m), 1.18 (3H, t, $J=7.3$ Hz) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$): δ 172.6, 171.9, 149.2, 148.6, 124.2, 123.9, 120.4, 111.0, 109.0, 108.1 (CH), 67.3 (C), 62.1, 56.2, 55.9, 32.0, 29.9 (CH_2), 14.0 (CH_3) ppm. LRMS (EI) *m*/*z* (rel int): 317 ($[M]^+$, 0.6), 244 ($[M-CO_2Et]^+$, 100); HRMS (ESI) *m*/*z*: found for $[M+H]^+$ 318.1332, Calcd for $C_{17}H_{20}O_5N$: 318.1341. Compound **3f** (80%), mp 145–147 °C (ethyl acetate). IR (film) ν_{max} 1733, 1667 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.14 (1H, d, $J=7.5$ Hz), 7.64 (1H, d, $J=8.1$ Hz), 7.50 (1H, t, $J=7.2$ Hz), 7.40 (1H, t, $J=7.2$ Hz), 7.30 (1H, d, $J=7.2$ Hz), 7.12 (1H, s), 6.56 (1H, s), 5.85 (1H, d, $J=8.1$ Hz), 4.05 (1H, d, $J=15.6$ Hz), 3.97 (3H, s), 3.97–3.88 (2H, m), 3.91 (3H, s), 3.65 (1H, d, $J=15.6$ Hz), 0.91 (3H, t, $J=6.9$ Hz) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$) δ 170.8, 162.4, 149.5, 148.1, 133.8, 127.8, 124.0, 122.7, 132.8, 128.7, 127.9, 127.4, 123.2, 109.9, 108.2, 107.8 (CH), 65.5 (C), 62.0, 56.3, 55.8, 38.1 (CH_2), 13.7 (CH_3) ppm. LRMS (EI) *m*/*z* (rel. int): 379 ($[M]^+$, 2), 306 ($[M-CO_2Et]^+$, 100); HRMS (ESI) *m*/*z*: found for $[M+H]^+$ 380.1492, Calcd for $C_{22}H_{22}O_5N$: 380.1498.

Compound **4a**, colorless gum. IR (film) ν_{max} 1734, 1666 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.32 (1H, s), 7.52 (1H, s), 6.97 (1H, s), 4.20–4.02 (4H, m), 3.97 (3H, s), 3.94 (3H, s), 2.47 (2H, t, $J=7.6$ Hz), 2.30–2.16 (2H, m), 1.35–1.20 (2H, m), 1.18 (3H, t, $J=6.2$ Hz), 1.12 (3H, t, $J=7.1$ Hz) ppm; ^{13}C NMR (DEPT) (100 MHz, $CDCl_3$) δ 173.8, 172.8, 169.4, 154.5, 149.8, 139.3, 122.8, 70.6 (C), 159.6, 107.2, 106.9 (CH), 62.4, 60.2, 38.6, 33.6, 18.5 (CH_2), 56.3, 56.1, 14.1, 13.7 (CH_3) ppm. LRMS (FAB) *m*/*z* (rel. int): 392 ($[M+H]^+$, 100). Compound **4c**, colorless gum. IR (film) ν_{max} 1734, 1666 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 8.30 (1H, s), 7.51 (1H, s), 6.96 (1H, s), 4.18–4.00 (4H, m), 3.97 (3H, s), 3.89 (3H,s), 2.60–2.30 (2H, m), 2.15 (2H, t, $J=7.8$ Hz), 1.65–1.40 (2H, m), 1.24–1.06 (6H, m), 1.05–0.63 (2H, m) ppm; ^{13}C NMR (DEPT) (50 MHz, $CDCl_3$): δ 173.8, 173.1, 169.6, 154.5, 149.7, 139.6, 122.8, 70.6 (C), 159.3, 107.0, 106.9 (CH), 62.3, 60.1, 39.3, 33.8, 24.5, 22.5 (CH_2), 56.3, 56.1, 14.1, 13.8 (CH_3) ppm; LRMS (EI) *m*/*z* (rel. int): 332 ($[M-CO_2Et]^+$, 100). Compound **4d**, colorless gum, IR (film) ν_{max} 1735, 1665 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.40 (1H, s), 7.57 (1H, s), 7.25–7.15 (3H, m), 7.05 (2H, d, $J=6.9$ Hz), 7.02 (1H, s), 4.23–4.07 (2H, m), 3.97 (6H, s), 2.86 (1H, dd, $J=4.8$, 11.7 Hz), 2.63 (1H, dd, $J=4.5$, 11.7 Hz), 2.40 (1H, dd, $J=4.5$, 12.9 Hz), 2.16 (1H, dd, $J=$ 4.5, 12.3 Hz), 1.17 (3H, t, $J=7.2$ Hz) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$) δ 173.8, 169.5, 154.5, 149.8, 140.8, 139.5, 122.9, 70.6 (C), 139.5, 128.4, 128.3, 120.6, 107.1, 107.0 (CH), 62.5, 41.5, 29.7 (CH_2), 56.4, 56.2, 13.8 (CH_3) ppm. LRMS (EI) *m*/*z* (rel. int): 381 ($[M]^+$, 4), 308 ($[M-CO_2Et]^+$); HRMS (ESI) *m*/*z*: $[M+H]^+$ found 382.1657, Calcd for $C_{22}H_{24}O_5N$, 382.1654.

2.1.2. Preparation of compound 7 from 3b. Compound **3b** (330 mg, 1.0 mmol) in 5 mL of ethanol was hydrogenated over 40 mg of 10% palladium on charcoal at ca. 1.2 atm hydrogen pressure (balloon) at room temperature for 4 h. The reaction mixture was filtered through a pad of Celite and washed with ethyl acetate thoroughly. The resulting filtrate was concentrated in vacuo to afford the pure hydrogenated product **6** quantitatively as a colorless gum. IR (film) ν_{max} 1730, 1695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.85 (1H, s), 6.56 (1H, s), 4.31–4.26 (1H, m), 4.17–4.11 (2H, m), 3.32 (1H, dt, $J=4.9$, 12.3 Hz), 3.84 (3H, s), 3.82 (3H, s), 3.05–2.95 (1H, m), 2.90–2.75 (1H, m), 2.70–2.60 (1H, m), 2.55–2.45 (1H, m), 2.45–2.30 (1H, m), 2.10–2.00 (1H, m), 2.03 (3H, t, $J=6.9$ Hz) ppm; ^{13}C NMR (DEPT) (100 MHz, $CDCl_3$) δ 172.9, 172.3, 148.6, 147.9, 127.3, 125.7, 111.3, 108.9 (CH), 66.8 (C), 61.9, 55.9, 55.7, 35.7, 33.0, 30.6, 27.7 (CH_2), 13.9 (CH_3) ppm. LRMS (FAB) *m*/*z* 320 ($[M+H]^+$, 80); 246 ($[M-CO_2Et]^+$, 100); HRMS (ESI) *m*/*z*: found for $[M+H]^+$ 320.1489; Calcd for $C_{17}H_{22}O_5N$: 320.1498.

Compound **10** was prepared from **3f** similarly (100%). Mp 144 °C (ethyl acetate). ^1H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, d, $J=7.5$ Hz), 7.44 (1H, t, $J=7.8$ Hz), 7.37 (1H, t, $J=$ 7.8 Hz), 7.28 (1H, d, $J=8.0$ Hz), 7.23 (1H, s), 6.69 (1H, s), 5.14–5.01 (1H, m), 4.03–3.93 (2H, m), 3.94 (3H, s), 3.95 (3H, s), 3.84 (1H, d, $J=15$ Hz), 3.40–3.20 (1H, m), 3.15 (1H, d, $J=15$ Hz), 3.05–2.90 (1H, m), 2.90–2.75 (1H, m), 0.97 (3H, t, $J=6.9$ Hz) ppm.

To a cooled (-78 °C) solution of compound **6** (479 mg, 1.5 mmol) in dry CH_2Cl_2 (18 mL), was added under nitrogen, DIBAL-H solution (3.0 mL, 1.0 M in toluene, 3.0 mmol) dropwise over 10 min. After the addition, the reaction mixture was stirred for an additional 1 h at -78 °C, quenched with brine (50 mL), and warmed to 20 °C over 30 min. The aqueous layer was separated, extracted with ethyl acetate (3×200 mL). The combined organic phases were washed with brine (2×25 mL), dried and concentrated. The crude product was purified by silica gel column chromatography (EtOAc/petroleum ether 1:1) to give the corresponding aldehyde **6a** (299 mg, 72%) as a colorless gum. IR (film) ν_{max} 1726, 1688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.66 (1H, s), 6.66 (1H, s), 6.63 (1H, s), 4.40–4.35 (1H, m), 3.66 (6H, s), 3.22–3.19 (1H, m), 2.94–2.84 (2H, m), 2.74–2.68 (1H, m), 2.50–2.46 (1H, m), 2.08–2.03 (1H, m) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$) δ 173.3, 148.9, 148.5, 126.9, 123.8, 196.1, 111.9, 108.2 (CH), 70.3 (C), 56.1, 55.9 (CH_3), 36.0, 30.1, 28.9, 27.4 (CH_2) ppm. LRMS (FAB) *m*/*z* 276 ($[M+H]^+$, 100).

To a cooled (-20 °C) solution of the above aldehyde (550 mg, 2.0 mmol) in THF (40 mL) was added under nitrogen, a solution of allylmagnesium bromide (3.0 mL, 0.7 M in diethyl ether, 2.1 mmol) dropwise over 20 min. After the addition, the reaction mixture was stirred for 20 min at -20 °C, quenched with brine (10 mL), dried and concentrated. The crude product was purified by silica gel column chromatography (EtOAc/petroleum ether 1:1) to afford the allylated product **7** (mixture of diastereomers, 507 mg, 80%) as a colorless gum. IR (film) ν_{max} 3366, 1670 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.68 (1H, s), 6.57 (1H, s), 5.88–5.76 (1H, m), 5.16–5.01 (2H, m), 4.39–4.16 (1H, m), 3.84 (6H, s), 3.89–3.65 (3H, m), 3.0–2.5 (5H, m), 2.50–1.85 (2H, m) ppm; LRMS (EI) *m*/*z* (rel int): 246 ($[M-CO_2Et]^+$, 100).

2.1.3. Preparation of alcohol 11 from compound 10. Compound **10** was reduced to the corresponding aldehyde **10a** by DIBAL-H as described above (72%). To a methanol (5 mL) solution of **10a** (339 mg, 1.0 mmol) was added $NaBH_4$ (38 mg, 1 mmol) portionwise at 0 °C. After stirred for 30 min, 2 mL of water was added. The mixture was extracted with $CHCl_3$. The combined organic phases were washed with brine, dried and concentrated in vacuo. The crude residue was purified by chromatography on silical gel eluting with EtOAc/petroleum ether (1:2) to give **11** (325 mg, 95%) as a colorless oil. IR (film) ν_{max} 3378, 1631 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, d, $J=$ 7.5 Hz), 7.45 (1H, t, $J=7.8$ Hz), 7.35 (1H, t, $J=7.5$ Hz), 7.25 (1H, t, $J=7.5$ Hz), 6.97 (1H, s), 6.70 (1H, s), 5.04–4.92 (1H, m), 3.93 (3H, s), 3.90 (3H, s), 3.25 (2H, s), 3.44 (1H, d, $J=15.9$ Hz), 3.40–3.26 (1H, m), 3.16 (1H, d, $J=16.2$ Hz), 3.02–2.90 (1H, m), 2.86–2.71 (1H, m), 2.16–2.01 (1H, m) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$) δ 163.8, 148.1, 147.8, 135.4, 129.5, 128.5, 127.7, 61.1 (C), 135.4, 128.3, 127.2, 111.6, 108.8 (CH), 67.9, 38.7, 37.6, 29.3 (CH_2), 56.2, 55.8 (CH_3) ppm.

2.1.4. Preparation of benzazepine intermediates 8 and 12. To a mixture of **7** (400 mg, 1.26 mmol) in dry CH_2Cl_2 (20 mL) and pyridine (5 mL) was added Et_3N (0.87 mL, 6.3 mmol) dropwise followed by addition of SO_2Cl_2 (0.22 mL, 2.72 mmol) dropwise at -78 °C under argon. The reaction mixture was allowed to warm to rt over 0.5 h and stirred overnight at ambient temperature. The reaction mixture was quenched with satd aqueous $NaHCO_3$, extracted with $CHCl_3$. The combined organic phases were washed with brine, dried and concentrated. The residue was chromatographed on silical gel by elution with EtOAc/petroleum ether (1:2) to give compound **8** (300 mg, 80%) as a colorless gum. IR (film) ν_{max} 1711, 1629 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.96 (1H, s), 6.58 (1H, s), 6.17–5.91 (1H, m), 5.23–5.10 (2H, m), 3.90–3.65 (2H, m), 3.87 (3H, s), 3.82 (3H, s), 3.22–3.20 (2H, m), 2.90–2.80 (4H, m), 2.58–2.49 (2H, m) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$) δ 175.3, 147.3, 146.8, 137.5, 136.4, 132.8, 130.1, 111.7, 110.9 (CH), 115.2, 107.9 (C), 55.9, 55.8 (CH_3), 46.0, 38.1, 34.4, 28.6, 24.3 (CH_2) ppm; LRMS (EI) *m*/*z* (rel int):

299 ($[M]^+$, 100). HRMS *m*/*z*: found for $[M+H]^+$ 300.1597, Calcd for $C_{18}H_{22}O_3N$: 300.1600.

Compound **12**, Colorless gum. IR (film) ν_{max} 1651, 1603 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (1H, d, $J=7.1$ Hz), 7.55 (1H, t, $J=7.1$ Hz), 7.45–7.38 (2H, m), 6.63 (1H, s), 6.66 (1H, s), 6.44 (1H, s), 4.81 (2H, t, $J=6.0$ Hz), 4.11 (2H, s), 3.89 (3H, s), 3.83 (3H, s), 3.25 (2H, t, $J=6.0$ Hz) ppm; ^{13}C NMR (DEPT) (100 MHz, $CDCl_3$) δ 162.5, 148.3, 147.2, 142.9, 136.9, 127.6, 124.7 (C), 132.1, 128.0, 126.0, 125.3, 113.4, 113.3, 104.2 (CH), 40.1, 39.7, 33.3 (CH_2), 56.0, 55.9 (CH_3) ppm; HRMS (SIMS) *m*/*z*: found for M^+: 321.1354, Calcd for $C_{20}H_{19}O_3N$: 321.1359.

2.1.5. Preparation of benzazepine 9 from compound 8. A mixture of $PdCl_2$ (6 mg) and $CuCl_2\cdot 2H_2O$ (10 mg) in DMF (2 mL) and H_2O (0.5 mL) was stirred at 35–40 °C for 0.5 h, to which a solution of compound **8** (22 mg, 0.07 mmol) in DMF (1 mL) was added dropwise at 35–40 °C. The reaction mixture was stirred for 3 h at the same temperature under oxygen atmosphere. The reaction mixture was then diluted with water (10 mL), extracted with $CHCl_3$ (2×30 mL), dried and concentrated in vacuo. The crude residue was purified by silica gel chromatography to furnish **9** (14 mg, 67%) as a clear gum. IR (film) ν_{max} 1718, 1638 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.56 (2H, s), 3.84 (3H, s), 3.79 (3H, s), 3.88–3.65 (2H, m), 3.54 (2H, s), 2.89–2.84 (2H, m), 2.77–2.70 (2H, m), 2.58–2.49 (2H, m), 2.11 (3H, s) ppm; ^{13}C NMR (DEPT) (75 MHz, $CDCl_3$) δ 207.0, 175.3, 147.5, 147.2, 138.9, 132.9, 129.2, 112.0, 110.6 (CH), 104.8 (C), 56.0, 55.8 (CH_3), 49.2, 46.3, 34.2, 28.9, 28.4, 24.9 (CH_2) ppm; LRMS (EI) *m*/*z* (rel int., %): 315 ($[M]^+$, 2), 272 ($[M-43]^+$, 100); HRMS (ESI) *m*/*z*: found for $[M+H]^+$ 316.1547, Calcd for $C_{18}H_{22}O_4N$: 316.1549.

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References and notes

1. Li, W.-D. Z.; Wang, Y.-Q. *Org. Lett.* **2003**, *5*, 2931–2934.
2. Prepared according to: Fang, F. G.; Maier, M. E.; Danishefsky, S. J.; Schulte, G. *J. Org. Chem.* **1990**, *55*, 831–838.
3. For other 3,4-dihydroisoquinoline annulations, see: (a) Cushman, M.; Gentry, J.; Dekow, F. W. *J. Org. Chem.* **1977**, *42*, 1111–1116. (b) Marsden, R.; MacLean, D. B. *Tetrahedron Lett.* **1983**, *24*, 2063–2066. (c) Marsden, R.; MacLean, D. B. *Can. J. Chem.* **1984**, *62*, 1392–1399. For recent examples, see: (d) Roy, A.; Nandi, S.; Ila, H.; Junjappa, H. *Org. Lett.* **2001**, *3*, 229–232. (e) Ploypradith, P.; Mahidol, C.; Sahakitpichan, P.; Wongbundit, S.; Ruchirawat, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 866–868.
4. The yield of this oxidative product is variable run by run. For example, a less strictly degassed DMF led to an decreased yield of the desired annulation product with more production of oxidative by-product in a relatively small scale reaction.
5. For a review, see: Popp, F. D. *Heterocycles* **1973**, *1*, 165–180. For a recent example, see: Takamura, M.; Funabashi, K.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc.* **2001**, *123*, 6801–6908 and Ref. 4 therein cited.
6. For a review on Ring Annulations via Reissert Compounds, see: Popp, F. D.; Uff, B. C. *Heterocycles* **1985**, *23*, 731–740.
7. Gibson, H. W.; Chesney, D. K.; Popp, F. D. *J. Heterocycl. Chem.* **1972**, 541–544.
8. Cf.: Malhotra, S. K.; Moakley, D. F.; Johnson, F. *J. Am. Chem. Soc.* **1967**, *89*, 2794–2795.
9. Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734–740.
10. Cf.: Hahn, J. T.; Kant, J.; Popp, F. D.; Chhabra, S. R.; Uff, B. C. *J. Heterocycl. Chem.* **1992**, 1165–1176.
11. For example, the reaction in THF is slow and sluggish.
12. Cf.: Suffness, M.; Cordell, G. A. In Brossi, A., Ed.; Protoberberine Alkaloids in The Alkaloids: Chemistry and Pharmacology; Academic: New York, 1985; Vol. 25, pp 188–198; see also page 338.
13. For recent synthetic studies, see: (a) Shimizu, K.; Takimoto, M.; Mori, M. *Org. Lett.* **2003**, *5*, 2323–2325. (b) Itoh, T.; Nagata, K.; Yokoya, M.; Miyazaki, M.; Kameoka, K.; Nakamura, S.; Ohsawa, A. *Chem. Pharm. Bull.* **2003**, *51*, 951–955. (c) Katritzky, A. R.; Mehta, S.; He, H.-Y. *J. Org. Chem.* **2001**, *66*, 148–152. (d) Padwa, A.; Hennig, R.; Kappe, C. O.; Reger, T. S. *J. Org. Chem.* **1998**, *63*, 1144–1155. (e) Garcia, E.; Arrasate, S.; Ardeo, A.; Lete, E.; Sotomayor, N. *Tetrahedron Lett.* **2001**, *42*, 1511–1513. (f) Allin, S. M.; James, S. L.; Martin, W. P.; Smith, T. A. D.; Elsegood, M. R. J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3029–3036. (g) Allin, S. M.; James, S. L.; Martin, W. P.; Smith, T. A. D. *Tetrahedron Lett.* **2001**, *42*, 3943–3946.
14. For the conversion of the 3,4-dimethoxy grouping into the desired 3,4-methylenedioxy grouping at the later stage of CET synthesis, see: Yasuda, S.; Yamada, T.; Hanaoka, M. *Tetrahedron Lett.* **1986**, *27*, 2023–2026. Furthermore, it has also been demonstrated in our study that the 3,4-methylenedioxy analog of dihydroisoquinoline **1** is equally effective for this annulation process.
15. Cf.: Koseki, Y.; Sato, H.; Watanabe, Y.; Nagasaka, T. *Org. Lett.* **2002**, *4*, 885–888.
16. Cf.: Koseki, Y.; Kusano, S.; Sakata, H.; Nagasaka, T. *Tetrahedron Lett.* **1999**, *40*, 2169–2172. For other related methods, see also: Padwa, A.; Beall, L. S.; Eidell, C. K.; Worsencroft, K. J. *J. Org. Chem.* **2001**, *66*, 2414–2421 and references cited therein.
17. For recent studies on the synthesis of berberine alkaloids, see: (a) Chikaoka, S.; Toyao, A.; Ogasawara, M.; Tamura, O.; Ishibashi, H. *J. Org. Chem.* **2003**, *68*, 312–318. (b) Allin, S. M.; James, S. L.; Elsegood, M. R. J.; Martin, W. P. *J. Org. Chem.* **2002**, *67*, 9464–9467. (c) Kaoudi, T.; Miranda, L. D.; Zard, S. Z. *Org. Lett.* **2001**, *3*, 3125–3127. (d) Pearson, W. H.; Fang, W.-K. *J. Org. Chem.* **2000**, *65*, 7158–7174. (e) Orito, K.; Satoh, Y.; Nishizawa, H.; Harada, R.; Tokuda, M. *Org. Lett.* **2000**, *2*, 2535–2537. (f) Lee, Y. S.; Cho, D. J.; Kim, S. N.; Choi, J. H.; Park, H. *J. Org. Chem.* **1999**, *64*, 9727–9730.
18. Cf.: (a) Reimann, E.; Grasberger, F.; Polborn, K. *Monatsh. Chem.* **2003**, *134*, 991–1014. (b) Reimann, E.; Grasberger, F.;

Polborn, K. *Monatsh. Chem.* **2000**, *131*, 73–84 and previous papers in this series.

19. For other formal (stepwise) annulative methods from Reissert compounds, see: (a) Ruchirawat, S.; Lertwanawatana, W.; Thepchumrune, P. *Tetrahedron Lett.* **1980**, *21*, 189–192. (b) Tyrell, J. A., III; McEwen, W. E. *J. Org. Chem.* **1981**, *46*, 2476–2479. For Heck-type intramolecular reaction, see: (c) Grigg, R.; Sridharan, V.; Stevenson, P. *Tetrahedron* **1990**, *46*, 4003–4018. (d) Sanchez-Sancho, F.; Mann, E.; Herradon, B. *Synlett* **2000**, 509–513. For radical cyclization, see: (e) Nimgirawath, S.; Ponghusabun, O. *Aust. J. Chem.* **1994**, *47*, 951–955. For photochemical methods, see: (f) Lenz, G. R. *J. Org. Chem.* **1974**, *39*, 2839–2845. (g) Lenz, G. R. *J. Org. Chem.* **1977**, *42*, 1117–1122. For other related formal annulative methods, see: (h) Cuevas, J.-C.; Snieckus, V. *Tetrahedron Lett.* **1989**, *30*, 5837–5840. (i) Kametani, T.; Terasawa, H.; Ihara, M.; Fukumoto, K. *Heterocycles* **1977**, *6*, 37–41.

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Unusual asymmetric oxidation of sulfide; the diastereoselective oxidation of prochiral sulfide-chiral acid salt with hydrogen peroxide without metal

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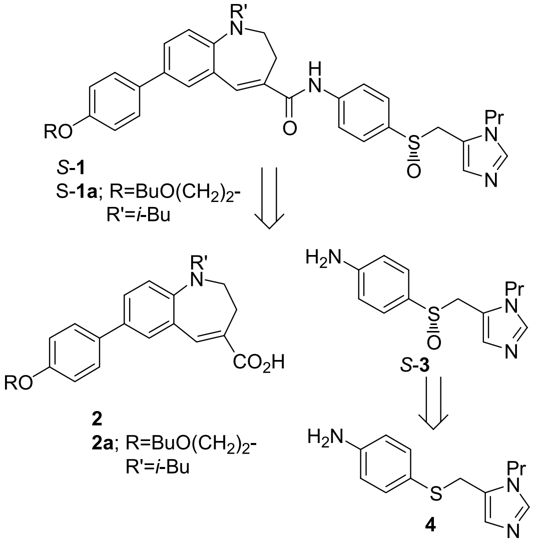
Abstract—The sulfide **4** was treated with chiral acid in a mixture of toluene and methyl *iso*-butylketone to precipitate the salt, which reacted with 30% H_2O_2 for 3 weeks at rt. The resulting crystals were collected followed by recrystallization to give the salt of enantiometrically pure sulfoxide and chiral acid **7** in 72% yield and 98.1% de, which was led to chiral sulfoxide *S*-**3** after neutralization. Sulfoxide *S*-**3** was led to *S*-**1a** as the candidate for an orally active HIV-1 therapeutic agent.

1. Introduction

Over the past three decades, many asymmetric synthetic methods have been developed in the pharmaceutical and agricultural industries because of switching to chiral compounds from racemic compounds.[1] For asymmetric oxidation, especially for enantioselective oxidation of sulfide, there have been many reports because of the increasing use of chiral sulfoxides both as biological products[2] and chiral auxiliaries.[3] Although the asymmetric oxidation of prochiral sulfides with chiral metal complexes such as Ti/diol, V, Ti and Mn/salen, and V-Schiff bases are generally used,[4] the Kagan method and its improvements are mainly used in preparations for a large scale.[5] However, these preparations have some drawbacks: (1) difficulty in removing or recovering the expensive auxiliaries, and (2) metals remaining in drug substances.

Recently, Seto et al. have reported that benzazepines having the chiral sulfoxide moiety *S*-**1** could be candidates for an orally active HIV-1 therapeutic agent, showing CCR5 antagonist activities.[6] In an early synthesis, *S*-**1** was prepared by the separation of *rac*-**1** using a chiral column by HPLC.[7] Therefore, the preparation of *S*-**1** amerable a large scale was required to support pharmacological and toxicological evaluations. To accomplish this goal, sulfide **4** was enantiometrically oxidated to give *S*-**3**, which led to *S*-**1** (Scheme 1).



Scheme 1.

2. Results and discussion

First, the preparation of *S*-**3** was conducted using the optical resolution of *rac*-**3**, which was produced by the alkylation of

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Keywords: Diastereoselective sulfoxidation; Benzazepine; CCR5 antagonist.

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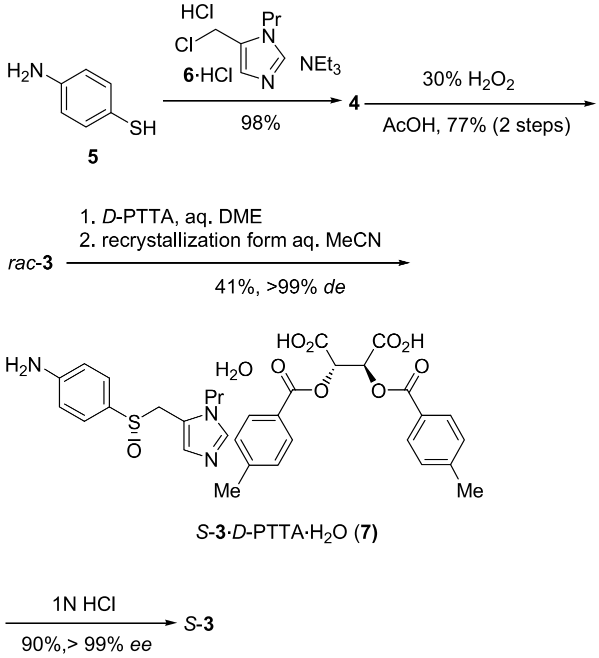


Scheme 2.

4-aminothiophenol **5** with 5-(chloromethyl)-1-propyl-1*H*-imidazole hydrochloride **6**·HCl[6a] and triethylamine in aqueous 2-propanol followed by the oxidation with 30% H_2O_2 in AcOH in 77% yield (2 steps) as shown in Scheme 2. Di-*p*-toluoyl-D-tartaric acid (D-PTTA) was found as a suitable acid of optical resolution by the screening of various acids. The optical resolution of *rac*-**3** was carried out with D-PTTA in aqueous 1,2-dimethoxyethane (DME) followed by recrystallization from aqueous MeCN to give *S*-**3**·D-PTTA monohydrate (**7**) in 41% yield and >99% de, which gave *S*-**3** in 90% yield and >99% ee after neutralization. The absolute configuration of **7** was determined by X-ray crystallographic analysis (Fig. 1).[8]

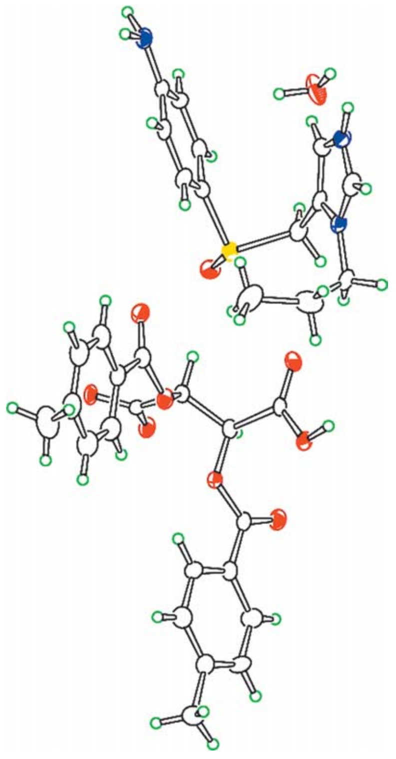
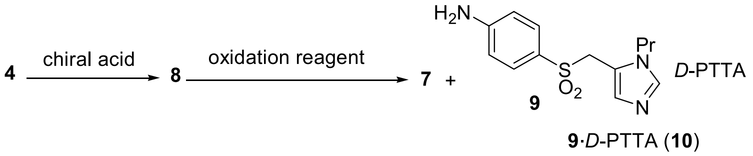


Figure 1. Molecular structure of **7**.

Next, the diastereoselective oxidation of the derivatives led from **4** was carried out. Namely, prochiral sulfide **4** yielded **8** by the salt formation with chiral acid, which was reacted with the oxidation reagent to afford the asymmetric oxidation (Table 1). Initially, D-PTTA as a chiral acid and 30% H_2O_2 as an oxidation reagent were chosen. The treatment of **4** was carried out with an equivalent of D-PTTA in methyl *iso*-butylketone (MIBK) to precipitate the salt of **4** and D-PTTA. Subsequently, 3 equiv of 30% H_2O_2 was added to the resulting mixture, and the suspension was stirred at rt for 24 h (Table 1). Surprisingly, the oxidation afforded the diastereoselective reaction, giving **7** in 42% conversion and 52% de, and sulfone **9** was not detected, while the reaction mixture was kept in suspension (run 1).[9] This result showed that the reaction of **8** with 30% H_2O_2 asymmetrically afforded the oxidation of sulfide, giving **7**, however, not the kinetic resolution of racemic sulfoxide to sulfone. Increasing the equivalent of H_2O_2 to 5 did not affect the conversion or diastereoselectivity (run 2). When *m*-chlorobenzoic peracid (*m*CPBA) was used, the oxidation afforded the diastereoselectivity, however, giving a large amount of sulfone **9** (run 3). A variety of solvents were also examined. The reactions in CH_2Cl_2 or toluene showed almost the same diastereoselectivities as that in MIBK, and gave an increased conversion (runs 4 and 5). The use of 2-propanol decreased both the conversion and diastereoselectivity (run 6). Mixed solvent, for instance, CH_2Cl_2/toluene and MIBK/toluene, increased the diastereoselectivity (runs 7 and 8). Using the conditions of run 8, **4** were reacted for 8 days at rt to give 92% conversion and 68% de (run 9). After the reaction was continued for 3 weeks, the resulting solid was filtered off followed by recrystallization from aqueous MeCN, giving **7** in 72% yield and 98.1% de (Scheme 3). On the other hand, the solution of **4** dissolved in a large amount of MIBK and toluene was reacted with 30% H_2O_2, showing lower selectivity (4% de, run 10). From these results, it was considered that the steric formation of **8** was held by deposition from the solution, which reacted heterogenously with H_2O_2 to afford the asymmetric oxidation. Moreover, the practical preparation was studied. After the reaction of **5** with **6**·HCl was completed, **4** was extracted with MIBK, and a solution of D-PTTA in toluene and 30% H_2O_2 were added to the solution. The mixture was stirred for 24 h at rt to give **7** in 60% conversion and 70% de. Subsequently, the addition of methanol to the reaction mixture followed by stirring for 8 h at 50 °C gave 98.5% conversion and 42% de, which was led to diastereometrically pure **7** (98.2% de) in 53% yield (2 steps) after the crystallization from the reaction solution.

Chiral sulfoxide *S*-**3** led to *S*-**1a** as one of the biologically active products (Scheme 4). In the previous report, **2a** was prepared in 3% from starting material using 10 steps.[6] We already reported the efficient synthesis of benzazepines.[11] According to our procedure, the synthesis of **2a** was carried out. The hydrolysis of 1-isobutylpyrrolidin-2-one with aqueous MsOH followed by neutralization and anilination of **11** gave butyric acid **12** in one-pot. After workup, **12** was

Table 1. Oxidation of **4** with D-PTTA[a]

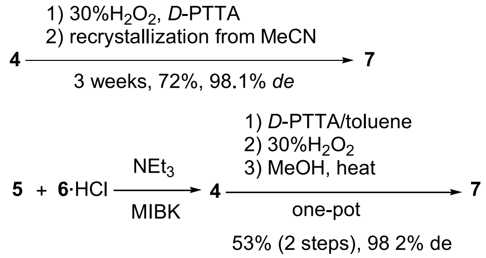


Entry	Solvent (v/w)	Chiral acid	Reagent (eq)	Conditions	Conversion (%)		
					7	**10**	de (%)[b]
1	MIBK (25)	D-PTTA	30% H_2O_2 (3)	rt, 24 h	42	—	52
2	MIBK (25)	D-PTTA	30% H_2O_2 (5)	rt, 24 h	48	—	47
3	MIBK (25)	D-PTTA	*m*CPBA (1.1)	rt, 24 h	31	41	47
4	CH_2Cl_2 (50)	D-PTTA	30% H_2O_2 (3)	rt, 24 h	68	—	49
5	Toluene (50)	D-PTTA	30% H_2O_2 (3)	rt, 24 h	75	3	42
6	2-Propanol (50)	D-PTTA	30% H_2O_2 (3)	rt, 24 h	11	—	28
7	CH_2Cl_2 (20)/toluene (40)	D-PTTA	30% H_2O_2 (3)	rt, 24 h	64	1	66
8	MIBK (25)/toluene (25)	D-PTTA	30% H_2O_2 (3)	rt, 24 h	40	—	76
9	MIBK (25)/toluene (25)	D-PTTA	30% H_2O_2 (3)	rt, 8 days	92	5	68
10[c]	MIBK (1000)/toluene (1000)	D-PTTA	30% H_2O_2 (3)	rt, 3 days	5	—	4

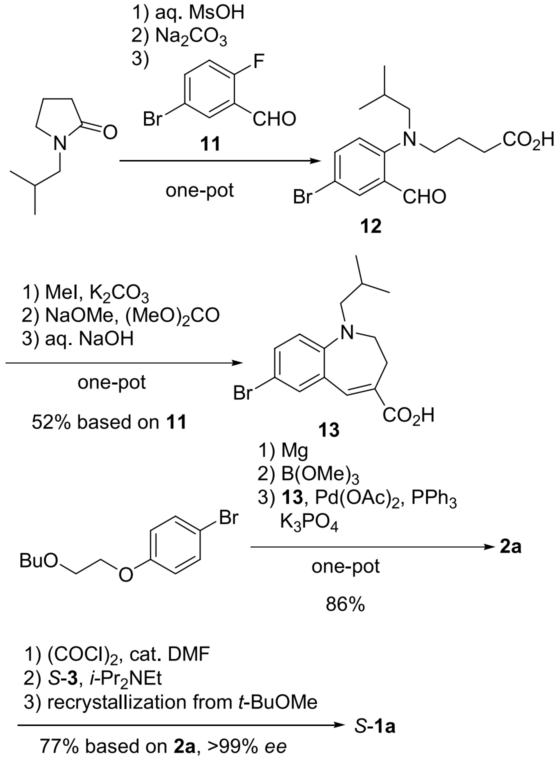
[a] To a mixture of **4** (99 mg, 0.4 mmol), D-PTTA (150 mg, 0.4 mmol) and solvent was added oxidation reagent (0.14 g, 1.2 mmol), and the suspension was stirred at rt.

[b] Determined by HPLC on a Daicel Chiralcel OD (85/15 *n*-hexane/ethanol).

[c] The reaction mixture was solution.



Scheme 3.



Scheme 4.

used in the next step without purification. Compound **12** was esterified with MeI and K_2CO_3, which was treated with the combination of NaOMe and dimethyl carbonate followed by hydrolysis to give **13** in 52% Yield (2 steps, one-pot). The Suzuki–Miyaura reaction of **13** with boronate complex led from aryl bromide gave **2a** in 86% yield. The treatment of carboxylic acid **2a** with 1.1 equiv of oxalyl chloride and a catalystic amount of DMF in THF, followed by amidation with 1.3 equiv of *S*-**3** and 3.5 equiv of *i*-Pr_2NEt, gave crude *S*-**1a** without the Pummerer rearrangement. Crude *S*-**1a** was recrystallized from *t*-BuOMe to give high quality *S*-**1a** (purity >99%, >99% *ee*) as a solvate of *t*-BuOMe in 77% yield (Scheme 5).[10]

3. Conclusion

A new type of asymmetric sulfoxidation has been developed. Treatment of prochiral sulfide **4** having imidazole moiety with tartaric acid derivative led to imidazole-sulfide having the chiral moiety, which was reacted with H_2O_2 to afford diastereometrically oxidation. Subsequently, the de of the resulting diastereomer sulfoxide **7** was increased by recrystallization, which was converted to high enantiometrically pure sulfoxide *S*-**3** after neutralization. For the procedure described here, it was easy to recover the tartaric acid derivative as an auxiliary, and this synthesis perfectly prevented contamination by metal.

4. Experimental

4.1. General

Melting points were recorded on a Büchi B-540 micro melting apparatus and were uncorrected. Optical rotation values were recorded on a JASCO DIP-370 polarimeter under standard conditions. IR spectra were recorded on a Horiba FT-210 spectrophotometer, and absorptions are reported in cm^{-1}. ^1H NMR (300 MHz) and ^{13}C NMR

(75.5 MHz) spectra were recorded on a Bruker DPX-300 spectrometer at ambient temperature. ^{1}H NMR spectra are reported as follows: Chemical shifts in ppm downfield from tetramethylsilane as an internal standard, multiplicity (s = singlet, d = doublet, t = triplet and m = mutiplet), coupling constants spectra (Hz) and integration. ^{13}C NMR spectra recorded in ppm relative to the central line for $CDCl_3$ at 77 ppm, DMSO-d_6 at 39.7 ppm, and CD_3OD at 49.0 ppm. The column chromatography was performed on Chromatorex (Fuji Silysia Chemical Ltd). Elemental analyses were performed at Takeda Analytical Research Laboratories, Ltd.

4.1.1. 4-{[(1-Propyl-1*H*-imidazol-5-yl)methyl]thio}phenylamine (4). The solution of **6**·HCl (3.90 g, 20 mmol) and water (2.5 mL) was added to a suspension of 4-aminothiophenol (**5**, 2.56 g, 20 mmol), NEt_3 (5.5 mL, 40 mmol) and 2-propanol (10 mL) at −10 to 0 °C, and stirred for 1 h. Water was added to a reaction mixture, and the remaining solution was extracted with methyl *iso*-butylketone (MIBK). The organic layer was washed with brine, dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by chromatography on silica-gel with AcOEt to give **4** as a white crystalline (4.85 g, yield 98%). Mp 49–51 °C. ^{1}H NMR ($CDCl_3$): δ 0.96 (3H, t, $J=$ 7.3 Hz), 1.76–1.88 (2H, m), 3.76 (2H, br s), 3.86–3.92 (4H, m), 6.54–6.59 (2H, m), 6.64 (1H, s), 7.06–7.11 (2H, m), 7.42 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.0, 24.0, 30.6, 46.3, 115.2, 121.0, 126.9, 128.8, 135.3, 137.5, 146.9. IR (KBr): 1633, 1600, 1496. Anal. Calcd for $C_{13}H_{17}N_3S\cdot0.1H_2O$: C, 62.67; H, 6.96; N, 16.86. Found: C, 62.74; H, 6.83; N, 16.77.

4.1.2. (*S*)-4-{[(1-Propyl-1*H*-imidazol-5-yl)methyl]sulfinyl}phenylammonium (2*S*,3*S*)-2,3-bis-[(4-methylbenzoyl)oxy]-butenoate hydrate (7) using asymmetric oxidation. The solution of **4** (1.24 g, 5.0 mmol) and MIBK (15 mL) was added to a solution of D-PTTA (1.94 g, 5.0 mmol), MIBK (15 mL) and toluene (30 mL) at rt. Subsequently, 30% H_2O_2 (1.73 g, 15.0 mmol) were added to the resulting suspension, and stirred for 3 weeks. The resulting solid was collected by filtration and recrystallized from a mixture of MeCN (9 mL) and water (9 mL) to give **7** as a white crystalline (2.4 g, yield 72%, 98.1% de). $[\alpha]_D^{25}=-31.5$ (*c* 1.014, MeOH). Mp 140–141 °C. ^{1}H NMR (CD_3OD): δ 0.89 (3H, t, $J=7.41$ Hz), 1.70–1.77 (2H, m), 2.40 (6H, s), 3.82 (2H, t, $J=7.53$ Hz), 4.18–4.38 (2H, m), 5.90 (2H, s), 6.7–6.74 (2H, m), 7.01 (1H, s), 7.18–7.14 (2H, m), 7.28–7.31 (4H, m), 7.98–8.00 (4H, m), 8.46 (1H, d, $J=1.2$ Hz). ^{13}C NMR (CD_3OD): δ 11.0, 21.7, 24.3, 50.6, 74.4, 115.4, 124.4, 126.7, 127.4, 128.1, 130.2, 131.0, 137.6, 145.6, 154.2, 167.2, 171.0. IR (KBr): 1708, 1592. Anal. Calcd for $C_{33}H_{35}N_3O_9S$: C, 61.00; H, 5.43; N, 6.47. Found: C, 59.26; H, 5.67; N, 6.18. HPLC conditions: column, Chiralcel OD, mobile phase: *n*-hexane/2-propanol (85/15), flow rate: 1.0 mL/min, detection: UV (277 nm), column temp.: 35 °C, retention time; 19.9 (substrate), 22.2 (*R*-product), 24.7 (sulfone product) and 28.5 (*S*-product) min.

4.1.3. Practical preparation of 7. The solution of **6**·HCl (3.90 g, 20 mmol) and water (2.5 mL) was added to a suspension of **5** (2.56 g, 20 mmol), NEt_3 (5.5 mL, 40 mmol) and 2-propanol (10 mL) at −15 to −10 °C, and stirred for 1 h. Water was added to a reaction mixture, and the remaining solution was extracted with methyl *iso*-butylketone (MIBK). The organic layer was washed with brine, dried with Na_2SO_4 and concentrated in vacuo. To the resulting residue were added MIBK (30 mL), D-PTTA (7.7 g, 20 mmol) in toluene/MIBK (90 mL/60 mL) and water (3.6 mL), and the mixture was stirred for 2 h at rt. Subsequently, 30% H_2O_2 (6.8 g, 60 mmol) were added to the resulting suspension, and stirred for 24 h. Methanol (30 mL) was added to the mixture, and the mixture was stirred for 8 h at 50 °C. Water (30 mL) was added to the reaction mixture, and the mixture was stirred for 5 h at rt. The resulting solid was collected by filtration to give **7** as a white crystalline (7.1 g, yield 53%, 98.2% de).

4.1.4. 4-{[(1-Propyl-1*H*-imidazol-5-yl)methyl]sulfinyl}-phenylamine (*rac*-3). The solution of **6**·HCl (0.78 g, 4.0 mmol) and water (0.5 mL) was added to a suspension of **5** (0.46 g, 3.64 mmol), NEt_3 (1.1 mL, 8.0 mmol) and MeOH (2 mL) at 0–5 °C, and stirred for 1 h. After warmed to rt, AcOH (1 mL) and 30% H_2O_2 (0.62 g, 5.46 mmol) were added to the resulting mixture, and stirred for 17 h. Sodium sulfite and 6 N NaOH (3 mL) were added to a reaction mixture at 0 °C, and stirred for 1 h. The remaining solution was extracted with a mixture of AcOEt and 2-propanol. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated in vacuo. To the resulting residue was added AcOEt (4 mL), and stirred for 1 h at rt. The resulting solid was collected by filtration to give *rac*-**3** monohydrate as a white crystalline (0.73 g, yield 71% from **5**). Mp 145–146 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, $J=7.4$ Hz), 1.69–1.78 (2H, m), 3.60–3.80 (2H, m), 3.97–4.08 (4H, m), 6.61 (1H, s), 6.67–6.72 (2H, m), 7.15–7.20 (2H, m), 7.44 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.1, 24.0, 46.5, 52.4, 114.7, 120.0, 126.2, 129.7, 131.3, 138.4, 149.9. IR (KBr): 3214, 1650, 1596, 1500. Anal. Calcd for $C_{13}H_{17}N_3OS$: C, 59.29; H, 6.51; N, 15.96. Found: C, 59.19; H, 6.63; N, 15.84.

4.1.5. Synthesis of 7 using optical resolution. To a solution of D-PTTA (14.7 g, 38.0 mmol), *rac*-**3** (10.0 g, 38.0 mmol) and DME (90 mL) was added water (90 mL) at rt, and stirred for 14 h. The resulting solid was collected by filtration and recrystallized from a mixture of MeCN (70 mL) and water (70 mL) to give **7** as a white crystalline (10.5 g, yield 41%, >99% de).

4.1.6. (*S*)-4-{[(1-Propyl-1*H*-imidazol-5-yl)methyl]sulfinyl}phenylamine (*S*-3). The suspension of **7** (2.7 g, 4.0 mmol, 99% de) and AcOEt (10 mL) was adjusted to below pH = 3 with 1 N HCl, and separated. The aqueous layer was adjusted to pH = 10 with 25% Na_2CO_3 solution, extracted with a mixture of AcOEt and 2-propanol (4:1, 15 mL × 3). The organic layer was washed with brine, dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was triturated with AcOEt and collected by filtration to give *S*-**3** as a white crystalline (0.79 g, 99% ee, yield 79%). $[\alpha]_D^{25}=-167.2$ (*c* 1.018, MeOH). Mp 141–142 °C. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, $J=7.4$ Hz), 1.69–1.79 (2H, m), 3.72–3.77 (2H, m), 3.97–4.08 (4H, m), 6.61 (1H, s), 6.60–6.71 (2H, m), 7.15–7.27 (2H, m), 7.44 (1H, s). ^{13}C NMR ($CDCl_3$): δ 11.1, 24.0, 46.5, 52.4, 114.7, 120.0, 126.2, 129.7, 131.3, 138.4, 149.9. IR (KBr): 3156, 1633, 1592,

1500. Anal. Calcd for $C_{13}H_{17}N_3OS$: C, 59.29; H, 6.51; N, 15.96. Found: C, 59.30; H, 6.60; N, 15.96.

4.1.7. 7-Bromo-1-isobuthyl-1-2,3-dihydro-1*H*-1-benzazepine-4-carboxylic acid (13). A solution of 1-isobutylpyrrolidin-2-one (28.0 g, 0.2 mol) in a mixture of MsOH (26 mL, 0.8 mol) and water (66 mL) was refluxed for 24 h. After the solution was cooled to rt, Na_2CO_3 (84.8 g, 0.8 mol) and water (30 mL) were added, and the mixture was stirred for 1 h. After addition of DMSO (30 mL) to the resulting mixture, a solution of **11** (12.2 g, 0.06 mol) in DMSO (66 mL) was added dropwise to the whole mixture under refluxing conditions. The mixture was refluxed for 9 h. After it was cooled to 30 °C, the mixture was treated with 6 N HCl until the pH was adjusted to 3 and extracted with AcOEt. The organic layer was washed with brine. The organic layer was extracted with 1 N NaOH. The aqueous layer was treated with 6 N HCl until the pH was adjusted to 3 and extracted with AcOEt. The organic layer was concentrated in vacuo to give crude **12** as a brown oil, which was used directly in further reactions without purification.

To a solution of crude **12** and K_2CO_3 (9.1 g, 0.066 mol) in DMF (50 mL) was added dropwise a solution of MeI (10.2 g, 0.072 mol) in DMF (10 mL) at rt, and the mixture was stirred for 1 h. After addition of dimethyl carbonate (120 mL) to the mixture, 28% NaOMe in MeOH (27.8 g, 0.144 mol) was added and the whole mixture was stirred for 1 h at 60 °C. After the mixture was cooled to 5 °C, 2 N HCl (100 mL) was added, and the whole mixture was concentrated in vacuo and extracted with toluene. The organic layer was washed successively with 1 N NaOH, brine and water, and concentrated in vacuo. To the resulting residue were THF (85 mL), MeOH (85 mL) and 1 N NaOH (120 mL, 0.12 mol), and the mixture was stirred for 1.5 h at 50 °C. After the mixture was concentrated in vacuo, the resulting solution was washed with toluene. The aqueous layer was treated with 6 N HCl until the pH was adjusted to 3 and extracted with AcOEt. The organic layer was washed with brine, and concentrated in vacuo. The residue was dissolved in MeOH (45 mL) at 60 °C, and water (9 mL) was added dropwise to the solution. The mixture was stirred for 8 h at rt, and stirred for 1 h at 0–5 °C. The resulting solid was collected by filtration and washed with a mixture of MeOH (15 mL) and water (15 mL) to give **13** as a yellow crystalline powder (10.1 g, yield 52% from **11**): Mp 144–145 °C. ^1H NMR ($CDCl_3$): δ 0.91 (6H, d, $J=6.60$ Hz), 1.90–2.25 (1H, m), 2.75–2.85 (2H, m), 3.12 (2H, d, $J=7.35$ Hz), 3.20–3.30 (2H, m), 6.72 (1H, d, $J=8.97$ Hz), 7.20–7.30 (1H, m), 7.44 (1H, s), 7.70 (1H, s). ^{13}C NMR ($CDCl_3$): δ 20.3, 26.5, 32.5, 51.9, 61.8, 109.6, 117.6, 124.1, 128.9, 132.5, 138.6, 140.9, 151.2, 173.6. IR (KBr): 1677, 1614, 1490. Anal. Calcd for $C_{15}H_{18}NO_2Br$: C, 55.57; H, 5.60; N, 4.32. Found: C, 55.52; H, 5.59; N, 4.13.

4.1.8. 7-[4-(2-Butoxyethoxy)phenyl]-1-isobuthyl-2,3-dihydro-1*H*−1-benzazepine-4-carboxylic acid (2a). Under an argon atmosphere, to a suspension of magnesium (2.25 g, 92 mmol) in THF (140 mL) was added dropwise a solution of 1-bromo-4-(2-butoxyethoxy)benzene (24.6 g, 90 mmol) in THF (40 mL) under refluxing conditions and the whole mixture was refluxed for 1 h. After the mixture was cooled to −10 °C, a solution of trimethylborate (10.0 mL, 90 mmol) in THF (40 mL) was added dropwise and the resulting mixture was stirred for 1 h at the same temperature. After the mixture was warmed to rt, $Pd(OAc)_2$ (27 mg, 0.12 mmol) and PPh_3 (126 mg, 0.048 mmol) were added and the whole mixture was stirred for 0.5 h at the same temperature. Next, **13** (19.4 g, 60 mmol) and a solution of K_3PO_4 (79.6 g, 375 mmol) in water (100 mL) were added, and the mixture was refluxed for 2 h. After it was cooled to rt, 3 N HCl was added and the mixture was separated. The aqueous layer was extracted with toluene. The organic layer was washed successively with 2 N NaOH and brine. Activated charcoal (2 g) and tri-*n*-butylphosphine (2 mL) were added to the organic layer and the mixture was stirred for 0.5 h. The charcoal was filtered off and washed with toluene. The filtrate and washing were combined and concentrated in vacuo. The residue was dissolved in *i*-Pr_2O under refluxing conditions. After it was cooled to 0 °C, the mixture was stirred for 1 h at the same temperature. The resulting solid was collected by filtration and washed with cold *i*-Pr_2O to give crude **2a** (23.7 g) as a yellow crystalline powder. Crude **2a** (2.0 g) was dissolved in 2-propanol (10 mL) under refluxing conditions, and tri-*n*-butylphosphine (0.2 mL) was added to the solution. After it was cooled to 0 °C, the mixture was stirred for 1 h at the same temperature. The resulting solid was collected by filtration and washed with cold 2-propanol to give crude **2a** as a yellow crystalline powder (1.9 g, yield 86%): Mp 126–127 °C. ^1H NMR ($CDCl_3$): δ 0.91–1.10 (9H, m), 1.35–1.50 (2H, m), 1.55–1.70 (2H, m), 2.00–2.15 (1H, m), 2.85–2.90 (2H, m), 3.20 (2H, d, $J=7.30$ Hz), 3.30–3.35 (2H, m), 3.58 (2H, d, $J=6.66$ Hz), 3.803.85 (2H, m), 4.15–4.20 (2H, m), 6.92 (1H, d, $J=8.75$ Hz), 7.01 (2H, d, $J=8.75$ Hz), 7.40–7.55 (4H, m), 7.91 (1H, s). ^{13}C NMR ($CDCl_3$): δ 13.8, 19.2, 20.4, 26.6, 31.7, 32.6, 52.0, 61.8, 67.5, 69.2, 71.4, 115.0, 116.2, 122.5, 127.2, 127.8, 128.4, 130.6, 133.0, 134.9, 142.5, 151.1, 157.8, 173.7. IR (KBr): 1668, 1608, 1500. Anal. Calcd for $C_{27}H_{35}NO_4$: C, 74.11; H, 8.06; N, 3.20. Found: C, 74.18; H, 8.33; N, 2.95.

4.1.9. 7-[4-(2-Butoxyethoxy)phenyl]-1-isobuthyl-1-*N*-(4-{[(1-propyl-1*H*-imidazol-5-yl)methyl]-(*S*)-sulfinyl}-phenyl)-2,3-dihydro-1*H*-1-benzazepine-4-carboxamide (*S*-1a). The suspension of **7** (5.0 g, 7.5 mmol, >99% de) and AcOEt (15 mL) was adjusted to below pH = 3 with 1 N HCl, and separated. The aqueous layer was adjusted to pH = 9 with 25% K_2CO_3 solution, extracted with a mixture of AcOEt and 2-propanol. The organic layer was washed with brine, dried with Mg_2SO_4 and concentrated in vacuo to give *S*-**3**. Oxalyl chloride (0.56 mL, 6.4 mmol) was dropped to a solution of **2a** (2.56 g, 5.9 mmol), DMF (1 drop) and THF (8 mL) at 0–5 °C, and stirred for 1 h. The resulting acid chloride was dropped to a mixture of *S*-**3**, NEt_3 (2.9 mL, 20.8 mmol) and THF (18 mL) below 10 °C and stirred for 1 h. Water was added to the reaction mixture, which was separated and extracted with AcOEt. The organic solution was washed with 10% acetic acid, 7% $NaHCO_3$ aqueous solution, and 10% NaCl aqueous solution. Activated carbon (0.4 g), silica-gel (4 g) and Na_2SO_4 (2 g) were added to an organic solution, and stirred for 10 min. Solid was filtered off, and the mother solution was concentrated in vacuo. The resulting residue was crystallized from a mixture of *t*-BuOMe (20 mL) and water (9 mL), and recrystallized from a mixture of *t*-BuOMe (31 mL) and EtOH (3 mL) to give *S*-**1a** as a yellow solid (3.5 g as the solvate of *t*-BuOMe, >99%*ee*, yield 78%). All analytical data of *S*-**1a** were determined as the solvate of *t*-BuOMe. $[\alpha]_D^{25} = -117.1$ (*c* 1.013, MeOH). Mp 94–96 °C. ^{1}H NMR (CDCl$_3$): δ 0.84–0.97 (12H, m), 1.19 (9H, s), 1.28–1.42 (2H, m), 1.53–1.75 (4H, m), 2.02–2.11 (1H, m), 2.88–2.94 (2H, m), 3.17–3.21 (2H, m), 3.21 (3H, s), 3.33–3.37 (2H, m), 3.53 (2H, t, $J=6.6$ Hz), 3.71–3.81 (4H, m), 3.95–4.10 (2H, m), 4.13–4.16 (2H, m), 6.55 (1H, s), 6.90–6.98 (3H, m), 7.32 (2H, d, $J=8.7$ Hz), 7.43–7.47 (5H, m), 7.75 (2H, d, $J=8.7$ Hz), 8.32 (1H, s). ^{13}C NMR (CDCl$_3$): δ 11.1, 13.9, 19.2, 20.5, 24.0, 26.7, 27.0, 31.7, 33.3, 46.6, 49.4, 52.0, 52.1, 61.7, 67.6, 69.1, 71.4, 72.8, 115.0, 116.3, 119.7, 120.1, 122.8, 125.3, 127.2, 127.8, 130.8, 131.3, 133.0, 133.6, 134.0, 136.0, 136.3, 138.6, 141.9, 150.7, 157.9, 168.3. IR (KBr): 2956, 1654, 1498, 1241. Anal. Calcd for $C_{40}H_{50}N_4O_4S \cdot C_5H_{12}O$: C, 70.10; H, 8.10; N, 7.27. Found: C, 69.82; H, 8.01; N, 7.31. HPLC conditions: column, Chiralcel OJ-R, mobile phase: 0.05 M KH_2PO_4/MeCN/MeOH (40/50/10), flow rate: 1.0 mL/min, detection: UV (295 nm), column temp.: 35 °C, retention time; 11.5 (*S*-product) and 14.1 (*R*-product) min.

Acknowledgements

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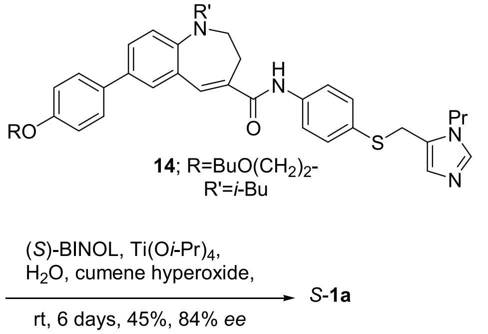
References and notes

1. *Chirality in Industry*; Collins, A. N., Sheldrake, G. N., Crosby, J., Eds.; Wiley: Chichester, UK, 1997.
2. (a) Tanaka, M.; Yamazaki, H.; Hakasui, H.; Nakamichi, N.; Sekino, H. *Chirality* **1997**, *9*, 17. (b) Von Unge, S.; Langer, V.; Sjölin, L. *Tetrahedron: Asymmetry* **1997**, *8*, 1967. (c) Holland, H. L.; Brown, F. M.; Larsen, B. G. *Tetrahedron: Asymmetry* **1994**, *5*, 1129. (d) Hutton, C. A.; White, J. M. *Tetrahedron Lett.* **1997**, *38*, 1643. (e) Morita, S.; Matsubara, J.; Otsubo, K.; Kitano, K.; Ohatani, T.; Kawano, Y.; Uchida, M. *Tetrahedron: Asymmetry* **1997**, *8*, 3707.
3. (a) Carrenõ, M. C. *Chem. Rev.* **1995**, *95*, 1717. (b) Solladie', G. *Synthesis* **1981**, 185. (c) Andersen, K. K. In *The Chemistry of Sulfones and Sulfoxides*; Patai, S., Rappoport, Z., Stirling, C. J. M., Eds.; Wiley: Chichester, UK, 1988; Chapter 16, pp 53–94. (d) Posner, G. H. In *The Chemistry of Sulfones and Sulfoxides*; Patai, S., Rappoport, Z., Stirling, C. J. M., Eds.; Wiley: Chichester, UK, 1988; Chapter 16, pp 823–849.
4. (a) Pitchen, P.; Dunãch, E.; Deshmukh, M. N.; Kagan, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 8188. (b) Di Furia, F.; Modena, G.; Seraglia, R. *Synthesis* **1984**, 325. (c) Palucki, M.; Hanson, P.; Jacobson, E. N. *Tetrahedron Lett.* **1992**, *33*, 7111. (d) Thakur, V. V.; Sudalai, A. *Tetrahedron: Asymmetry* **2003**, *14*, 407. (e) Noda, K.; Hosoya, N.; Yanai, K.; Irie, R.; Katsuki, T. *Tetrahedron Lett.* **1994**, *35*, 1887. (f) Nakajima, K.; Kijima, M.; Fujita, J. *Chem. Lett.* **1986**, 1483. (g) Bolm, C.; Bienewald, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2883. (h) Adam, W.; Korb, M. N.; Roschmann, K. J.; Moller, C. R. S. *J. Org. Chem.* **1998**, *63*, 3423. (i) Vetter, A. H.; Berkessel, A. *Tetrahedron Lett.* **1998**, *39*, 1741.
5. (a) Bowden, S. A.; Burke, J. N.; Gray, F.; McKown, S.; Moseley, J. D.; William, O.; Moss, W. O.; Murray, P. M.; Welham, M. J.; Young, M. J. *Org. Process Res. Dev.* **2004**, *8*, 33. (b) Nishi, T.; Nakajima, K.; Iio, Y.; Ishibashi, K.; Fukuzawa, T. *Tetrahedron: Asymmetry* **1998**, *9*, 2567. (c) Matsugi, M.; Fukuda, N.; Muguruma, Y.; Yamaguchi, T.; Minamikawa, J.-I.; Otsuka, S. *Tetrahedron* **2001**, *57*, 2739. (d) Pitchen, P. *Chem. Ind. (London)* **1994**, 636. (e) Cotton, H.; Elebring, T.; Larsson, M.; Li, L.; Sörensen, H.; von Unge, S. *Tetrahedron: Asymmetry* **2000**, *11*, 3819.
6. (a) Seto, M.; Miyamoto, N.; Aikawa, K.; Aramaki, Y.; Kanzaki, N.; Iizawa, Y.; Baba, M.; Shiraishi, M. *Bioorg. Med. Chem.* **2005**, *13*, 363. (b) Seto, M.; Aramaki, Y.; Okawa, T.; Miyamoto, N.; Aikawa, K.; Kanzaki, N.; Niwa, S.; Iizawa, Y.; Baba, M.; Shiraishi, M. *Chem. Pharm. Bull.*5**2004**, 577–590.
7. The asymmetric synthesis of *S*-**1a** as shown in Scheme 5 was also reported.[6a]



Scheme 5.

8. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center (CCDC 249650). Copies of data can be obtained free of charge via www.ccdc.cam.ac.uk/.
9. The reaction of **4** with an equivalent of (*R*)-1, 1′-binaphathyl-2,2′-diyl hydrogenphosphate and 3 equivalents of 30% H_2O_2 in CH_2Cl_2 for 24 h at rt gave the salt of *R*-3 and (*R*)-1, 1′-binaphathyl-2,2′-diyl hydrogenphosphate in 51% conversion and 31% de.
10. In a previous report[6], *S*-**1a** was isolated as a solvate of AcOEt.
11. (a) Ikemoto, T.; Ito, T.; Nishiguchi, A.; Miura, S.; Tomimatsu, K. *Tetrahedron Lett.* **2004**, *45*, 9335.(b) Ikemoto, T.; Ito, T.; Nishiguchi, A.; Miura, S.; Tomimatsu, K. Organic Process Research Development. 2005, published online February 3, 2005, doi; 10.1021/op0497916.

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Chlorodinitrophenylhydrazine, a useful crystalline agent for absolute configuration determination of various chiral ketones

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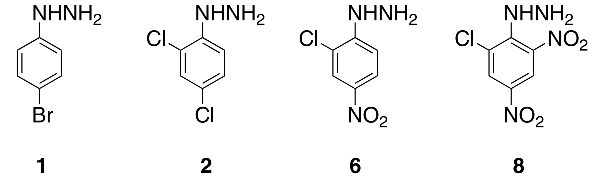
Abstract—For the determination of absolute configuration of various chiral ketones, we examined some hydrazines having a heavy atom as crystalline auxiliaries, and found that 2-chloro-4,6-dinitrophenylhydrazine is a useful crystalline agent for carbonyl compounds. Chiral hydrazones prepared from the hydrazine and various chiral ketones gave suitable single crystals for X-ray crystallographic analysis. The absolute configurations of the hydrazones were determined by X-ray crystallographic analysis using anomalous dispersion effect of the chlorine atom. The hydrazine is a useful crystalline agent for absolute configuration determination of various chiral ketones.

1. Introduction

Optically active α-substituted ketones (α-chiral ketones) are versatile intermediates for the synthesis of natural products, especially in pheromone synthesis.[1] A number of methods for the synthesis of α-chiral ketones have been reported, for example, stereoselective α-alkylation[2] and enantioselective protonation of enolates and enols.[3] Enzymatic transformations to obtain α-chiral ketones have also been reported, for example, hydrolysis of enol esters[4] and reduction of α,β-unsaturated carbonyl compounds (enones).[5] The enantioselective protonation of enol esters[6] and enol ethers[7] using a catalytic antibody has also been reported. We have developed the method for the asymmetric synthesis of α-chiral ketones by the reduction of enones with baker's yeast[8] or a carbon–carbon double bond reductase purified from baker's yeast.[9] However, few studies are found in the literature for the direct method to determine the absolute configurations of these chiral ketones. They have been determined by derivation to the known compounds[2g,6] or assumed from empirical rules.[2f,4c,10]

X-ray crystallographic analysis is the only method to determine the absolute configuration of a molecule. The anomalous dispersion effect of a heavy atom must be used for the determination of the absolute configuration. However, in most cases, the chiral ketones reduced from the corresponding enones are oily substances and contain no heavy atom, so that single crystals must be prepared with a certain crystalline auxiliary having a heavy atom. Hydrazines are well-known to be good crystalline agents for ketones. It is easy that a halogen atom as a heavy atom is introduced into a phenyl ring in the phenylhydrazine. Thus, a phenylhydrazine derivative having (a) halogen atom(s) is employed as a crystalline agent (Scheme 1). Recently, we reported some preliminary results on the determination of absolute configuration of chiral ketones.[11] This paper will describe the details and application of this method.



Scheme 1.

2. Results and discussion

2.1. Design and synthesis of crystalline agents

At first, commercial 4-bromophenylhydrazine (**1**) and 2,4-dichlorophenylhydrazine (**2**) were used as crystalline agents, and hydrazones of chiral ketones were prepared with them under acidic conditions. All chiral ketones here studied (**3a–e**) were obtained by baker's yeast reduction of enones (Scheme 2).[8c] The yields of hydrazone (**4**, **5c**,**e**) are

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Keywords: Phenylhydrazine; Chiral ketone; X-ray crystallography; Absolute configuration.

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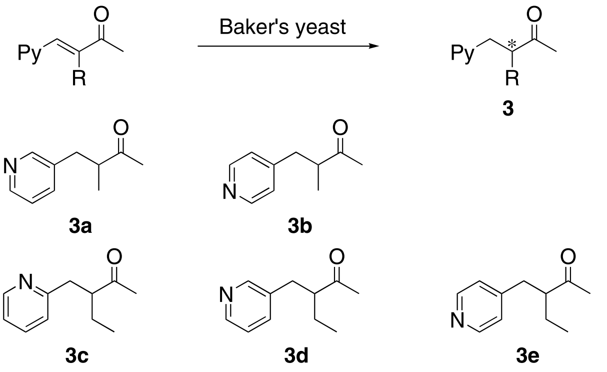


Scheme 2.

listed in Table 1. Unfortunately, these hydrazones did not give suitable single crystals for X-ray crystallographic analysis.

Table 1. Yields (%) of hydrazone

Ketone	Hydrazine			
	1	**2**	**6**	**8**
3a	**4** (70)			**10a** (64)
3b				**10b** (75)
3c		**5c** (63)	**7c** (40)	**10c** (31)
3d			**7d** (61)	**10d** (80)
3e		**5e** (35)	**7e** (24)	**10e** (86)
11				**14** (91)
12				**15** (59)
13				**16** (80)

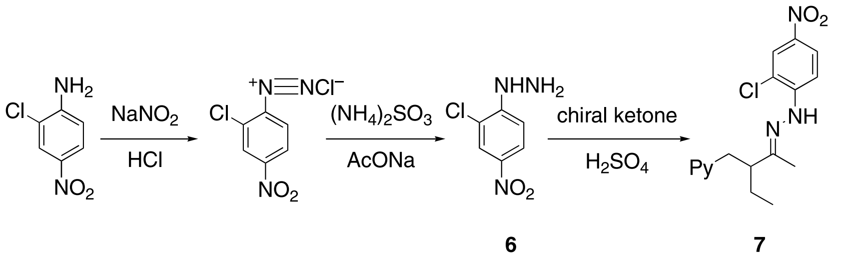
Since, a nitro group is also known to have a good crystallinity, introduction of a nitro group into the hydrazone is expected for more crystallinity. Therefore, 2-chloro-4-nitrophenylhydrazine (**6**) was designed and synthesized. It has a chlorine atom as a heavy atom and a nitro group for a crystallinity. Its synthesis was carried out in the conventional method (Scheme 3). 2-Chloro-4-nitrophenylhydrazones (**7**) were prepared from the corresponding hydrazine **6** and various chiral ketones (Table 1), but they did not give good single crystals as in the cases of **4** and **5**.

2-Chloro-4,6-dinitrophenylhydrazine (**8**) having two nitro groups was designed with the hope of more crystallinity.[12] 2-Chloro-4,6-dinitroaniline is a weak base because of two nitro groups and a chlorine atom, so that diazotization does not occur with a conventional way. Therefore, the aniline dissolved in hot acetic acid was diazotized with nitrosyl sulfate prepared from sodium nitrite and sulfuric acid,[13] and iodinated with potassium iodide to afford 1-chloro-2-iodo-3,5-dinitrobenzene (**9**). It was stirred with hydrazine monohydrate in methanol at room temperature to give the desired hydrazine **8** (Scheme 4).[14]

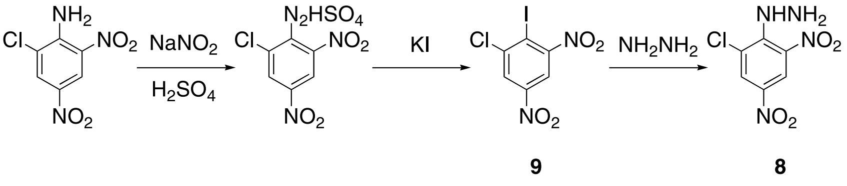
2.2. Determination of the absolute configuration of chiral ketones

The reaction of hydrazine **8** with chiral ketones in the presence of an acid catalyst gave chiral hydrazones **10** in good yields as shown in Table 1. The ketone **3a** and **3b** having a methyl group at the α-position of carbonyl group were racemized slowly under a strong acidic condition. The conditions to prevent the racemization were studied and found that ee of the chiral ketone kept with a catalytic amount of sulfuric acid. Therefore, syntheses of hydrazone with chiral ketones **3a** and **3b** having a methyl group at the α-position of carbonyl group were carried out under that condition (condition A). Syntheses with other ketones were done under a strong acid condition (condition B). Synthesized 2-chloro-4,6-dinitrophenylhydrazone derivatives (**10a–e**) were recrystallized from various solvents to obtain single crystals. Single crystals of the hydrazone were subjected to X-ray crystallographic analysis in order to elucidate their absolute configurations using the anomalous dispersion effect of a chlorine atom. The ORTEP drawings of **10a–e** are shown in Figures 1–5, respectively. Crystallographic data of the above analysis are listed in Table 2.

Except for **10c**, the structures of hydrazones exhibit (*E*)-forms at the carbon–nitrogen double bond, and the production of single isomer was confirmed by ^{1}H NMR experiments. As judged by ^{1}H NMR experiment, hydrazone **10c** consisted of two isomers, which should be (*E*)- and (*Z*)-forms. The ratio of isomers was about 1/1 estimated by



Scheme 3.



Scheme 4.

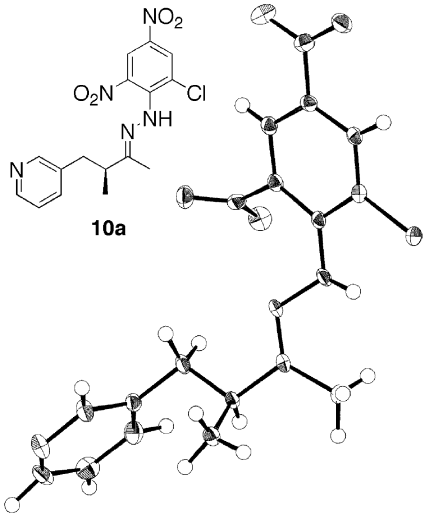


Figure 1. The ORTEP drawing of **10a** with displacement ellipsoids at 50% probability level. The asymmetric unit contains two independent molecules (only one molecule is shown).

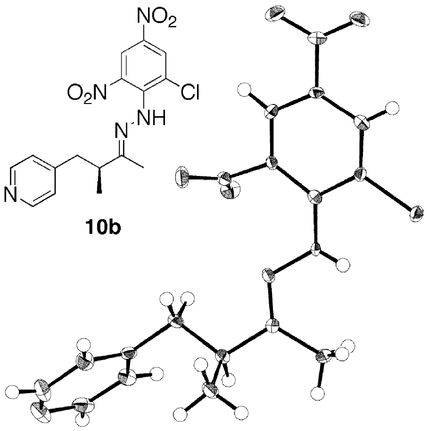


Figure 2. The ORTEP drawing of **10b** with displacement ellipsoids at 50% probability level. The asymmetric unit contains two independent molecules (only one molecule is shown).

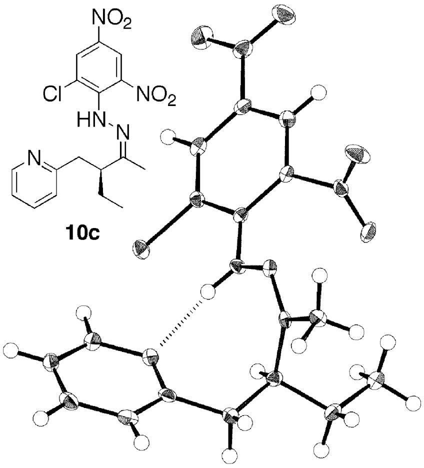


Figure 3. The ORTEP drawing of **10c** with displacement ellipsoids at 50% probability level.

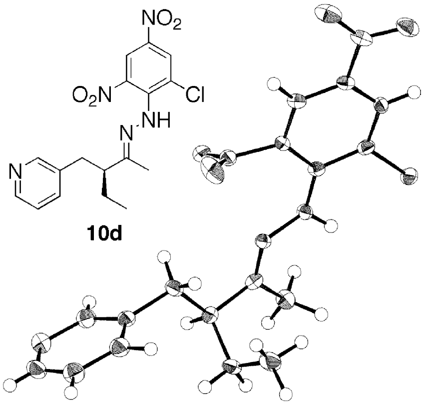


Figure 4. The ORTEP drawing of **10d** with displacement ellipsoids at 50% probability level.

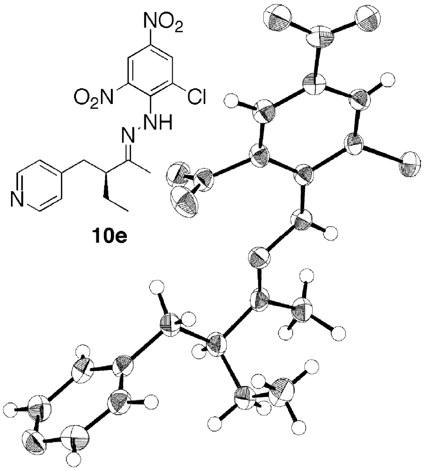


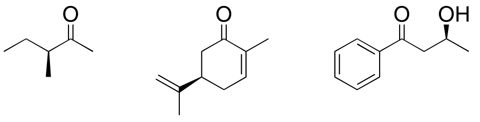
Figure 5. The ORTEP drawing of **10e** with displacement ellipsoids at 50% probability level.

integral curves in a ^{1}H NMR spectrum, but all the single crystals which subjected to X-ray crystallographic analysis were exhibited to be (*Z*)-form. The ORTEP drawing of (*Z*)-**10c** illustrated in Figure 3 shows that there exists a hydrogen bond between N in the pyridine ring and NH in the hydrazone moiety, the length of which is 2.91 Å. Because of the hydrogen bond, the (*Z*)-form should be stabilized and exhibit a good crystallinity. The absolute configurations of these hydrazones **10a–e** were determined to be *S* by Flack parameters[16] and intensities comparisons of Bijvoet pairs.[17] These configurations were determined by the anomalous dispersion effect of a chlorine atom in the crystalline auxiliary, 2-chloro-4,6-dinitrophenylhydrazine. Therefore, the absolute configurations of the chiral ketone obtained by baker's yeast reduction **3a–e** were determined to be *S*.

In order to apply this method to other types of chiral ketone, hydrazones were synthesized from a chiral alkyl ketone **11**, commercially available (*R*)-carvone **12**, and a chiral hydroxy ketone **13** (Scheme 5). The alkyl ketone **11** was supplied by Daicel Chemical Industries, Ltd and the hydroxy ketone **13** was obtained by baker's yeast reduction of the corresponding β-diketone.[15] The hydrazones **14–16** also gave single crystals which were subjected to X-ray crystallographic analysis in order to elucidate their absolute

Table 2. Crystallographic data of hydrazone **10a–e**

	10a	**10b**	**10c**	**10d**	**10e**
Formula	$C_{16}H_{16}ClN_5O_4$	$C_{16}H_{16}ClN_5O_4$	$C_{17}H_{18}ClN_5O_4$	$C_{17}H_{18}ClN_5O_4$	$C_{17}H_{18}ClN_5O_4$
Formula weight	377.79	377.79	391.81	391.81	391.81
Crystal system	Monoclinic	Orthorhombic	Orthorhombic	Monoclinic	Monoclinic
Space group	$C2$ (#5)	$P2_12_12$ (#18)	$P2_12_12_1$ (#19)	$P2_1$ (#4)	$P2_1$ (#4)
a (Å)	18.456 (10)	17.500 (2)	7.508 (4)	7.013 (7)	7.010 (4)
b (Å)	9.039 (5)	21.236 (2)	14.258 (7)	5.527 (5)	5.514 (2)
c (Å)	21.007 (11)	9.1634 (9)	16.590 (9)	23.38 (2)	23.89 (1)
β (°)	104.183 (6)			93.40 (2)	103.031 (5)
V (Å^3)	3397.6 (27)	3405.4 (5)	1776.0 (14)	904.6 (13)	899.7 (7)
Z value	8	8	4	2	2
No. observations	7616	7788	4045	3124	3899
No. variables	593	597	316	316	315
R_1 ($F^2 > 2.0\sigma(F^2)$)	0.050	0.050	0.038	0.049	0.060
wR_2 (all data)	0.128	0.085	0.066	0.133	0.168
S (all data)	1.04	1.01	1.00	1.08	1.10
Flack parameter	−0.03 (7) (S)	0.05 (5) (S)	0.03 (5) (S)	0.0 (1) (S)	0.0 (1) (S)



11 **12** **13**

Scheme 5.

configurations using the anomalous dispersion effect of a chlorine atom. The ORTEP drawings of **14–16** are illustrated in Figures 6–8. The crystallographic data of **14–16** are listed in Table 3. The absolute configurations were successfully determined by Flack parameter[16] and intensities comparison of Bijvoet pairs.[17] To improve the practical usefulness of this method, Cu Kα ($\lambda = 1.5418$ Å) radiation was tested as an alternative X-ray source. Structure determination of hydrazone **15** using Cu Kα radiation at room temperature also gave satisfactory results (Flack parameter[16] = 0.05 (2)).

In conclusion, we have developed a new method for the determination of the absolute configuration of chiral ketones. This is the simple and direct method and the potential of this methodology was clearly demonstrated. It should be noted that 2-chloro-4,6-dinitrophenylhydrazine (**8**) is a useful crystalline agent for this purpose. This method can be applied to both of aromatic and aliphatic chiral ketones, and is helpful for the determination of the absolute configuration of various chiral ketones.

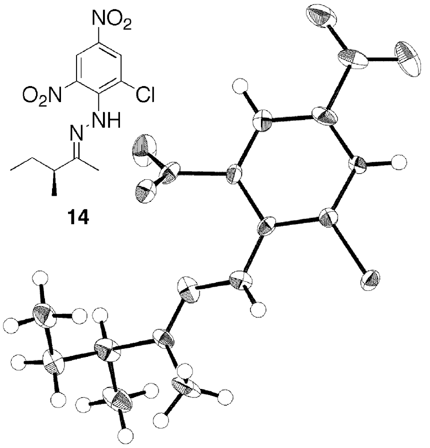


Figure 6. The ORTEP drawing of **14** with displacement ellipsoids at 50% probability level. The asymmetric unit contains two independent molecules (only one molecule is shown).

3. Experimental

3.1. General

Organic reagents and solvents were purchased from Nacalai Tesque, Inc., Wako Pure Chemical Ind., Ltd, Tokyo Kasei Kogyo Co., Ltd, and Aldrich Chemical Co. Optically active 3-methyl-2-pentanone (**11**) was supplied by Daicel Chemical Industries, Ltd. ^{1}H NMR spectra (300 MHz) were recorded on a JEOL AL-300 spectrometer on $CDCl_3$

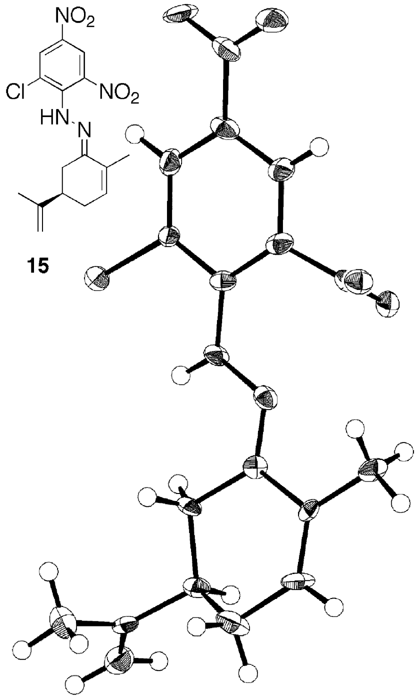


Figure 7. The ORTEP drawing of **15** with displacement ellipsoids at 50% probability level. The asymmetric unit contains two independent molecules (only one molecule is shown).

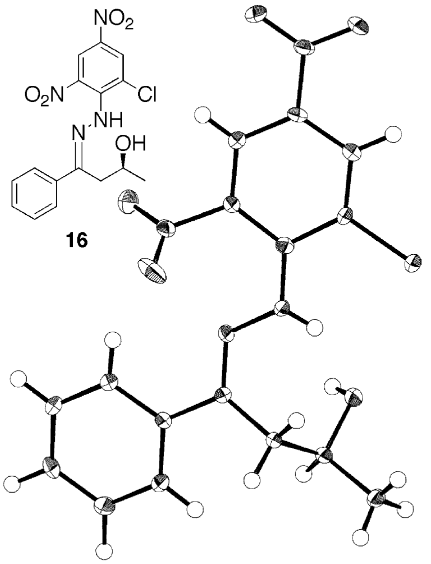


Figure 8. The ORTEP drawing of **16** with displacement ellipsoids at 50% probability level.

Table 3. Crystallographic data of hydrazone **14–16**

	14	**15**	**16**
Formula	$C_{12}H_{15}ClN_4O_4$	$C_{16}H_{17}ClN_4O_4$	$C_{16}H_{15}ClN_4O_5$
Formula weight	314.73	364.79	378.77
Crystal system	Monoclinic	Triclinic	Orthorhombic
Space group	$P2_1$ (#4)	$P1$ (#1)	$P2_12_12_1$ (#19)
a (Å)	7.090 (6)	8.146 (9)	7.0719 (5)
b (Å)	16.003 (12)	8.748 (10)	13.1074 (11)
c (Å)	12.855 (10)	12.388 (14)	17.718 (2)
α (°)		97.347 (13)	
β (°)	96.229 (11)	91.298 (10)	
γ (°)		108644 (14)	
V (Å^3)	1449.9 (17)	827.7 (16)	1642.3 (2)
Z value	4	2	4
No. observations	2813[a]	5013	3736
No. variables	379	456	295
R_1 ($F^2 > 2.0\sigma(F^2)$)	0.074[a]	0.074	0.028
wR_2 (all data)	0.184[a]	0.222	0.066
S (all data)	1.01[a]	1.24	1.00
Flack parameter	−0.1 (2) (S)	0.00 (11) (R)	−0.02 (5) (S)

[a] $F^2 > 3.0\sigma(F^2)$

with TMS as an internal reference. IR spectra were obtained on a HORIBA FT-720 spectrophotometer. Elemental analysis was performed with a Yanaco MT-5 elemental analyzer.

3.2. Preparation of hydrazine 8

3.2.1. 1-Chloro-2-iodo-3,5-dinitrobenzene (9). A slight modification of the reported method was adopted for the synthesis of this compound.[13,18] 2-Chloro-4,6-dinitroaniline (2.18 g, 10 mmol) in 27 mL of acetic acid was stirred at 70 °C until the aniline was dissolved. The solution was rapidly cooled below 20 °C. In another flask, 5.6 mL of sulfuric acid was cooled and vigorously stirred on an ice bath, sodium nitrite (0.80 g, 12 mmol) was added slowly. The mixture was heated at 70 °C until the solution was became clear, then the solution was cooled below 20 °C. With aniline solution stirring, nitrite solution was added dropwise to it keeping the temperature below 20 °C. The solution was stirred at 15–20 °C for 1 h. To the solution, potassium iodide (2.32 g, 14 mmol) dissolved in 20 mL of water was added slowly, then heated at 50 °C for 15 min. The solution was neutralized with aqueous sodium carbonate and the aqueous solution was extracted with ethyl acetate. The combined extracts were washed with aqueous sodium hydrogensulfite and brine, and dried over anhydrous magnesium sulfate. Removal of the solvent gave 1-chloro-2-iodo-3,5-dinitrobenzene quantitatively. ^1H NMR ($CDCl_3$, TMS) $\delta = 8.35$ (1H, d, $J = 2.4$ Hz), 8.49 (1H, d, $J = 2.4$ Hz). IR (KBr) 3082, 1554, 1534, 1364, 1342 cm^{-1}.

3.2.2. 2-Chloro-4,6-dinitrophenylhydrazine (8). A slight modification of the reported method was adopted for the synthesis of this compound.[14] 1-Chloro-2-iodo-3,5-dinitrobenzene (**9**, 3.28 g, 10 mmol) was dissolved in 30 mL of methanol. To the solution, hydrazine hydrate (1.03 g, 20.6 mmol) in 6 mL of methanol was added dropwise and stirred at room temperature, yellow precipitate was generated. After 28 h, the solvent and excess hydrazine were removed by suction filtration, and the residue was washed with methanol. The solid was recrystallized from ethyl acetate, 2-chloro-4,6-dinitrophenylhydrazine was obtained as yellow needles (1.36 g, 58.4%). ^1H NMR ($CDCl_3$, TMS) δ 4.08 (2H, br), 6.91 (1H, br), 8.32 (1H, d, $J = 2.4$ Hz), 8.47 (1H, d, $J = 2.4$ Hz). IR (KBr) 3353, 1654, 1540, 1508, 1324 cm^{-1}. Found: C, 30.76; H, 2.23; N, 23.86%. Calcd for $C_6H_5ClN_4O_4$: C, 30.98; H, 2.17; N, 24.09%.

3.3. Preparation of hydrazones

Method A. Phenylhydrazine derivative (0.43 mmol) was in 5 drops of sulfuric acid and 15 mL of ethanol, and heated on a boiling water bath until hydrazine was dissolved. After cooling, chiral ketone (0.43 mmol) in 2 mL of ethanol and 1 mL of water was added to the solution. After appropriate time, the solution was basified with saturated aqueous sodium carbonate and the aqueous solution was extracted with dichloromethane. The combine extracts were dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with ethyl acetate as an eluent to obtain phenylhydrazone derivative. The hydrazone was recrystallized to generate single crystals for X-ray crystallographic analysis. The yields of the isolated products, recrystallization solvents, and their spectra as well as physical data are listed below.

3.3.1. (*E*)-3-Methyl-4-(3-pyridyl)-2-butanone 2-chloro-4,6-dinitrophenylhydrazone (10a). Recrystallized from dichloromethane/ethyl acetate. Mp 146.5–147.8 °C; 64% yield; ^{1}H NMR ($CDCl_3$, TMS) δ 1.11 (3H, d, *J*=6.5 Hz), 1.96 (3H, s), 2.58–2.71 (2H, m), 2.95 (1H, dd, *J*=5.6, 13.1 Hz), 7.20–7.25 (1H, m), 7.49–7.51 (1H, m), 8.36–8.47 (4H, m); IR (KBr) 3333, 1598, 1545, 1518, 1327 cm^{-1}. Found: C, 50.71; H, 4.29; N, 18.46%. Calcd for $C_{16}H_{16}ClN_5O_4$: C, 50.87; H, 4.27; N, 18.54%.

3.3.2. (*E*)-3-Methyl-4-(4-pyridyl)-2-butanone 2-chloro-4,6-dinitrophenylhydrazone (10b). Recrystallized from dichloromethane/ethyl acetate. Mp 182.3–183.9 C; 75% yield; ^{1}H NMR ($CDCl_3$, TMS) δ 1.11 (3H, d, *J*=6.9 Hz), 1.96 (3H, s), 2.60 (1H, dd, *J*=8.4, 13.7 Hz), 2.67–2.79 (1H, m), 2.95 (1H, dd, *J*=6.0, 13.7 Hz), 7.10 7.12 (2H, m), 8.36–8.38 (2H, m), 8.46–8.51 (2H, m); IR (KBr) 3350, 1601, 1543, 1521, 1328 cm^{-1}. Found: C, 50.88; H, 4.40; N, 18.50%. Calcd for $C_{16}H_{16}ClN_5O_4$: C, 50.87; H, 4.27; N, 18.54%.

3.3.3. (*E*)-3-Methyl-2-pentanone 2-chloro-4,6-dinitrophenylhydrazone (14). Recrystallized from anisole/hexane. mp 61.2–63.3 °C; 91% yield; ^{1}H NMR ($CDCl_3$, TMS) δ 0.88 (3H, t, *J*=7.4 Hz), 1.08 (3H, d, *J*=6.8 Hz), 1.33–1.48 (1H, m), 1.52–1.66 (1H, m), 1.95 (3H, s), 2.34 (1H, sextet, *J*=6.8 Hz), 8.36 (1H, d, *J*=2.5 Hz), 8.40 (1H, br), 8.47 (1H, d, *J*=2.5 Hz); IR (KBr) 3347, 1602, 1542, 1521, 1333 cm^{-1}; HRMS *m/z* found: 314.0782. Calcd for $C_{12}H_{15}ClN_4O_4$: 314.0782.

3.3.4. (*R*)-Carvone 2-chloro-4,6-dinitrophenylhydrazone (15). Recrystallized from dichlorometane/ethanol. Mp 189.5–190.2 °C; 59% yield; ^{1}H NMR ($CDCl_3$, TMS) δ; 1.56 (3H, s), 1.81 (3H, s), 2.14–2.26 (2H, m), 2.33–2.39 (1H, m), 2.50–2.57 (1H, m), 2.75 (1H, dd, *J*=3.9, 15.3 Hz), 4.83 (1H, br), 4.88 (1H, br), 6.19 (1H, br), 8.37 (1H, d, *J*=2.5 Hz), 8.47 (1H, d, *J*=2.5 Hz), 8.69 (1H, br); IR (KBr) 3348, 1600, 1550, 1517, 1323 cm^{-1}. Found: C, 52.41; H, 4.70; N, 15.20%. Calcd for $C_{16}H_{17}ClN_4O_4$: C, 52.68; H, 4.70; N, 15.36%.

3.3.5. (*S*)-3-Hydroxy-1-phenylbutan-1-one 2-chloro-4,6-dinitrophenylhydrazone (16). Recrystallized from dichlorometane/ethanol. Mp 204.5–205.8 °C; 80% yield; ^{1}H NMR ($CDCl_3$, TMS) δ; 1.47 (3H, d, *J*=6.1 Hz), 2.30 (1H, br), 3.00 (1H, d, *J*=14.5 Hz), 3.09 (1H, dd, *J*=9.2, 14.5 Hz), 4.41–4.45 (1H, m), 7.39–7.42 (3H, m), 7.64–7.66 (2H, m), 8.34 (1H, d, *J*=2.4 Hz), 8.44 (1H, d, *J*=2.4 Hz), 10.59 (1H, br); IR (KBr) 3519, 1598, 1539, 1507, 1319 cm^{-1}. Found: C, 50.51; H, 3.70; N, 14.60%. Calcd for $C_{16}H_{15}ClN_4O_5$: C, 50.74; H, 3.99; N, 14.79%.

Method B. Phenylhydrazine derivative (0.23 mmol) was dissolved in 0.25 mL of sulfuric acid and 0.37 mL of water, then 1 mL of ethanol were added. To the solution, chiral ketone (0.23 mmol) in 1 mL of ethanol was added, and heated on boiling water for 3 min. After appropriate time, the solution was basified with 2 M sodium hydroxide and the aqueous solution was extracted with dichloromethane. The combined extracts were dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with ethyl acetate as an eluent to obtain six chloro-2.4-dinitrophenylhydrazone derivatives. The hydrazone was recrystallized to generate single crystals for X-ray crystallographic analysis. The yields of the isolated products, recrystallization solvents, and their spectra as well as physical data are listed below.

3.3.6. 3-(2-Pyridylmethyl)-2-pentanone 2-chloro-4,6-dinitrophenylhydrazone (10c)

Recrystallized from ethyl acetate. Mp 149.8–155.9 °C (dec); 31% yield; ^{1}H NMR of (*E*)- and (*Z*)-forms mixture ($CDCl_3$, TMS) δ 0.89 (3H, t, *J*=7.4 Hz), 1.03 (3H, t, *J*=7.4 Hz), 1.56–1.73 (2H×2, m), 1.81 (3H, s), 1.93 (3H, s), 2.90–3.02 (2H×2, m), 3.28 (1H, dd, *J*=4.2, 17.1 Hz), 3.76–3.88 (1H, m), 7.07–7.17 (2H×2, m), 7.55–7.64 (1H×2, m), 8.33–8.57 (3H×2+1H, m), 10.81 (1H, br); IR (KBr) 2959, 1601, 1534, 1517, 1324 cm^{-1}. Found: C, 52.13; H, 4.64; N, 17.75%. Calcd for $C_{17}H_{18}ClN_5O_4$: C, 52.11; H, 4.63; N, 17.83%.

3.3.7. (*E*)-3-(3-Pyridylmethyl)-2-pentanone 2-chloro-4,6-dinitrophenylhydrazone (10d)

Recrystallized from hexane/ethyl acetate. Mp 108.5–110.0 °C; 80% yield; ^{1}H NMR ($CDCl_3$, TMS) δ 0.89 (3H, t, *J*=7.5 Hz), 1.54–1.63 (2H, m), 1.88 (3H, s), 2.54–2.63 (1H, m), 2.77–2.82 (2H, m), 7.19–7.24 (1H, m), 7.48–7.52 (1H, m), 8.35–8.47 (5H, m); IR (KBr) 3342, 1603, 1539, 1517, 1341 cm^{-1}. Found: C, 52.05; H, 4.57; N, 17.96%. Calcd for $C_{17}H_{18}ClN_5O_4$: C, 52.11; H, 4.63; N, 17.83%.

3.3.8. (*E*)-3-(4-Pyridylmethyl)-2-pentanone 2-chloro-4,6-dinitrophenylhydrazone (10e)

Recrystallized from methanol/chloroform/ethyl acetate. Mp 129.7–129.9 °C; 86% yield; ^{1}H NMR ($CDCl_3$, TMS) δ 0.89 (3H, t, *J*=7.4 Hz), 1.53–1.60 (2H, m), 1.88 (3H, s), 2.60–2.67 (1H, m), 2.72–2.86 (2H, m), 7.09–7.12 (2H, m), 8.35–8.47 (5H, m); IR (KBr) 3342, 1603, 1539, 1517, 1340 cm^{-1}; HRMS *m/z* found: 391.1035. Calcd for $C_{17}H_{18}ClN_5O_4$: 391.1047.

3.4. Crystallographic studies

A prismatic single crystal was mounted on a glass fiber. The lattice parameters and intensity data were measured on a Rigaku R-CCD/III diffractometer and radiation was Mo Kα (λ=0.71070 Å). The data were collected at a temperature of −180 °C using the ω scan technique to a maximum 2θ value of 55.0 degree. A total of 1200 oscillation images were collected. All the structures were solved by direct method SIR97[19] and expanded Fourier techniques.[20] All non-hydrogen atoms were refined anisotropically and hydrogen atoms were refined isotropically by full-matrix least square method. All calculations were performed using CrystalClear software package of Rigaku Corporation.[21,22] The ORTEP drawings are presented in Figures 1–8, and the crystallographic parameters are listed in Tables 2 and 3.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposit with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 172429 for **10a**, CCDC 172430 for **10b**, CCDC 172427 for **10c**, CCDC 172428 for **10d**, CCDC 172432 for **10e**, CCDC 172431 for **14**, CCDC 262899 for **15**, CCDC 262900 for **16**. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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References and notes

1. (a) Riley, R. G.; Silverstein, R. M.; Moser, J. C. *Science* **1974**, *183*, 760–762. (b) Mori, K.; Masuda, S.; Suguro, T. *Tetrahedron* **1981**, *37*, 1329–1340. (c) Mori, K.; Nomi, H.; Chuman, T.; Kohno, M.; Kato, K.; Noguchi, M. *Tetrahedron* **1982**, *38*, 3705–3711. (d) Mori, K.; Ebata, T. *Tetrahedron* **1986**, *42*, 4421–4426. (e) Ebata, T.; Mori, K. *Agric. Biol. Chem.* **1987**, *51*, 2925–2928.
2. (a) Yamashita, T.; Mitsui, H.; Watanabe, H.; Nakamura, N. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 961–962. (b) Murakata, M.; Nakajima, M.; Koga, K. *J. Chem. Soc., Chem. Commun.* **1990**, 1657–1658. (c) Sonnet, P. E.; Heath, R. R. *J. Org. Chem.* **1980**, *45*, 3137–3139. (d) Evans, D. A.; Takacs, J. M. *Tetrahedron Lett.* **1980**, *21*, 4233–4236. (e) Helmchen, G.; Weirchowski, R. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 60–61. (f) Mayers, A. I.; Williams, D. R.; Erickson, G. W.; White, S.; Druelinger, M. *J. Am. Chem. Soc.* **1981**, *103*, 3081–3087. (g) Mayers, A. I.; Williams, D. R.; White, S.; Erickson, G. W. *J. Am. Chem. Soc.* **1981**, *103*, 3088–3093. (h) Tomioka, K.; Ando, K.; Takemasa, Y.; Koga, K. *J. Am. Chem. Soc.* **1984**, *106*, 2718–2719. (i) Enders, D.; Eicheauer, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 549–551. (j) Enders, D.; Eichenauer, H.; Baus, U.; Schubert, H.; Kremer, K. A. M. *Tetrahedron* **1984**, *40*, 1345–1359.
3. Fehr, C. *Angew. Chem., Int. Ed.* **1996**, *35*, 2566–2587.
4. (a) Ohta, H.; Matsumoto, K.; Tsutsumi, S.; Ihori, T. *J. Chem. Soc., Chem. Commun.* **1989**, 485–486. (b) Matsumoto, K.; Tsutsumi, S.; Ihori, T.; Ohta, H. *J. Am. Chem. Soc.* **1990**, *112*, 9614–9619. (c) Kume, Y.; Ohta, H. *Tetrahedron Lett.* **1992**, *33*, 6367–6370. (d) Sakai, T.; Matsuda, A.; Tanaka, Y.; Korenaga, T.; Ema, T. *Tetrahedron: Asymmetry* **2004**, *15*, 1929–1932.
5. (a) Leuenberger, H. G. W.; Boguth, W.; Widmer, E.; Zell, R. *Helv. Chim. Acta* **1976**, *59*, 1832–1849. (b) Kergomard, A.; Renard, M. F.; Veschambre, H. *Tetrahedron Lett.* **1978**, *52*, 5197–5200. (c) Utaka, M.; Konishi, S.; Takeda, A. *Tetrahedron Lett.* **1986**, *27*, 4737–4740. (d) Sakai, T.; Matsumoto, S.; Hidaka, S.; Imajo, N.; Tsuboi, S.; Utaka, M. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3473–3475.
6. Fujii, I.; Lerner, R. A.; Janda, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 8528–8529.
7. Sinha, S. C.; Keinan, E. *J. Am. Chem. Soc.* **1995**, *117*, 3653–3654.
8. (a) Kawai, Y.; Saitou, K.; Hida, K.; Ohno, A. *Tetrahedron: Asymmetry* **1995**, *6*, 2143–2144. (b) Kawai, Y.; Saitou, K.; Hida, K.; Dao, D. H.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2633–2638. (c) Kawai, Y.; Hayashi, M.; Tokitoh, N. *Tetrahedron: Asymmetry* **2001**, *12*, 3007–3013.
9. Kawai, Y.; Hayashi, M.; Inaba, Y.; Saitou, K.; Ohno, A. *Tetrahedron Lett.* **1998**, *39*, 5225–5228.
10. Hirata, T.; Shimoda, K.; Kawano, T. *Tetrahedron: Asymmetry* **2000**, *11*, 1063–1066.
11. Kawai, Y.; Hayashi, M.; Tokitoh, N. *Tetrahedron Lett.* **2002**, *43*, 465–467.
12. Kawai, Y.; Hayashi, M.; Tokitoh, N. *Acta Crystallogr. Sect. E* **2002**, *58*, o1098–o1099.
13. Hodgson, H. H.; Walker, J. *J. Chem. Soc.* **1933**, 1620–1621.
14. Borsche, W. *Ber.D. Chem. Gesellschaft* **1921**, *54*, 669–684.
15. Ohta, H.; Ozaki, K.; Tsuchihashi, G. *Agric. Biol. Chem.* **1986**, *50*, 2499–2502.
16. Flack, H. D. *Acta Crystallogr. Sect. A* **1983**, *39*, 876–881.
17. (a) Peerdeman, A. F.; Bijvoet, J. M. *Acta Crystallogr.* **1956**, *9*, 1012–1015. (b) Ibers, J. A.; Hamilton, W. C. *Acta Crystallogr.* **1964**, *17*, 781–782.
18. Sandin, R. B.; Cairns, T. L. In *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. II, pp 604–605.
19. Altomare, A. M. B.; Camalli, M.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
20. Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Gelder, R. D.; Israel, R.; Smits, J.M.M. *The DIRDIF-99 program system*, Technical Report of the Crystallography Laboratory.
21. Rigaku Corporation 1999, CrystalClear Software User's Guide, Molecular Structure Corporation.
22. Pflugrath, J. W. *Acta Crystallogr. Sect. D* **1999**, *D55*, 1718–1725.

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Formal total synthesis of (+)-wortmannin using catalytic asymmetric intramolecular aldol condensation reaction

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Abstract—A catalytic process for the synthesis of optically active C4-substituted tetrahydroindandiones using an asymmetric intramolecular aldol condensation reaction was developed. When 30 mol% of phenylalanine and 50 mol% of pyridinium *p*-toluenesulfonate were used under highly concentrated conditions, a variety of C4-substituted tetrahydroindandiones and octahydronaphthalenediones were obtained in high yield (up to 89% yield) and high enantiomeric excess (up to 94% ee). One of the products was successfully transformed into the key intermediate for the synthesis of the phosphatidylinositol 3-kinase inhibitor wortmannin, achieving formal total synthesis of (+)-wortmannin.

1. Introduction

Phosphatidylinositol 3-kinase (PI 3-kinase) is an important enzyme that functions in signal transduction pathways, and its inhibitors are often used as a biological tool in cell molecular biology.[1] These agents have contributed greatly to studies of intracellular signaling pathways in diabetes and cancer research. Wortmannin (**1**) was isolated from *Penicillium wortmanniii*[2] and *Myrothecium roridium*[3] as an antifungal antibiotic, and later determined to be a potent and specific covalent inhibitor of PI 3-kinase (Fig. 1).[4–6] In addition, it has potent inhibitory activity on smooth muscle light chain kinase,[7] anti-inflammatory activity comparable to that of indomethacin,[8,9] and other biological activities.[10,11]

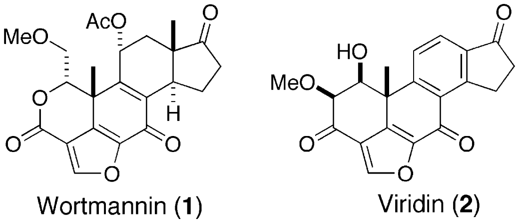
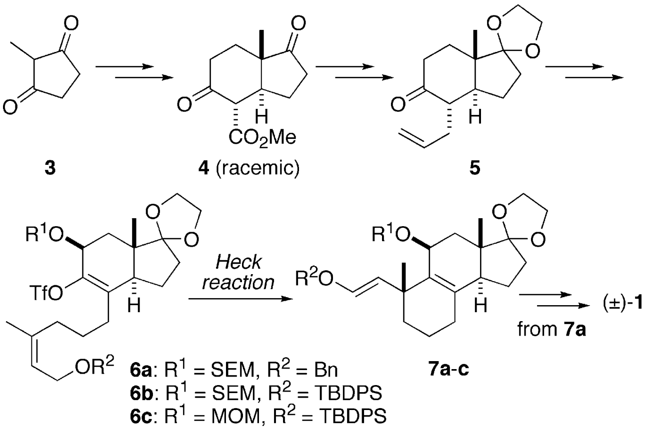


Figure 1. Structures of wortmannin (**1**) and viridin (**2**).

The challenging molecular structure of **1** (a bisallylic quaternary carbon center and a highly reactive furanocyclohexadienone lactone unit on the steroid backbone)[3,12,13] coupled with its exciting biological actions, prompted us to attempt its total synthesis. In 1996, we succeeded in the first chemical synthesis of **1** from hydrocortisone.[14] Later, the first direct total synthesis of (±)-**1** from simple cyclic diketone **3** was also achieved by our group using an intramolecular Heck reaction of **6a** and a diosphenol Claisen rearrangement as key steps (Scheme 1).[15] Recently, Sorensen and co-workers reported a racemic total synthesis of the related natural product viridin (**2**), using rhodium-catalyzed cyclotrimerization, a



Scheme 1.

Keywords: Enantioselective synthesis; (+)-Wortmannin; Intramolecular aldol condensation reaction.

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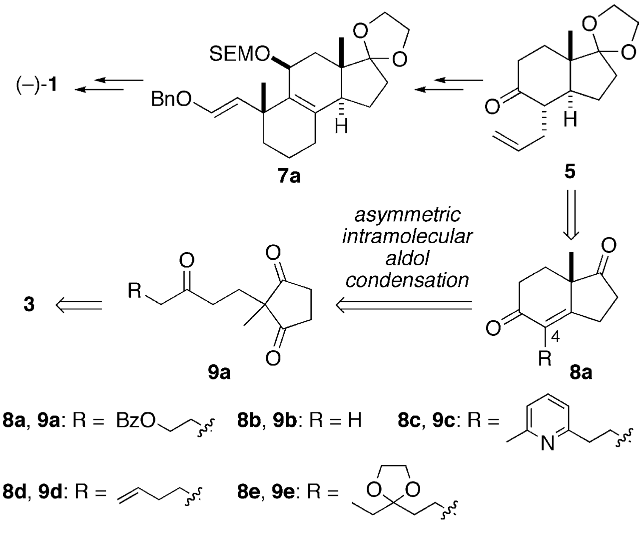
thermal electrocyclic rearrangement, and Donohoe dihydroxylation.[16]

From a medicinal viewpoint, syntheses of optically active wortmannin (**1**) as well as its derivatives, which possess more potent inhibitory activity and have less toxicity, are highly desirable for the development of new antitumor drugs. Other than our chemical transformation from (+)-hydrocortisone, asymmetric synthesis of **1** has not been reported, and a new versatile and direct method for the asymmetric synthesis of **1** is in high demand. To address this issue, we previously examined a kinetic resolution using an asymmetric Heck reaction of **6** with several chiral ligands.[17] All trials of this strategy, unfortunately, resulted in low chemical yield although the enantiomeric excess of **7c** was excellent. These unsatisfactory results[18] led us to search for a more direct route to optically active **7a**, namely an asymmetric intramolecular aldol condensation reaction of triketone **9a** to **8a** (Scheme 2).



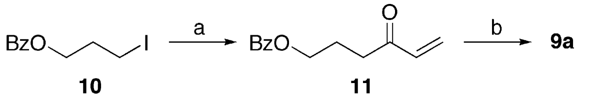
Scheme 2.

The asymmetric intramolecular aldol condensation reaction is one of the most powerful methods to synthesize the tetrahydroindandione skeleton corresponding to the steroidal C-D ring. In the early-to-mid 1970s, Hajos et al.[19] and Wiechart et al.[20] independently developed an asymmetric intramolecular aldol condensation reaction using a catalytic amount of proline to provide C4-unsubstituted tetrahydroindandione **8b** (R = H).[21] Recently, this proline-catalyzed asymmetric reaction was successfully extended to several intermolecular reactions such as the direct aldol reaction.[22] On the other hand, the use of stoichiometric amounts of amino acids is indispensable to construct C4-substituted tetrahydroindandione. Danishefsky et al. reported asymmetric synthesis of C4-substituted tetrahydroindandione **8c**, which was utilized for the total synthesis of estrone and 19-norsteroids, using 1.2 equiv of L-phenylalanine and 0.5 equiv of 1 N $HClO_4$ (82% yield, 86% ee).[23] Tsuji et al. reported similar conditions (1 equiv of L-phenylalanine and 0.4 equiv of 1 N $HClO_4$) for the synthesis of **8d** (85% yield, 76% ee), which was utilized for the total synthesis of (+)-19-nortestosterone.[24] Corey et al. also reported the asymmetric synthesis of the protostenediols synthetic intermediate **8e**, in which 50 mol% of camphorsulfonic acid was used instead of 1 N $HClO_4$ with 1 equiv of L-phenylalanine (77% yield, 95% ee).[25] These stoichiometric reactions prompted us to develop a more atom economical catalytic process for the synthesis of C4-substituted tetrahydroindandione **8**. Herein, we describe the catalytic asymmetric intramolecular aldol reaction of triketone **9** to **8** with broad substrate generality. The concentrations, additive, and sonication were very important to improve the reactivity of the reaction. Moreover, the product **8a** was successfully converted to key intermediates **5** and **7a**, achieving formal total synthesis of (+)-wortmannin.

2. Results and discussion

2.1. Catalytic asymmetric intramolecular aldol condensation reaction

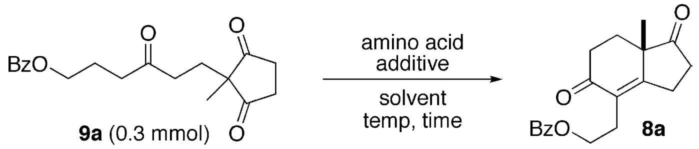
The substrate **9a** for the aldol condensation was synthesized in the following two steps (i) a palladium coupling reaction of the alkylzinc prepared from **10**[26] with acryloyl chloride, and (ii) a Michael reaction of diketone **3** to **10** (53% yield for two steps) (Scheme 3).



Scheme 3. Reagents and conditions: (a) Zn–Cu, THF, reflux, then $Pd(OAc)_2$ (1 mol%), PPh_3 (2.5 mol%), acryloyl chloride, 0 °C to rt; (b) **3**, NEt_3 (30 mol%), DMF, 0 °C to rt, 53% for two steps.

As a preliminary experiment, Danishefsky's condition was first applied to the substrate **9a** and the desired product **8a** was obtained in 47% yield and 58% ee (Table 1, Entry 1). We first screened a variety of natural and synthetic amino acids, including proline (Entry 2), and phenylalanine was best in terms of total efficiency. Next, we investigated additive effects. By changing the additive from $HClO_4$ to pyridinium *p*-toluenesulfonate (PPTS), the reactivity was greatly improved to afford **8a** in 78% yield within 10 h (Entry 3). Enantioselectivity was also improved to 75% ee. The use of other pyridinium salt derivatives of *p*-toluenesulfonic acid, such as salts of picoline, lutidine, collidine, and 2,6-di-*tert*-butyl-4-methylpyridine, did not improve the results. The effects of concentration were then examined. Highly concentrated conditions improved not only the chemical yield but also the enantioselectivity (Entries 4 to 6). The best yield was obtained under solvent free conditions (Entry 6, 90% yield, 83% ee). Although Swaminathan et al. previously reported a solvent-free asymmetric aldol condensation of **9f** (R = Me) using L-phenylalanine and *d*-camphorsulfonic acid, in their case phenylalanine did not work as a catalyst (59% yield and 79% ee using 1 equiv of phenylalanine).[27] Under this solvent free condition, however, there was some difficulty performing the reaction due to the high viscosity of the medium. The addition of 1 equiv of DMSO solved this problem and gave better selectivity (Entry 7). This optimized condition was effective for the catalytic process,

Table 1.



Entry	Amino acid (mol%)	Additive (mol%)	Solvent	Temp (°C)	Time (h)	Yield (%)	ee (%)
1	L-Phe (100)	1 N $HClO_4$ (50)	CH_3CN (0.5 M)	80	168	47	58
2	L-Pro (100)	1 N $HClO_4$ (50)	CH_3CN (0.5 M)	80	168	13	38
3	L-Phe (100)	PPTS (100)	CH_3CN (0.5 M)	80	10	78	75
4	L-Phe (100)	PPTS (100)	CH_3CN (1.0 M)	80	10	80	78
5	L-Phe (100)	PPTS (100)	CH_3CN (2.0 M)	80	10	84	80
6	L-Phe (100)	PPTS (100)	—	80	10	90	83
7	L-Phe (100)	PPTS (100)	DMSO (1 equiv)	80	10	81	85
8	L-Phe (30)	PPTS (50)	DMSO (1 equiv)	60	8	78	86
9	L-Phe (30)	PPTS (50)	DMSO (1 equiv)	50	24	55	94
10[a]	L-Phe (30)	PPTS (50)	DMSO (1 equiv)	50	24	64	94
11[a,b]	L-Phe (30)	PPTS (50)	DMSO (1 equiv)	50	24	73	94

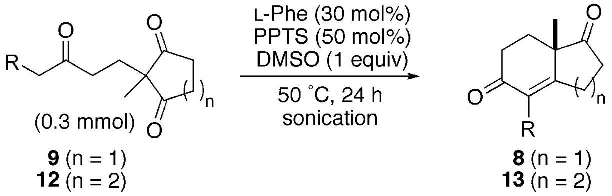
[a] Sonication was used.

[b] 58 mmol scale.

and using 30 mol% of the catalyst, **8a** was obtained in 78% yield and 86% ee (Entry 8). Moreover, the enantiomeric excess was increased to 94% at 50 °C although the chemical yield was moderate (Entry 9). The chemical yield was then improved to 64% using sonication (Entry 10). In addition, the chemical yield of **8a** was improved to 73% in large scale (58 mmol) without loss of enantiomeric excess (Entry 11). The fact that the reaction was performed under highly concentrated conditions with minimal waste makes this process desirable in terms of practicality and environmental consciousness. When L-phenylalanine was used as a catalyst, absolute configuration of the obtained product **8a** was determined to be the *S* configuration, as shown in Scheme 2, by chemical transformation to the known compound.[28]

After determining the optimized conditions, we examined the scope and limitations using a variety of triketones **9** ($n=1$) and **12** ($n=2$) (Table 2). The present catalytic asymmetric process was applicable to the synthesis of various C4-substituted tetrahydroindandiones **8** and octahydronaphthalenediones **13** (Entries 1–7). As shown in entry 5, Tsuji's synthetic intermediate **8d** for (+)-19-nortestosterone was obtained in better enantimomeric excess (90% ee using 30 mol% of phenylalanine) compared to the original result (76% ee using 100 mol% of phenylalanine).[24] Furthermore, when **9h** was used as a substrate, scale effects were again observed without loss of enantiomeric excess (Entry 7). In the cases of **9b** and **12b** (R=H), suitable substrates for the proline-catalyzed reaction to provide C4-unsubstituted products **8b** and **13b**,[19,20] very low enantiomeric excess was obtained (Entries 8 and 9).

Table 2.



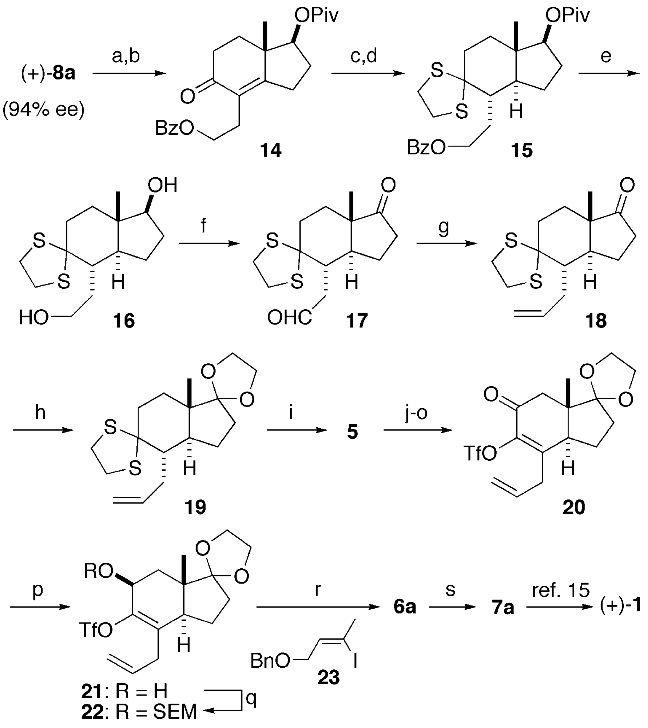
Entry	Substrate	Yield (%)	ee (%)
1	**9f**: R=Me, $n=1$	87	93[a]
2	**12f**: R=Me, $n=2$	83	87[a]
3	**9g**: R=Et, $n=1$	79	91
4	**12g**: R=Et, $n=2$	57	80
5	**9d**: $R=CH_2CH_2CH=CH_2$, $n=1$	69	90[a]
6	**9h**: $R=(CH_2)_3OBz$, $n=1$	65	91
7[b]	**9h**: $R=(CH_2)_3OBz$, $n=1$	89	90
8	**9b**: R=H, $n=1$	73	11[a]
9	**12b**: R=H, $n=2$	75	9[a]

[a] The absolute configuration was determined to be *S*.

[b] 3 mmol scale.

2.2. Formal total synthesis of (+)-wortmannin

We then focused on the transformation of tetrahydroindandione **8a** to the key intermediate of wortmannin synthesis (Scheme 4). Chemo- and stereoselective reduction of **8a** (94% ee) with $NaBH_4$ at low temperature and the following esterification of the resulting hydroxyl group with pivaloyl chloride gave **14** as a sole product. Next, we examined the conversion of **14** to *trans*-hydrindane.[29] Although Pd–C hydrogenation is a common method for this purpose, in our system the hydrogenation suffered from considerable over-reduction of the carbonyl group to methylene and low reproducibility. These problems were not overcome, even after intensive studies to examine the effects of altering temperature, solvent, and additive. The Ni boride reduction, previously utilized in a similar system by Molander et al., successfully promoted the selective reduction of enone **14** to *trans*-hydrindane in reasonable yield (ca. 50–60%).[28] After conversion of ketone to thioacetal (ca. 70–80%), the mixture of desired **15** and inseparable impurity derived from the Ni boride reduction was further purified by recrystallizaton to provide chemically and optically pure **15** (21% yield from **14**) as a white needle crystalline (mp 114–115 °C, $[\alpha]_D$ +17.9, $CHCl_3$). The reduction of **15** with $LiAlH_4$, Swern oxidation, and Wittig reaction of the resulting aldehyde using NaHMDS led to terminal alkene **18** in



Scheme 4. Reagents and conditions: (a) $NaBH_4$, EtOH, −78 °C, 92%; (b) PivCl, pyridine, DMAP (30 mol%), 0 °C to rt, 90%; (c) $NiCl_2\cdot 6H_2O$, $NaBH_4$, MeOH, −78 °C; (d) ethanedithiol, $BF_3\cdot Et_2O$ (30 mol%), CH_2Cl_2, 0 °C to rt, 21% for two steps after recrystallization (>99% ee); (e) $LiAlH_4$, THF, 0 °C to rt, 99%; (f) oxalyl chloride, DMSO, CH_2Cl_2, −78 to −40 °C, 91%; (g) CH_3PPh_3Br, NaHMDS, THF, −78 to 0 °C, 93%; (h) 2-ethyl-2-methyl-1,3-dioxolane, PTSA (10 mol%), rt, 78% (15% of **18** was recovered); (i) $PhI(CF_3CO_2)_2$, CH_3CN–H_2O, rt, 84%; (j) KHMDS, TMSCl, THF, −78 °C; (k) $KHCO_3$, mCPBA, CH_2Cl_2, −20 °C; (l) 1 N NH_4F, MeOH, 0 °C, 42% for three steps; (m) $Cu(OAc)_2$, MeOH, 0 °C to rt; (n) DBU, CH_2Cl_2, 0 °C; (o) Tf_2O, *i*-Pr_2NEt, CH_2Cl_2, −78 °C, 47% for three steps; (p) $NaBH_4$, $CeCl_3\cdot 7H_2O$, MeOH, 0 °C, 65%; (q) SEMCl, 2,6-lutidine, TBAI (10 mol%), CH_2Cl_2, 40 °C, 87%; (r) 9-BBN, THF, 0 °C then **23**, K_3PO_4, $PdCl_2(dppf)$ (3 mol%), THF–DMF, 60 °C, 64%; (s) $Pd(OAc)_2$ (10 mol%), DPPP (20 mol%), K_2CO_3, TBAB, toluene, 100 °C, 65% (α:β = 1:18).

excellent yield. When KHMDS or *n*-BuLi was used instead of NaHDMS, the chemical yield was greatly decreased because of dimer formation through the intermolecular aldol reaction. Synthesis of the reported key intermediate **5** in an optically pure manner was completed by acetal formation under mild conditions using 2-ethyl-2-methyl-1,3-dioxolane, followed by transformation of thioacetal to ketone using freshly prepared [bis(trifluoroacetoxy)iodobenzene],[30] resulting in formal total synthesis of (+)-wortmannin (**1**). Compound **5** was further converted to the more advanced intermediate **7a** by following our racemic synthesis. Compound **5** was converted to enol triflate **20** through (i) α-hydroxylation of ketone, (ii) α-keto enol formation by Cu(II) oxidation and DBU treatment, and (iii) triflation of the resulting enol.[14] The subsequent Luche reduction of **20** provided allylic alcohol **21** (α:β = ca. 1:3, 64% yield of isolated β-isomer) and the following SEM-ether formation afforded compound **22**. After hydroboration of **22** with 9-BBN, a Suzuki–Miyaura cross coupling reaction was conducted with alkyl iodide **22** to provide **6a**. Finally, an intramolecular Heck reaction of **6a** gave the optically pure tricyclic intermediate **7a** ($[\alpha]_D$ +28.0, $CHCl_3$).

3. Conclusion

A versatile asymmetric catalysis for the synthesis of C4-substituted tetrahydroindandione **8** using catalytic amounts of L-phenylalanine and PPTS was developed. Furthermore, formal total synthesis of (+)-wortmannin was achieved.

4. Experimental

4.1. General

All reactions were performed under an argon atmosphere with dry solvents, unless otherwise stated. Reagents were purified by the usual methods. Reactions were monitored using thin-layer chromatography with silica gel Merck 60 (230–400 mesh ASTM). Infrared (IR) spectra were recorded on a JASCO FT/IR 410 Fourier transform infrared spectrophotometer. NMR spectra were recorded on a JEOL JNM-LA500 spectrometer, operating at 500 MHz for ^1H NMR and 125.65 MHz for ^{13}C NMR and calibrated using residual undeuterated solvent as an internal reference. Optical rotation was measured on a JASCO P-1010 polarimeter. ESI mass spectra were measured on a Waters micromass ZQ with a Waters 2695 Separation Module (for LC-MS). HR-MS spectra were measured on a JEOL JMS-MS700V in positive ion mode.

4.2. Substrate synthesis for catalytic asymmetric intramolecular aldol condensation reaction

4.2.1. Synthesis of 9a. A suspension of **10** (50 g, 0.172 mol) and Zn–Cu (17 g, 0.259 mol) in THF (170 mL) was stirred under reflux conditions for 90 min, affording alkylzinc reagent. After cooling the reaction mixture to 0 °C, PPh_3 (386 mg, 4.31 mmol) and $Pd(OAc)_2$ (1.13 g, 1.72 mmol) were added. Acryloyl chloride (15.4 mL, 0.190 mol) was then slowly added to the mixture while maintaining the reaction temperature below 10 °C. After stirring for 90 min at room temperature, the reaction was quenched by the addition of saturated aqueous NH_4Cl solution, concentrated under reduced pressure, and extracted with AcOEt. The organic extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give crude enone **11**. The slow addition of 2-methyl-1,3-cyclopentanedione (**3**) (13 g, 0.172 mol) to a stirred solution of the crude enone **11** and NEt_3 (7.2 mL, 51.7 mmol) in DMF (180 mL) maintained the reaction temperature below 10 °C (rapid addition of **3** promoted intramolecular aldol reaction to afford racemic **9a**). After stirring for 24 h at room temperature, the reaction was quenched by the slow addition of water while maintaining the temperature below 10 °C (rapid addition of water promoted the intramolecular aldol reaction). The mixture was then extracted with AcOEt and the organic extract was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (AcOEt–Hexane = 1:3) to give **9a** (30.4 g, 53% for 2 steps) as a pale yellow oil. FT-IR (neat) ν_{max} 2962, 1718, 1681, 1616, 1601, 1584, 1452, 1402, 1315, 1274, 1176, 1115, 1070, 1026, 983, 916, 712, 687, 674 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.10 (s, 3H), 1.90 (t, 2H, J = 7.1 Hz), 1.98–2.04 (m, 2H), 2.45 (t, 2H, J = 7.1 Hz), 2.53 (t, 2H, J = 7.1 Hz), 2.71–2.87 (m, 4H), 4.30 (t, 2H, J = 6.2 Hz), 7.44 (t, 2H, J = 7.6 Hz), 7.56 (br-dt, 1H), 8.01 (br-d, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.1, 22.7, 27.8, 34.7, 36.7, 39.1, 55.1, 64.0, 128.4, 130.2, 133.0, 166.5, 208.9, 215.7; MS [ESI(+)] m/z 353 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{19}H_{23}O_5^+$ ($M+H^+$): 331.1540; Found 331.1544.

4.2.2. Synthesis of 9g. Triketone **9g** was synthesized in 65% yield by an intermolecular Michael addition of **3** to 1-hexene-3-one, similar to **9a**. Pale yellow oil; FT-IR (neat) ν_{max} 2963, 2933, 1721, 1455, 1418, 1372, 1299, 1126, 1061, 993 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (t, 3H, J = 7.2 Hz), 1.05 (s, 3H), 1.45–1.53 (m, 2H), 1.83 (t, 2H, J = 7.2 Hz), 2.28 (t, 2H, J = 7.2 Hz), 2.37 (t, 2H, J = 7.2 Hz), 2.64–2.86 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.5, 17.0, 19.0, 27.8, 34.6, 36.3, 44.7, 55.1, 210.2, 215.7; MS [ESI(+)] m/z 232 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{12}H_{19}O_3^+$ ($M+H^+$): 211.1329; Found 211.1331.

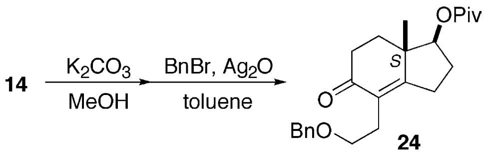
4.2.3. Synthesis of 12g. Triketone **12g** was synthesized in 73% yield by an intermolecular Michael addition of 2-methyl-1,3-cyclohexanedione to 1-hexene-3-one, similar to **9a**. Pale yellow oil; FT-IR (neat) ν_{max} 2962, 1694, 1457, 1024 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77–0.82 (m, 3H), 1.14–1.15 (m, 3H), 1.43–1.51 (m, 2H), 1.77–2.00 (m, 4H), 2.20–2.28 (m, 4H), 2.51–2.69 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.5, 17.0, 17.4, 19.6, 29.5, 37.2, 37.6, 44.5, 64.2, 209.6, 209.9; MS [ESI(+)] m/z 247 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{13}H_{21}O_3^+$ ($M+H^+$): 225.1485; Found 225.1495.

4.2.4. Synthesis of 9h. Triketone **9h** was synthesized similar to **9a**. Starting from 4-iodobutyl benzoate,[31] an I/Zn exchange reaction followed by a palladium-catalyzed coupling reaction with acryloyl chloride afforded the corresponding enone in 52% yield as a colorless oil. FT-IR (neat) ν_{max} 2954, 1717, 1682, 1276, 1116, 713 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.76–1.79 (m, 4H), 2.53–2.56 (m, 2H), 4.30–4.34 (m, 2H), 5.82 (d, 1H, J = 10.4 Hz), 6.22 (d, 1H, J = 17.7 Hz), 6.35 (dd, 1H, J = 10.4, 17.7 Hz), 7.43 (t, 2H, J = 7.4 Hz), 7.55 (t, 1H, J = 7.4 Hz), 8.03 (d, 2H, J = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.3, 28.2, 38.9, 64.5, 128.1, 128.3, 129.5, 130.3, 132.8, 136.4, 166.6, 200.2; MS [ESI(+)] m/z 255($M+Na^+$). Then, intermolecular Michael addition of **3** to the enone gave **9h** in 80% yield as a white powder. Mp 58–59 °C; FT-IR (neat) ν_{max} 2931, 1718, 1282, 710 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.10 (s, 3H), 1.65–1.77 (m, 4H), 1.90 (t, 2H, J = 7.2 Hz), 2.39–2.46 (q, 4H, J = 7.3 Hz), 2.71–2.89 (m, 4H), 4.31 (t, 2H, J = 6.3 Hz), 7.44 (t, 2H, J = 7.4 Hz), 7.56 (t, 1H, J = 7.4 Hz), 8.03 (d, 2H, J = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$)) δ 19.2, 20.0, 27.8, 28.1, 34.7, 36.5, 42.2, 55.1, 64.5, 128.3, 129.5, 130.3, 132.8, 166.6, 209.6, 215.8; MS [ESI(+)] m/z 367 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{20}H_{25}O_5^+$ ($M+H^+$): 345.1702; Found 345.1711.

Triketones **9b**,[32] **12b**,[32] **9f**,[33] **12f**,[34] and **9b**[24] are other known compounds.

4.3. Catalytic asymmetric intramolecular aldol condensation reaction

4.3.1. General procedure. A mixture of **9a** (19.0 g, 57.5 mmol), L-phenylalanine (2.84 g, 17.2 mmol), PPTS (7.2 g, 28.8 mmol), and DMSO (4.4 mL, 57.5 mmol) was stirred at room temperature for 1 min and then sonicated at 50 °C for 24 h. The reaction mixture was diluted with AcOEt, poured into saturated aqueous $NaHCO_3$ solution, and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:3) to give **8a** (11.6 g, 73%, 94% ee) as a pale yellow oil. $[\alpha]_D^{25}$ +180.8 (c 0.52, $CHCl_3$, 94% ee) FT-IR (neat) ν_{max} 2963, 1744, 1716, 1664, 1600, 1451, 1354, 1314, 1273, 1174, 1114, 1070, 1025, 713 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.23 (s, 3H), 1.83 (td, 1H, J = 6.0, 13.4 Hz), 2.06 (ddd, 1H, J = 2, 5, 13.4 Hz), 2.15–2.23 (m, 1H), 2.49–2.57 (m, 2H), 2.60–2.69 (m, 1H), 2.71–2.81 (m, 3H), 2.99 (ddd, 1H, J = 2, 13, 17.2 Hz), 4.38–4.45 (m, 2H), 7.43 (t, 2H, J = 7.6 Hz), 7.56 (br-dt, 1H), 7.97 (br-dd, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.1, 24.6, 25.4, 28.7, 32.8, 35.5, 49.0, 63.1, 128.5, 129.4, 130.0, 130.1, 133.1, 165.2, 166.4, 197.1, 217.0; MS [ESI (+)] m/z 335 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{19}H_{21}O_4^+$ ($M+H^+$): 313.1434; Found 313.1448. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AD-H, i-PrOH/Hexane 1/9, flow rate 1.0 mL/min, t_R 16.0 min for (R)-isomer and t_R 18.5 min for (S)-isomer, detected at 254 nm]. The absolute configuration of **8a** was determined to be S by transformation to the known compound **24**[28] via **14**, as shown below. $[\alpha]_D^{25}$ +11.4 (c 1.02, $CHCl_3$, 94% ee); Reported data: $[\alpha]_D^{25}$ −11.6 (c 1.03, $CHCl_3$, >99% ee) for the R isomer.



4.3.2. Data for 8g.[35] White powder; $[\alpha]_D^{23}$ +135.1 (c 1.96, $CHCl_3$, 86% ee) FT-IR (neat) ν_{max} 2963, 2672, 1711, 1666, 1606, 1460, 1422, 1358, 1336, 1306, 1262, 1239, 1202, 1161, 1143, 1114, 1065, 1015, 945, 884, 859, 832, 729 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, 3H, J = 7.4 Hz), 1.28 (s, 3H), 1.82 (dt, 1H, J = 5.8, 13.7 Hz), 2.04–2.07 (m, 1H), 2.27 (q, 2H, J = 7.4 Hz), 2.37–2.84 (m, 3H), 2.88–2.95 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.5, 18.5, 21.2, 24.0, 28.7, 32.9, 35.5, 48.7, 135.9, 162.1, 197.3, 217.7; MS [ESI(+)] m/z 215 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{12}H_{17}O_2{}^+$ ($M+H^+$): 193.1229; Found 193.1232. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK OJ-H, i-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 12.8 min for minor isomer and t_R 14.3 min for major isomer, detected at 254 nm].

4.3.3. Data for 13g. White powder; $[\alpha]_D^{23}$ +58.8 (c 1.15, $CHCl_3$, 75% ee) FT-IR (neat) ν_{max} 2966, 2932, 2872, 1744, 1664, 1448, 1371, 1354, 1311, 1260, 1173, 1120, 1058, 1009, 902, 853, 748 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90–0.94 (dt, 3H), 1.43 (s, 3H), 1.58 (d, 1H, J = 2 Hz), 1.69–1.76 (m, 1H), 2.02–2.19 (m, 3H), 2.34 (m, 2H), 2.41–2.53 (m, 3H), 2.65–2.72 (m, 1H), 2.90 (br-dt, 1H); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 14.0, 18.8, 22.3, 23.5, 26.6, 29.5, 33.6, 37.3, 50.8, 136.9, 158.0, 197.3, 212.1; MS [ESI(+)] *m/z* 229 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{13}H_{19}O_2^+$ ($M+H^+$): 207.1385; Found 207.1384. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK OJ-H, *i*-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 10.4 min for minor isomer and t_R 11.5 min for major isomer, detected at 254 nm].

4.3.4. Data for 8h. Pale yellow oil; $[\alpha]_D^{23}$ +153 (*c* 1.10, $CHCl_3$, 86% ee) FT-IR (KBr) ν_{max} 2960, 1745, 1716, 1664, 1275, 1116, 715 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.27 (s, 3H), 1.81 (dd, 1H, $J=5.8$, 13.8 Hz), 1.84–1.91 (m, 2H), 2.07 (ddd, 1H, $J=2.1$, 5.2, 13.4 Hz), 2.34–2.60 (m, 5H), 2.72 (dd, 1H, $J=2.8$, 10.8 Hz), 2.77–2.84 (m, 1H), 2.94 (dd, 1H, $J=2.8$, 17.1 Hz), 4.29 (t, 2H, $J=7.4$ Hz), 7.45 (t, 2H, $J=7.4$ Hz), 7.57 (t, 1H, $J=7.4$ Hz), 8.04 (d, 2H, $J=7.4$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.2, 21.9, 24.3, 27.7, 28.9, 32.9, 35.5, 48.9, 64.3, 128.4, 129.5, 130.2, 132.9, 133.1, 163.6, 166.5, 197.3, 217.2; MS [ESI(+)] *m/z* 335 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{20}H_{23}O_4^+$ ($M+H^+$): 327.1597; Found 327.1583. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AD-H, *i*-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 20.1 min for major isomer and t_R 22.8 min for minor isomer, detected at 254 nm].

Other products shown in Table 2 are known compounds and the absolute configuration of those compounds was determined to be *S* by comparison of optical rotation.[27]

4.3.5. HPLC analysis of 8b, 13b, 8f, 13f, and 8d. *Compound* **8b**. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AD-H, *i*-PrOH/Hexane 1:50, flow rate 1.0 mL/min, t_R 30.0 min for (*R*)-isomer and t_R 34.9 min for (*S*)-isomer, detected at 254 nm].

Compound **13b**. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK OD-H, *i*-PrOH/Hexane 1:50, flow rate 1.0 mL/min, t_R 25.8 min for (*R*)-isomer and t_R 28.6 min for (*S*)-isomer, detected at 254 nm].

Compound **8f**. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AS-H, *i*-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 20.0 min for (*R*)-isomer and t_R 28.8 min (*S*)-isomer), detected at 254 nm].

Compound **13f**. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AS-H, *i*-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 17.4 min for (*R*)-isomer and t_R 20.4 min for (*S*)-isomer, detected at 254 nm]. $[\alpha]_D^{23}$ +120.7 (*c* 0.49, $CHCl_3$, 80% ee).

Compound **8d**. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AS-H, *i*-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 15.7 min (*R*)-isomer] and t_R 22.1 min (*S*)-isomer), detected at 254 nm]. $[\alpha]_D^{23}$ +219.3 (*c* 1.67, $CHCl_3$, 90% ee).

4.4. Formal total synthesis of (+)-wortmannin

4.4.1. Synthesis of 14. $NaBH_4$ (1.00 g, 26.3 mmol) was added over 30 min to a stirred solution of **8a** (31.4 g, 0.101 mol, 94% ee) in absolute EtOH (500 mL) at −78 °C. After stirring for 1 h at the same temperature, the reaction mixture was poured into saturated aqueous NH_4Cl solution, concentrated under reduced pressure, and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated to give crude β-alcohol. DMAP (3.70 g, 30.3 mmol) and pivaloyl chloride (25 mL, 0.202 mol) were added to a stirred solution of the crude β-alcohol in pyridine (200 mL) at 0 °C. After stirring for 48 h at room temperature, the reaction mixture was poured into water and extracted with AcOEt. The organic extract was washed with water, 1 N aqueous solution of HCl and brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:3) to afford pivaloyl ester **14** (33.0 g, 82% for 2 steps) as a pale yellow oil. $[\alpha]_D^{28}$ +17.0 (*c* 2.0, $CHCl_3$, 94% ee) FT-IR (neat) ν_{max} 2973, 1722, 1665, 1601, 1479, 1453, 1420, 1396, 1375, 1355, 1314, 1274, 1154, 1114, 1069, 1027, 1008, 937, 891, 769 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.07 (s, 3H), 1.14 (s, 9H), 1.66–1.90 (m, 3H), 2.09–2.15 (m, 1H), 2.33–2.37 (m, 1H), 2.45–2.64 (m, 5H), 4.25–4.33 (m, 2H), 4.64 (dd, 1H, $J=7.6$, 10.3 Hz), 7.36 (t, 2H, $J=7.6$ Hz), 7.48 (t, 1H, $J=7.3$ Hz), 7.91 (d, 2H, $J=7.6$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.5, 25.4, 25.5, 26.2, 27.0, 32.9, 33.7, 38.7, 44.7, 62.7, 80.4, 128.2, 129.2, 130.0, 132.7, 166.1, 168.3, 177.7, 197.2; MS [ESI(+)] *m/z* 421 ($M+Na^+$); Anal. Calcd for $C_{24}H_{30}O_5$: C 72.34, H 7.59; Found C 72.05, H 7.61.

4.4.2. Synthesis of 15. $NiCl_2\cdot6H_2O$ (2.15 g, 9.03 mmol) was added to a stirred solution of **14** (720 mg, 1.81 mmol) in MeOH (18 mL) at room temperature. After $NiCl_2\cdot6H_2O$ was dissolved in MeOH, $NaBH_4$ (682 mg, 18.1 mmol) was added over 30 min at −78 °C. After stirring for 20 min at room temperature, silica gel was added to the reaction mixture, which was then filtered and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:9) to afford *trans*-hydrindane compound with inseparable byproducts. Ethanedithiol (0.10 mL, 1.21 mmol) was added to a stirred solution of the residue in CH_2Cl_2 (3.6 mL), followed by the addition of $BF_3\cdot Et_2O$ (0.076 mL, 0.606 mmol) at 0 °C. After stirring for 18 h at room temperature, the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:20) to give **15** with inseparable byproducts, which was then further purified by recrystallization (ether–hexane) to give chemically and optically pure **15** (180 mg, 21% from **14**, >99% ee) as a white needle crystalline. Mp 114–115 °C; $[\alpha]_D^{28}$ +11.4 (*c* 2.6, $CHCl_3$, >99% ee) FT-IR (KBr) ν_{max} 3421, 2970, 2921, 2851, 1723, 1654, 1601, 1583, 1478, 1455, 1421, 1395, 1364, 1334, 1314, 1282, 1164, 1123, 1071, 1024, 991, 972, 936, 898 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (s, 3H), 1.19 (s, 9H), 1.39–1.66 (m, 5H), 1.69–1.76 (m, 1H), 1.80–1.85 (m, 1H), 1.92–1.97 (m, 1H), 2.11 (td, 1H $J=3.4$, 14.4 Hz), 2.17–2.25 (m, 2H), 2.33–2.39 (m, 1H), 3.18–3.33

(m, 4H), 4.25–4.30 (m, 1H), 4.39–4.44 (m, 1H), 4.61 (dd, 1H, $J=7.7$, 9.5 Hz), 7.44 (t, 2H, $J=7.7$ Hz), 7.54–7.57 (m, 1H), 8.06–8.08 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.0, 24.9, 27.2, 27.4, 30.6, 36.0, 38.7, 38.8, 39.0, 42.2, 42.5, 42.9, 51.1, 64.9, 75.5, 81.5, 128.3, 129.6, 130.4, 132.8, 166.6, 178.4; MS [ESI(+)] *m/z* 499 ($M+Na^+$); Anal. Calcd for $C_{26}H_{36}O_4S_2$: C 65.51, H 7.61; Found C 65.23, H 7.52. The enantiomeric excess was determined by chiral stationary-phase HPLC analysis [DAICEL CHIRALPAK AD-H, *i*-PrOH/Hexane 1:9, flow rate 1.0 mL/min, t_R 8.1 min for major isomer and t_R 9.6 min for minor isomer, detected at 254 nm].

4.4.3. Synthesis of 16. Compound **15** (1.04 g, 2.19 mmol) was added to a stirred suspension of $LiAlH_4$ (311 mg, 6.56 mmol) in THF (10 mL) at 0 °C. After stirring for 90 min at room temperature, the reaction mixture was quenched by the addition of water (0.3 mL) at 0 °C, followed by 4 N aqueous solution of NaOH (0.3 mL) and water (0.9 mL), which was then filtered and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:1) to afford **16** (689 mg, 99%) as a white powder. $[\alpha]_D^{28}$ +29.4 (*c* 0.36, $CHCl_3$, >99% ee) FT-IR (KBr) ν_{max} 3292, 2920, 2876, 1637, 1445, 1428, 1383, 1353, 1278, 1241, 1205, 1160, 1135, 1033, 1018 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.57 (s, 3H), 1.24–1.30 (m, 1H), 1.38–1.59 (m, 8H), 1.92 (ddd, 1H, $J=2.3$, 6.6, 11.8 Hz), 2.03–2.24 (m, 4H), 3.15–3.35 (m, 4H), 3.51–3.57 (m, 1H), 3.66–3.73 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.9, 24.5, 30.1, 34.6, 35.7, 38.7, 38.8, 42.3, 42.4, 43.0, 51.4, 62.5, 75.9, 80.9; MS [ESI(+)] *m/z* 311 ($M+Na^+$); HR-MS [EI(+)] Calcd for $C_{14}H_{24}O_2S_2$ (M^+): 288.1218; Found 288.1230.

4.4.4. Synthesis of 17. A solution of DMSO (0.74 mL, 10.4 mmol) in CH_2Cl_2 (4.3 mL) was added to a stirred solution of oxalyl chloride (0.45 mL, 5.21 mmol) in CH_2Cl_2 (4.3 mL) at −78 °C. After stirring for 20 min at the same temperature, a solution of **16** (500 mg, 1.74 mmol) in CH_2Cl_2 (9 mL) was added to the mixture. After stirring for 1 h at −40 °C, triethylamine (2.4 mL) was added and the reaction mixture was stirred for 20 min at the same temperature. The reaction mixture was poured into saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:4) to give **17** (449 mg, 91%) as a white powder. $[\alpha]_D^{28}$ +109.8 (*c* 1.3, $CHCl_3$, >99% ee) FT-IR (KBr) ν_{max} 3443, 3416, 2997, 2919, 2883, 2858, 2841, 2716, 1710, 1473, 1462, 1427, 1398, 1377, 1351, 1336, 1305, 1281, 1262, 1243, 1225, 1206, 1161, 1130,1119, 1088, 1071, 1053, 1031, 1013 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.97 (s, 3H), 1.53–1.62 (m, 3H), 1.72–1.80 (m, 2H), 2.05–2.12 (m, 1H), 2.16–2.27 (m, 2H), 2.41–2.54 (m, 2H), 2.71–2.76 (m, 1H), 2.96 (ddd, 1H, $J=1.9$, 5, 17.4 Hz), 3.10–3.28 (m, 4H), 9.83 (t, 1H, $J=1.9$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.5, 22.9, 30.7, 35.5, 38.9, 39.0, 41.2, 41.4, 44.9, 47.6, 50.1, 74.1, 200.3, 219.7; MS [ESI(+)] *m/z* 307 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{14}H_{21}O_2S_2^+$ ($M+H^+$): 285.0977; Found 285.0990.

4.4.5. Synthesis of 18. A 1.0 M THF solution of NaHMDS (2.3 mL, 2.28 mmol) was added to a stirred suspension of Ph_3PCH_3Br (1.08 g, 3.04 mmol, dried at 100 °C for 1 h under reduced pressure prior to use) in THF (10 mL) at 0 °C. After stirring for 30 min at the same temperature, a solution of **17** (431 mg, 1.52 mmol) in THF (10 mL) was added at −78 °C, which was gradually warmed to 0 °C within 4 h. The reaction mixture was then quenched by the addition of saturated aqueous NH_4Cl at −78 °C and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:20) to give **18** (400 mg, 93%) as a white powder. $[\alpha]_D^{28}$ +111.5 (*c* 2.1, $CHCl_3$, >99% ee) FT-IR (KBr) ν_{max} 3454, 3071, 2962, 2924, 2857, 1732, 1637, 1473, 1457, 1431, 1407, 1378, 1282, 1251, 1089, 1039, 1011, 994, 923 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.93 (s, 3H), 1.57–1.65 (m, 3H), 1.69–1.73 (m, 1H), 1.99–2.12 (m, 3H), 2.15–2.25 (m, 3H), 2.37–2.44 (m, 1H), 2.75–2.79 (m, 1H), 3.15–3.34 (m, 4H), 4.95–5.08 (m, 2H), 5.84–5.92 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.6, 23.7, 30.9, 35.3, 35.8, 39.0, 39.1, 41.9, 46.2, 47.8, 51.7, 75.1, 115.2, 138.9, 219.6; MS [ESI(+)] *m/z* 305 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{15}H_{23}OS_2^+$ ($M+H^+$): 283.1185; Found 283.1179.

4.4.6. Synthesis of 19. *p*-Toluenesulfonic acid (181 mg, 0.954 mmol) was added to a stirred solution of **18** (2.70 g, 9.54 mmol) in 2-ethyl-2-methyl-1,3-dioxolane at room temperature. After stirring for 48 h at the same temperature, the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:20) to give **19** (2.43 g, 78%) as a colorless oil with recovery of **18** (398 mg, 15%). $[\alpha]_D^{21}$ +4.6 (*c* 0.70, $CHCl_3$, >99% ee) FT-IR (neat) ν_{max} 3071, 2972, 2945, 2878, 1637, 1457, 1435, 1380, 1309, 1279, 1228, 1187, 1163, 1103, 1035, 995, 957, 907, 855 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (s, 3H), 1.25–1.40 (m, 2H), 1.71–1.97 (m, 6H), 2.07–2.21 (m, 3H), 2.54 (brdd, 1H), 3.14–3.33 (m, 4H), 3.80–3.96 (m, 4H), 4.90 (d, 1H, $J=10.1$ Hz), 5.01 (d, 1H, $J=17.1$ Hz), 5.83–5.91 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 24.3, 30.0, 34.2, 36.0, 38.9, 39.0, 42.2, 46.2, 46.7, 51.0, 64.7, 65.2, 75.6, 114.5, 118.4, 139.6; MS [ESI(+)] *m/z* 349 ($M+Na^+$); HR-MS [FAB(+)] Calcd for $C_{17}H_{27}O_2S_2^+$ ($M+H^+$): 327.1447; Found 327.1449.

4.4.7. Synthesis of 5. Freshly prepared $PhI(OCOCF_3)_2$ (79 mg, 0.184 mmol) was added to a stirred solution of **19** (30.0 mg, 0.0920 mmol) in CH_3CN (0.8 mL) and H_2O (0.1 mL) at room temperature. After stirring for 1 min at the same temperature, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:9) to give **5** (19.3 mg, 84%) as a colorless oil. $[\alpha]_D^{22}$ +17.9 (*c* 1.96, $CHCl_3$, >99% ee) FT-IR (neat) ν_{max} 3078, 2975, 2878, 1708, 1434, 1308, 1177 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.13 (s, 3H), 1.37–1.45 (m, 1H), 1.52–1.56 (m, 1H), 1.73–1.80 (m, 1H), 1.83–1.89 (m, 1H), 1.92–2.02 (m, 2H), 2.03–2.09 (m, 1H), 2.21–2.44 (m, 5H), 3.81–3.92 (m, 4H), 4.93–5.02 (m, 2H), 5.80 (ddt, 1H);

^{13}C NMR (125 MHz, $CDCl_3$) δ 13.9, 23.2, 28.9, 30.9, 34.4, 37.4, 45.8, 48.2, 50.7, 64.5, 65.3, 116.1, 117.7, 136.5, 211.3; MS [EI(+)] *m/z* 250 (M^+); HR-MS [EI(+)] Calcd for $C_{15}H_{22}O_3^+$ (M^+): 250.1569; Found 250.1571.

4.4.8. Synthesis of 20. A solution of **5** (135 mg, 0.540 mmol) in THF (2.7 mL) was added dropwise to a stirred solution of potassium hexamethyldisilazide (324 mg, 1.619 mmol) in THF (2.7 mL) at −78 °C. After stirring for 30 min at the same temperature, TMSCl (0.14 mL, 1.08 mmol) was added at −78 °C. After additional stirring for 20 min at the same temperature, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution and extracted twice with Et_2O. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to give crude enol silyl ether. *m*-Chloroperbenzoic acid (219 mg, 0.756 mmol) was added by portions to a stirred suspension of the residual oil and $KHCO_3$ (270 mg, 2.70 mmol) in CH_2Cl_2 (2.7 mL) at −20 °C. After stirring for 2 h at the same temperature, the reaction mixture was diluted with CH_2Cl_2 and poured into saturated aqueous $NaHCO_3$ solution to give white precipitate. Water was added to dissolve the precipitate and extracted with CH_2Cl_2. The organic extract was washed with saturated aqueous $Na_2S_2O_3$ solution and brine, dried over Na_2SO_4, and concentrated. A mixture of the residual oil and 3 N aqueous solution of NH_4F (0.54 mL) in MeOH (2.7 mL) was stirred at 0 °C for 15 min, and then about half of the MeOH was removed under reduced pressure. The residual mixture was poured into a saturated aqueous $NaHCO_3$ solution, and extracted with EtOAc. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash silica gel column chromatography (AcOEt–hexane = 1:4) to give α-hydroxy ketone (61 mg, 42%) as a colorless oil. $Cu(OAc)_2 \cdot H_2O$ (91 mg, 0.456 mmol) was added to a solution of the α-hydroxy ketone in MeOH (1.1 mL) at 0 °C. After stirring for 15 h at room temperature, the reaction mixture was quenched by the addition of water. After additional stirring for 30 min, the mixture was extracted with CH_2Cl_2. The organic extract was successively washed with brine, 10% aqueous citric acid solution and brine, dried over Na_2SO_4, and concentrated. A solution of the residual oil and DBU (0.034 mL, 0.228 mmol) in CH_2Cl_2 (1 mL) was stirred at 0 °C for 10 min, which was then quenched by the addition of saturated aqueous NH_4Cl solution, and extracted with CH_2Cl_2. Organic extract was washed with brine, dried over Na_2SO_4, and concentrated to give crude diosphenol. Diisopropylethylamine (0.048 mL) and Tf_2O (0.038 mL) were added to a solution of the residual oil in CH_2Cl_2 (1 mL) −78 °C. After stirring for 15 min at the same temperature, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ solution and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt–hexane = 1:20) to give **20** (42 mg, 47%) as a colorless oil. $[\alpha]_D^{22}$ +38.8 (*c* 0.17, $CHCl_3$, >99% ee) FT-IR (neat) ν_{max} 1747, 1697, 1418, 1210, 1140, 1017, 865 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.99 (s, 3H), 1.71–1.80 (m, 1H), 1.93–2.12 (m, 3H), 2.45 (d, 1H, J = 16.5 Hz), 2.75 (d, 1H, J = 16.5 Hz), 2.96 (dd, 1H, J = 7.2, 13.5 Hz), 3.22 (dd, 1H, J = 7.2, 13.5 Hz), 3.32 (dd, 1H, J = 5.8, 14.3 Hz), 3.82–3.96 (m, 4H), 5.19 (d, 1H, J = 10.5 Hz), 5.20 (d, 1H, J = 16.0 Hz), 5.73 (ddt, 1H, J = 7.2, 10.5, 16.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.6, 21.2, 33.2, 33.3, 45.0, 45.9, 48.5, 64.6, 65.6, 118.5 (q, coupling with F), 119.3, 131.1, 141.3, 150.6, 190.0; MS [EI(+)] *m/z* 386 (M^+); HR-MS [EI(+)] Calcd for $C_{16}H_{20}O_6F_3S^+$ (M+): 397.0932; Found 397.0939.

4.4.9. Synthesis of 21. $CeCl_3 \cdot 7H_2O$ (699 mg) was added to a solution of **20** (620 mg, 1.56 mmol) in MeOH (10 mL) at room temperature and the mixture was stirred at the same temperature until $CeCl_3 \cdot 7H_2O$ was dissolved. Then $NaBH_4$ (77 mg, 2.03 mmol) was added to the mixture at 0 °C. After stirring for 1 h at the same temperature, the reaction mixture was quenched by the addition of acetone followed by water, and extracted with AcOEt. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (AcOEt–hexane = 5:1) to give **21** (403 mg, 65%) and the diastereomer (about 130 mg) as white solids. $[\alpha]_D^{23}$ +43.8 (*c* 0.16, $CHCl_3$, >99% ee) FT-IR (neat) ν_{max} 3471, 2974, 2889, 1408, 1209, 1141, 1039 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.06 (s, 3H), 1.57–1.66 (m, 1H), 1.81 (d, 1H, J = 14.1 Hz), 1.83–1.96 (m, 2H), 2.11 (d, 1H, J = 6.4 Hz), 2.12–2.22 (m, 2H), 2.71–2.80 (m, 2H), 3.16 (dd, 1H, J = 6.9, 14.6 Hz), 3.84–3.94 (m, 4H), 4.53 (t, 1H, J = 6.4 Hz), 5.09 (dd, 1H, J = 1.5, 10.5 Hz), 5.14 (dd, 1H, J = 1.5, 17.1 Hz), 5.68–5.76 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.2, 21.0, 32.1, 34.8, 37.0, 44.2, 46.2, 64.5, 65.4, 65.9, 117.4, 117.6, 132.8, 133.1, 143.3; MS [FAB(+)] *m/z* 381 ($M - H_2O + H^+$); HR-MS [FAB(+)] Calcd for $C_{16}H_{20}O_5F_3S^+$ (M^+): 381.983; Found 381.994.

Spectroscopic data except for optical rotation of **22**, **24**, and **7a** and experimental procedure for the syntheses of those compounds were previously reported.[15]

4.4.10. Optical rotation of 22, 24 and 7a. *Compound* **22**: $[\alpha]_D^{23}$ +83.3 (*c* 0.12, $CHCl_3$, >99% ee).

Compound **24**: $[\alpha]_D^{21}$ +4.6 (*c* 0.70, $CHCl_3$, >99% ee).

Compound **7a**: $[\alpha]_D^{22}$ +28.0 (*c* 0.10, $CHCl_3$, >99% ee, diastreomixture, $\alpha:\beta$ = 1:18).

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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.2005.03.038

References and notes

1. Hanson, J. R. *Nat. Prod. Rep.* **1995**, *12*, 381–384.
2. Brian, P. W.; Curtis, P. J.; Hemming, H. G.; Norris, G. L. F. *Trans. Br. Mycol. Soc.* **1957**, *40*, 365.
3. Petcher, T. J.; Weber, H.-P.; Kis, Z. *J. Chem. Soc., Chem. Commun.* **1972**, 1063.
4. Kanai, F.; Ito, K.; Todaka, M.; Hayashi, H.; Kamohara, S.; Ishii, K.; Okada, T.; Hazeki, O.; Ui, M.; Ebina, Y. *Biochem. Biophys. Res. Commun.* **1993**, *195*, 762–768.
5. Yano, H.; Nakanishi, S.; Kimura, K.; Hanai, M.; Saitoh, Y.; Fukui, Y.; Nonomura, Y.; Matsuda, Y. *J. Biol. Chem.* **1993**, *268*, 25846–25856.
6. Arcaro, A.; Wymann, M. P. *Biochem. J.* **1993**, *296*, 297–301.
7. Nakanishi, S.; Kakita, S.; Takahashi, K.; Kawahara, E.; Tsukada, E.; Sano, T.; Tomoyuki, Y.; Yamada, K.; Yoshida, M.; Kase, H. *J. Biol. Chem.* **1992**, *267*, 2157.
8. For anti-inflammatory activity, see: Knol, E. F.; Koenderman, L.; Mul, P. J.; Verhoeven, A. J.; Roos, D. *Eur. J. Immunol.* **1991**, *21*, 881–885.
9. Wiesinger, D.; Guvler, H. U.; Haefliger, W.; Hauser, D. *Experimentia* **1974**, *30*, 135.
10. For vasodilatory preparations, see; Nakanishi, S., Sano, T., Yamada, K., Kase, H., Kakita, S., Matsuda, Y., Japanese Patent 3215423,1991 (*Chem. Abstr.*, **1992**, *116*, 34574).
11. For block the induction of a respiratory burst during phagocytosis, see: Baggiolini, M.; Dewald, B.; Schnyder, J.; Ruch, W.; Cooper, P. H.; Payne, T. G. *Exp. Cell Res.* **1987**, *169*, 408.
12. MacMillan, J.; Simpson, T. J.; Yeboah, S. K. *J. Chem. Soc., Chem. Commun.* **1972**, 1063.
13. MacMillan, J.; Vanstone, A. E.; Yeboah, S. K. *J. Chem. Soc. Perkin* **1972**, *I*, 2898–2903.
14. Sato, S.; Nakada, M.; Shibasaki, M. *Tetrahedron Lett.* **1996**, *37*, 6141–6144.
15. Mizutani, T.; Honzawa, S.; Tosaki, S.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 4680–4682.
16. Anderson, E. A.; Alexanian, E. J.; Sorensen, E. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 1998–2001.
17. Honzawa, S.; Mizutani, T.; Shibasaki, M. *Tetrahedron Lett.* **1999**, *40*, 311–314.
18. All attempts to achieve the enantioselective synthesis of **3** using asymmetric intramolecular aldol condensation reaction were unsatisfactory in terms of isolated yield and enantioselectivity.
19. (a) Hajos, Z. G.; Parrish, D. R.; Asymmetric Synthesis of Optically Active Polycyclic Organic Compounds. German Patent DE 2102623, July 29, 1971. (b) Hajos, Z. G.; Parrish, D. R. *J. Org. Chem.* **1974**, *39*, 1615–1621.
20. (a) Eder, U.; Sauer, G.; Wiechert, R. Optically active 1,5-indanone and 1,6-naphthalenedione. German Patent DE 2014757, Oct. 7, 1971. (b) Eder, U.; Sauer, G.; Wiechert, R. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 496–497.
21. For a recent example, see: Inomata, K.; Barrague, M.; Paquette, L. A. *J. Org. Chem.* **2005**, *70*, 533–539.
22. For a general review, see: List, B. *Tetrahedron* **2002**, *58*, 5573–5590.
23. Danishefsky, S.; Cain, P. *J. Am. Chem. Soc.* **1976**, *98*, 4975–4982.
24. Shimizu, I.; Naito, Y.; Tsuji, J. *Tetrahedron Lett.* **1980**, *21*, 487–490.
25. Corey, E. J.; Virgil, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 6429–6431.
26. Using the reported procedure in Ref. 17, we unfortunately faced some reproducibility problems. Thus, in this manuscript, the reproducible value of isolated yields and experimental procedures for steps j–o are described.
27. Rajagopal, D.; Narayanan, R.; Swaminathan, S. *Proc. Indian Acad. Sci.* **2001**, *3*, 197–213.
28. Molander, G. A.; Quimbach, M. S.; Silva, L. F., Jr; Spencer, K. C.; Balsell, J. *Org. Lett.* **2001**, *3*, 2257–2260.
29. For excellent review: Jankowski, P.; Marczak, S.; Wicha, J. *Tetrahedron* **1998**, *54*, 12071–12150.
30. Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287–290.
31. Oku, A.; Harada, T.; Kita, K. *Tetrahedron Lett.* **1982**, *23*, 681–684.
32. Dauben, W. G.; Bunce, R. A. *J. Org. Chem.* **1983**, *48*, 4642–4648.
33. Banerjee, D. K.; Kasturi, T. R.; Sarkar, A. *Proc. Indian Acad. Sci.* **1983**, *92A*, 181–187.
34. Favaloro, F. G., Jr; Honda, T.; Honda, Y.; Gribble, G. W.; Suh, N.; Risingsong, R.; Sporn, M. B. *J. Med. Chem.* **2002**, *45*, 4801–4805.
35. Binda, J. S.; Neyyarapally, A. T.; Gupta, R. C.; Kamboj, V. P.; Anand, N. *J. Med. Chem.* **1975**, *18*, 921–925.

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A new method for synthesis of α,α-disubstituted carbonyl compounds from carbonyl compounds with one-carbon homologation through β-oxido carbenoid rearrangement as the key reaction

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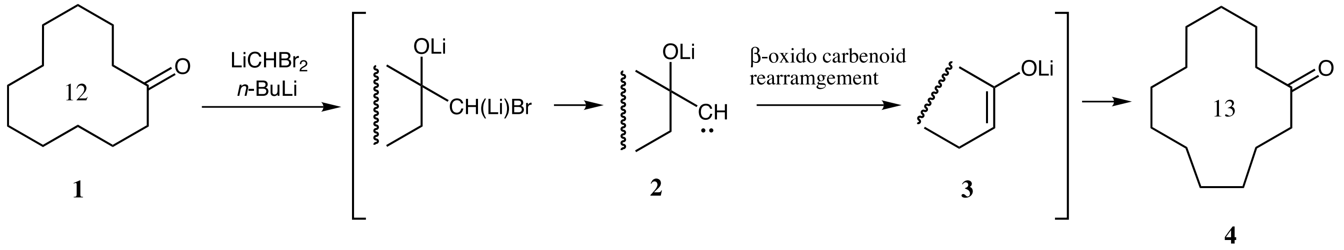
Abstract—The addition reaction of carbonyl compounds with lithium α-sulfinyl carbanions of 1-chloroalkyl *p*-tolyl sulfoxides gave adducts, α-chloro β-hydroxy sulfoxides, in high to quantitative yields. The adducts were first treated with a base to give alkoxides, which were treated with *i*-PrMgCl or *t*-BuLi to give β-oxido carbenoids via a sulfoxide–metal exchange reaction. The β-oxido carbenoid rearrangement then took place to afford the enolates with one-carbon elongation. The enolate intermediates were found to be able to be trapped with electrophiles such as aldehydes, ethyl chloroformate, benzoyl chloride, haloalkanes to give α,α-disubstituted carbonyl compounds in moderate to good yields. This method provides a new and efficient way for synthesis of α,α-disubstituted carbonyl compounds from carbonyl compounds with one-carbon homologation in only two synthetic operations.

1. Introduction

Carbonyl compounds are undoubtedly of utmost importance in organic and synthetic organic chemistry and innumerable studies have been published for their chemistry and synthesis. Homologation of carbonyl compounds from lower carbonyl compounds by carbon–carbon coupling is an important and extensively used method for preparation of the desired carbonyl compounds.[1] One-carbon ring-expansion[2] or one-carbon homologation of ketones or aldehydes via a β-oxido carbenoid is one example of the homologation and a few methods have been reported.[3–5] For example, as shown in Scheme 1, Taguchi and Nozaki reported in 1974 a one-carbon ring enlargement of cyclododecanone **1** to cyclotridecanone **4** with dibromomethyllithium through the β-oxido carbenoid **2**.[3a] This reaction was expected to proceed via an enolate **3**. Cohen and co-workers used bis(phenylthio)methyllithium as a source for the β-oxido carbenoid.[4] We used lithium α-sulfinyl carbanion of 1-chloroalkyl aryl sulfoxides as the source for the β-oxido carbenoids.[5] These reactions were all presumed to proceed via enolates (lithium enolate **3** in Taguchi and Nozaki's case).



Scheme 1.

Keywords: Sulfoxide; Sulfoxide–metal exchange; β-Oxido carbenoid; One-carbon homologation; α,α-Disubstituted carbonyl compound.

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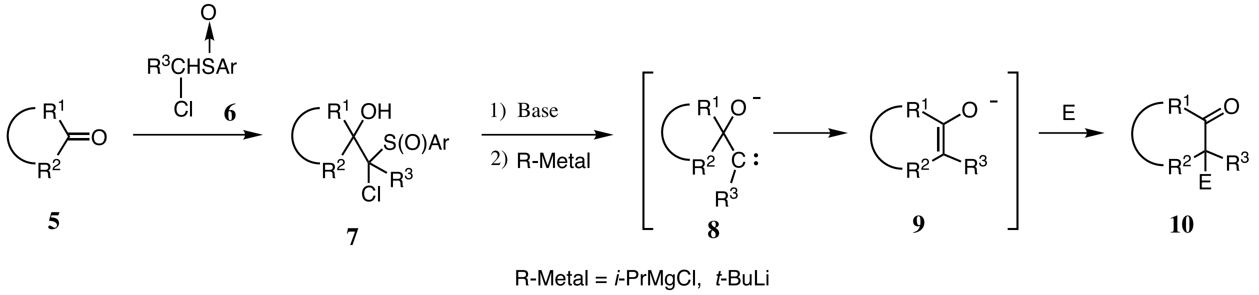


Scheme 2.

Enolates are the most useful reactive intermediates for the reaction of carbonyl compounds. If the enolates generated from the β-oxido carbenoid rearrangement mentioned above could be trapped with electrophiles, the reaction would afford a new method for synthesis of α-substituted carbonyl compounds from lower carbonyl compounds with one-carbon homologation. However, no such investigation has been reported.

Previously, we reported a new method for synthesis of α-substituted carbonyl compounds **10** (E=H) from carbonyl compounds **5** (Scheme 2).[5] Thus, addition reaction of the lithium α-sulfinyl carbanion of 1-chloroalkyl aryl sulfoxides **6** with the carbonyl compounds **5** gave the adducts **7** in high to quantitative yields. The adduct **7** was first converted to alkoxide and then treated with an alkylmetal. The sulfoxide–metal exchange reaction[6] took place to give β-oxido carbenoid **8**. The β-oxido carbenoid rearrangement of **8** proceeded to give the enolate **9**, which was quenched with H_2O to give the one-carbon homologated α-substituted carbonyl compound **10** in good yield.

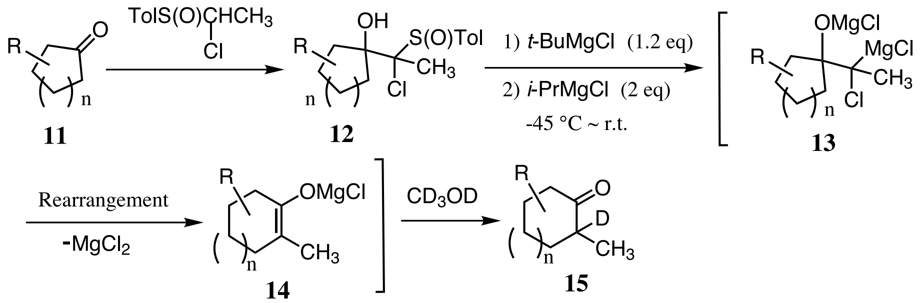
Recently, we reinvestigated our reaction. Especially, we focused our attention on trapping the enolate intermediates **9**. Namely, we thought that if we could trap the enolate intermediates **9** with electrophiles, the whole sequence was a new method for synthesis of α,α-disubstituted carbonyl compounds **10** from carbonyl compounds **5** with one-carbon homologation. This idea was investigated, and, indeed, we were able to obtain the α,α-disubstituted carbonyl compounds **10**.[7] In this paper details of the results from this study are reported.

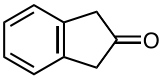
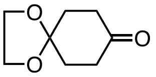
2. Results and discussion

2.1. Synthesis of α-deuterio α-methyl cyclic ketones from cyclic ketones with one-carbon ring expansion

First, we reinvestigated our one-carbon ring expansion of cyclic ketones **11** with 1-chloroethyl *p*-tolyl sulfoxide as the one-carbon homologating agent through the adducts **12** with various bases and alkylmetals, and the magnesium β-oxido carbenoids **13** were found to give better yields compared with the former conditions (LDA and *t*-BuLi).[5] The representative reaction is as follows (Table 1). To a solution

Table 1. Synthesis of 2-deuterio-2-methylcycloalkanones **15** from cycloalkanones **11** with one-carbon ring expansion



Entry	Cycloalkanone **11**	Adduct **12**	**15**		
				Yield/%	D-content/%[a]
1		**12a**	**15a**	70	93
2		**12b**	**15b**	75	95
3	Cyclooctanone	**12c**	**15c**	54	97
4	Cyclodecanone	**12d**	**15d**	52	65
5	Cyclododecanone	**12e**	**15e**	82	51
6	Cyclopentadecanone	**12f**	**15f**	78	88

[a] The deuterium incorporation was measured by ^1H NMR.

of the adduct **12**, which was synthesized from ketone **11** and lithium α-sulfinyl carbanion of 1-chloroethyl *p*-tolyl sulfoxide at −70 °C in almost quantitative yield, in THF at −45 °C was added *t*-BuMgCl (1.2 equiv) and the reaction mixture was stirred for 10 min. By this treatment, magnesium alkoxide was formed. To this solution was added 2 equiv of *i*-PrMgCl and the temperature of the reaction mixture was allowed to warm to room temperature. The sulfoxide–magnesium exchange reaction[6] took place to afford the magnesium carbenoid **13**. The β-oxido carbenoid rearrangement of the magnesium carbenoid **13** proceeded to afford the magnesium enolate **14** with concomitant elimination of magnesium chloride. Finally, the reaction was quenched by adding excess CD_3OD to give 2-deuterio-2-methylcycloalkanone **15** in good chemical yield. The study was carried out by using six kinds of cyclic ketones and the results are summarized in Table 1.

As shown in Table 1, the adducts **12** derived from 5-membered ketone to 15-membered ketone gave the 2-methylcycloalkanones with one-carbon expansion in 52–82% chemical yields. The results for the quenching of this reaction with CD_3OD are quite interesting. In the cases in entries 1–3 and 6, 2-deuterated cyclic ketones **15** in good to high deuterium incorporation were obtained. No regioisomer was obtained in these cases. From these results it was anticipated that when this reaction is quenched with some electrophiles, 2,2-disubstituted ketones could be obtained. In contrast to these results, the reaction shown in entries 4 and 5 did not give satisfactory deuterium incorporation. The results were found to be reproducible and not to be experimental error. We investigated the proton source for these reactions; however, at this time the real reason for this low deuterium incorporation is obscure.

2.2. Synthesis of 2-methyl-2-(substituted)cycloalkanones 16 and enol carbonates 17 from cycloalkanones with one-carbon ring expansion

With new and efficient method for the formation of enolates with one-carbon ring expansion in hand, we next tried to trap the enolates with aldehydes, benzoyl chloride, and ethyl chloroformate, and the results are summarized in Table 2.

We selected three adducts **12a**, **12b** and **12f** (see Table 1), because, as mentioned above, these adducts gave the deuterated and expanded cycloalkanones in good yields with high deuterium incorporation. First, **12** was treated with 1.2 equiv of *t*-BuMgCl at −45 °C followed by *i*-PrMgCl (2 equiv) and the reaction mixture was slowly allowed to warm to room temperature. To this reaction mixture was added the electrophiles (2 equiv) and the reaction mixture was stirred at room temperature for 30 min. Propionaldehyde and benzaldehyde reacted with the enolate intermediates to give the adducts **16a**, **16b**, **16c** and **16e** (entries 1, 3, 4, and 7); however, the yields were found to be somewhat variable. The reaction with acetone did not give any adduct and only the corresponding protonated ketones (**16**: E = H) were obtained.

Benzoyl chloride was also found to be effective in this procedure to give the benzoylated product in up to 71% yield (entries 6 and 9). The reaction with ethyl chloroformate needs some comment. In the case of the reaction with **12a** and **12b**, prepared from 5- and 6-membered ketones, the products were found to be the enol carbonates **17a** and **17b** (entries 2 and 5). No expected ketones having an ethoxycarbonyl group at the α-position were obtained. However, the reaction with **12f** prepared from 15-membered ketone gave the 16-membered ketone having an ethoxycarbonyl group at the α-position **16f**, though the yield was somewhat low (entry 8). The enol carbonates and α-ethoxycarbonylated ketones were easily distinguished from their IR spectrum. The carbonyl absorption of the enol carbonates appears at about 1750–1760 cm^{-1}.

The results in entries 1 and 2 are interesting. If one treats 2-tetralone with a base followed by some electrophiles, the electrophiles must be substituted on the carbon between the carbonyl carbon and the phenyl ring, because the hydrogen on the carbon is more acidic than the hydrogen on the carbon present on the other side of the carbonyl carbon. Compounds **16a** and **17a** in entries 1 and 2 are fairly difficult to synthesize from 2-tetralone by other methods.

2.3. Synthesis of 2-ethyl- and 2-(4-phenylbutyl)-2-(substituted)cycloheptanones from cyclohexane-1,4-dione *mono*-ethylene ketal with one-carbon ring expansion

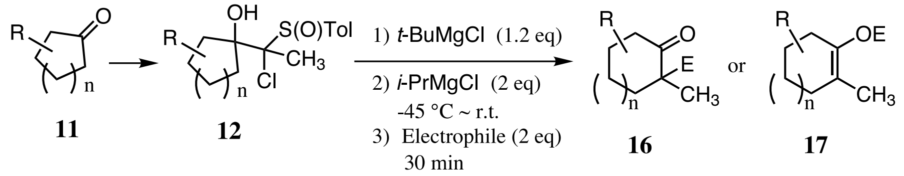
Encouraged by the results described above, we investigated the reaction with 1-chloroalkyl *p*-tolyl sulfoxides having a longer carbon chain other than the methyl group (Scheme 3). The adduct **19**, derived from 1,4-cyclohexanedione *mono*-ethylene ketal **18** and 1-chloropropyl *p*-tolyl sulfoxide in quantitative yield, was treated with *i*-PrMgCl under the conditions described in Table 2. However, we obtained the desired product **20** (35%) with an allylic alcohol **21** (65%) as a main product.

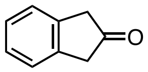
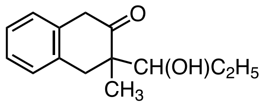
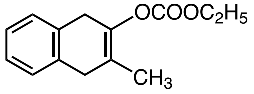
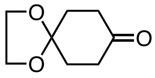
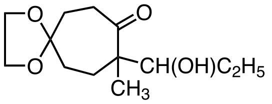
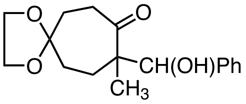
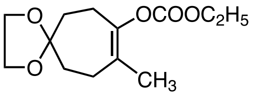
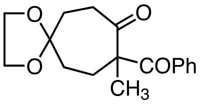
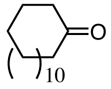
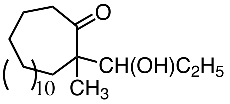
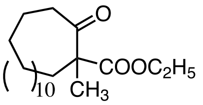
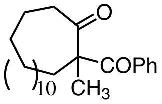
This result implied that when the magnesium carbenoid has a β-hydrogen, the hydrogen migration to give an olefin is a faster reaction than the β-oxido carbenoid rearrangement. Therefore, we applied our former conditions (LDA-*t*-BuLi)[5] to **19** and the desired product **20** was obtained in 64% yield without the allylic alcohol **21**.

By using the former conditions,[5] the effect of the alkyl substituents in this procedure was investigated and the results are summarized in Table 3. The adduct **19** having an ethyl group and the adduct **22** having a 4-phenylbutyl group as R were used. As shown in Table 3, entries 1–4, 2,2-disubstituted cycloheptanones having an ethyl group were obtained from 38–64% yields, which indicated the effectiveness of this procedure. However, comparing these results (entries 2–4) with those in Table 2, entries 3–6, the yields are somewhat lower. In these cases, the reaction with ethyl chloroformate gave the ketones having an ethoxycarbonyl group at the α-position (**23d** and **23g**) without the enol carbonates.

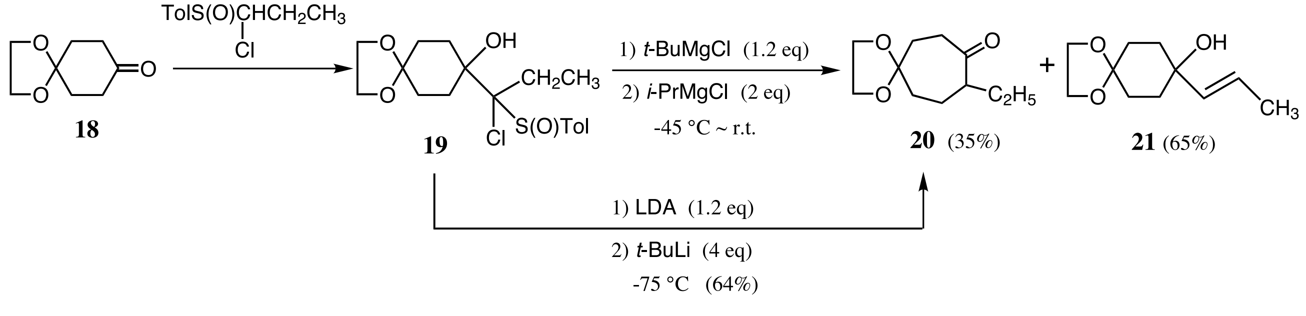
Entries 6 and 7 in Table 3 show that the yield of one-carbon expanded 2,2-disubstituted cycloheptanones having a 4-phenylbutyl group was markedly diminished. These results could be explained by the steric hindrance of the 4-phenylbutyl group.

Table 2. Synthesis of 2-methyl-2-(substituted)cycloalkanones **16** and enol carbonates **17** from cycloalkanones **11** with one-carbon ring expansion



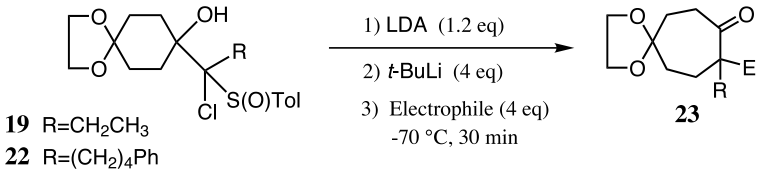
Entry	Cycloalkanone **11**	Electrophile	Product		Yield/%
1		CH_3CH_2CHO	**16a**		38[a]
2		$ClCOOC_2H_5$	**17a**		40
3		CH_3CH_2CHO	**16b**		71[a]
4		PhCHO	**16c**		64[a]
5		$ClCOOC_2H_5$	**17b**		74
6		PhCOCl	**16d**		63
7		CH_3CH_2CHO	**16e**		73[a]
8		$ClCOOC_2H_5$	**16f**		33
9		PhCOCl	**16g**		71

[a] A single isomer.

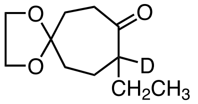
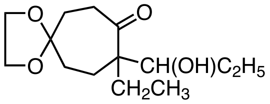
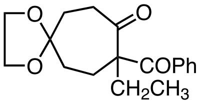
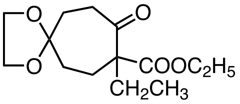
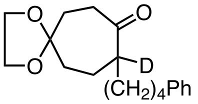
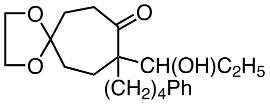
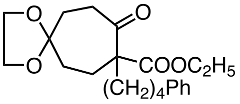


Scheme 3.

Table 3. Synthesis of 2-alkyl-2-(substituted)cycloalkanones **23** from the adduct **19** and **22** with one-carbon ring expansion



19 R=CH_2CH_3
22 R=$(CH_2)_4Ph$
23

Entry	**19** or **22** (R)	Electrophile	**23**		Yield/%
1	CH_3CH_2	CD_3OD	**23a**		64 (D=94%)
2	CH_3CH_2	CH_3CH_2CHO	**23b**		61[a]
3	CH_3CH_2	PhCOCl	**23c**		38
4	CH_3CH_2	$ClCOOC_2H_5$	**23d**		46
5	$Ph(CH_2)_4$	CD_3OD	**23e**		66 (D=94%)
6	$Ph(CH_2)_4$	CH_3CH_2CHO	**23f**		13[b]
7	$Ph(CH_2)_4$	$ClCOOC_2H_5$	**23g**		44

[a] About 4:1 mixture of two diastereomers.
[b] A single isomer.

2.4. Synthesis of 2,2-dialkylcycloheptanones from cyclohexane-1,4-dione *mono*-ethylene ketal with one-carbon ring expansion

Treatment of enolates with haloalkanes is the most well-known procedure for the alkylation of carbonyl compounds at the α-position. We investigated the trapping of the enolate intermediates mentioned above with haloalkanes and the results are summarized in Table 4.

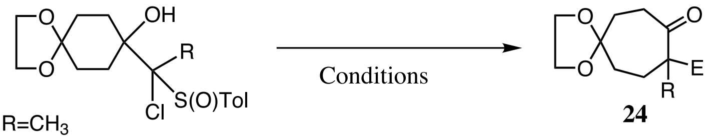
The representative procedure is as follows (Table 4, entry 1). The adduct **12b** in THF at −45 °C was treated with *t*-BuMgCl followed by *i*-PrMgCl and the temperature of the reaction mixture was allowed to warm to room temperature. HMPA (4 equiv)[8] followed by iodomethane (4 equiv) was added to the reaction mixture and the reaction mixture was stirred at room temperature overnight to give the desired 2,2-dimethylcycloheptanone **24a** in 73% yield (condition A). When this reaction was carried out without HMPA, almost no **24a** was obtained. Benzyl bromide and allyl iodide reacted with the enolate intermediate to give the desired 2,2-dialkylated cyclohepanones (**24b** and **24c**) in good yields. However, this reaction with iodobutane did not afford any desired alkylated product.

In the case of the adduct having an ethyl group (**19**), the enolate was generated with LDA-*t*-BuLi as mentioned in Scheme 3 and Table 3 (condition B). The trapping of the enolate intermediate was tried with iodomethane and allylic iodide; however, the yields of the desired products were not satisfactory (entries 4 and 5).

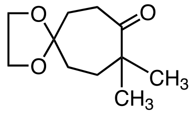
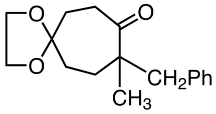
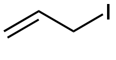
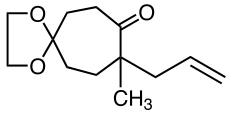
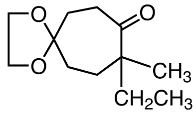
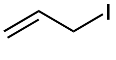
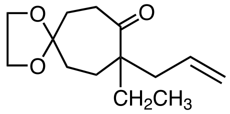
2.5. Extension of this procedure to aldehydes

Next, this procedure was extended to aldehydes. The addition reaction of 1-chlorobutyl *p*-tolyl sulfoxide to *p*-anisaldehyde **25a** and 3-phenylpropanal **25b** afforded two adducts **26** and **27** in quantitative yields (see Table 5).[5] Previously, we treated these adducts with LDA followed by *t*-BuLi and found that both **26a** and **26b** gave ketones **28a** and **28b** stereospecifically in high yields (Table 5; entries 1 and 3). Interestingly, the treatment of **27a** gave the aldehyde **29a** in good yield

Table 4. Synthesis of 2,2-dialkylcycloalkanones **24** from the adduct **12b** and **19** with one-carbon ring expansion



12b R=CH_3
19 R=CH_2CH_3
24

Entry	**12b** or **19** (R)	Haloalkane	Conditions	**24**		Yield/%
1	CH_3	CH_3I	A	**24a**		73
2	CH_3	$PhCH_2Br$	A	**24b**		75
3	CH_3		A	**24c**		59
4	CH_3CH_2	CH_3I	B	**24d**		38
5	CH_3CH_2		B	**24e**		29

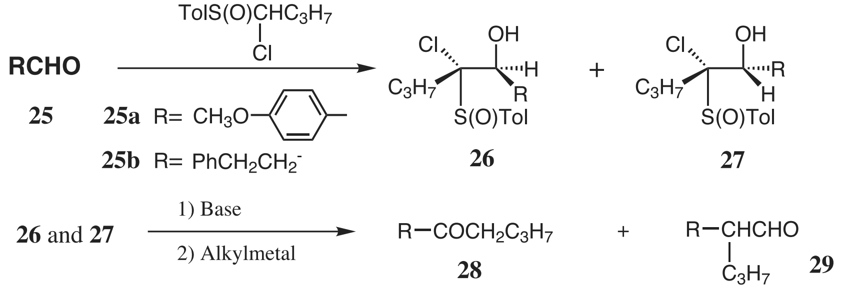
Conditions: **A**: *t*-BuMgCl (1.2 equiv), *i*-PrMgCl (2 equiv), −45 °C ~ rt, then HMPA (4 equiv) and haloalkane (4 equiv), stirred at room temperature overnight. **B**: LDA (1.2 equiv), *t*-BuLi (4 equiv), −70 °C then HMPA (4 equiv) and haloalkane (4 equiv), stirred at −70 °C for 30 min.

with high stereospecificity (entry 5). The treatment of **27b** gave mainly ketone **28b** with aldehyde **29b** (entry 7).[5]

We reinvestigated this reaction with *t*-BuMgCl followed by *i*-PrMgCl (under the conditions shown in Table 1). The results are summarized in Table 5, entries 2, 4, 6 and 8. As shown in entries 1–4, the reaction with **26a** and **26b** was better conducted with *i*-PrMgCl than with *t*-BuLi (compare entries 1 and 3 with entries 2 and 4). In the cases of the reaction with the diastereomers **27a** and **27b**, the yields and the specificity were found to be variable (entries 5–8).

We tried to trap the enolate intermediate of the reaction of

Table 5. Treatment of the adducts **26** and **27** derived from aldehydes with *t*-BuLi and *i*-PrMgCl



Entry	Adduct	Base	Alkylmetal	Product (**28** and **29**)	
				Yield/%	**28**:**29**
1	**26a**	LDA (1.2 equiv)	*t*-BuLi (4 equiv)	80	99:1
2	**26a**	*t*-BuMgCl (1.2 equiv)	*i*-PrMgCl (2 equiv)	99	99:1
3	**26b**	LDA (1.2 equiv)	*t*-BuLi (4 equiv)	89	96:4
4	**26b**	*t*-BuMgCl (1.2 equiv)	*i*-PrMgCl (2 equiv)	88	99:1
5	**27a**	LDA (1.2 equiv)	*t*-BuLi (4 equiv)	76	1:99
6	**27a**	*t*-BuMgCl (1.2 equiv)	*i*-PrMgCl (2 equiv)	67	31:69
7	**27b**	LDA (1.2 equiv)	*t*-BuLi (4 equiv)	89	77:23
8	**27b**	*t*-BuMgCl (1.2 equiv)	*i*-PrMgCl (2 equiv)	50	96:4

26a, **27a**, and **26b** with CD_3OD, propionaldehyde, and ethyl chloroformate and the results are summarized in Table 6. Considerably good results were obtained from the trial with **26a** and α,α-disubstituted ketones were produced from 67 to 80% (entries 1–5). Again, the product in this reaction with ethyl chloroformate was found to be the enol carbonate **30** (entries 4 and 5). The same results were obtained with *t*-BuLi (condition A) and *i*-PrMgCl (condition B) (entries 2, 3 and 4, 5).

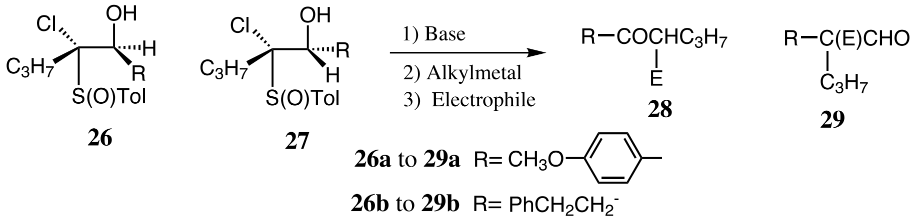
In contrast to these results, although quenching of the intermediate of the reaction of **27a** with CD_3OD gave the deuterated aldehyde **29b** (entry 6), the reaction with propionaldehyde did not give the desired adduct but instead protonated aldehyde **29a** (E=H) in 59% yield (entry 7). The reaction of **26b** under the conditions A and B was carried out and the enolate intermediate was trapped with propionaldehyde (entries 8 and 9). These reactions gave the desired adduct **28c** and it was found that the magnesium carbenoid gave much better yield (entry 9).

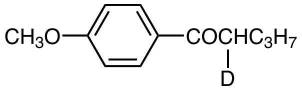
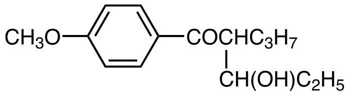
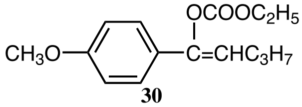
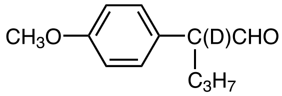
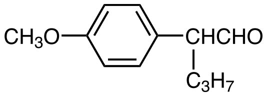
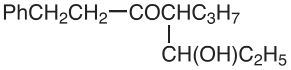
Finally, trapping the enolate intermediate from the adducts **26a** and **26b** with haloalkanes was investigated and the results are summarized in Table 7. As mentioned above in Table 4, alkylation of the enolate intermediates with haloalkanes was carried out better with magnesium enolate than lithium enolate. The adducts **26a** and **26b** were treated with *t*-BuMgCl followed by *i*-PrMgCl and the reaction mixture was allowed to warm to room temperature. To the reaction mixture was added HMPA followed by iodomethane, benzyl bromide, and allyl iodide. As shown in Table 7 these reactions gave the desired α-alkylated ketones **31** in good to quantitative yields.

2.6. Synthesis of silyl enol ethers from carbonyl compounds with one-carbon elongation

Silyl enol ethers are important compounds in organic synthesis.[9] We tried to trap the enolate intermediates with chlorotrialkylsilane and the results are shown in Scheme 4. At first, the adduct **12b** was treated with *t*-BuMgCl followed by *i*-PrMgCl and the generated enolate was treated with

Table 6. Synthesis of α-substituted ketones **28** and α-substituted aldehydes **29** from aldehydes **25** through the adducts **26** and **27** with one-carbon elongation



Entry	Adduct	Conditions[a]	Electrophile	Product	Yield/%
1	**26a**	A	CD_3OD	 **28a**	80 (D=95%)
2	**26a**	A	CH_3CH_2CHO	 **28b**	75[b]
3	**26a**	B	CH_3CH_2CHO	**28b**	75[b]
4	**26a**	A	$ClCOOC_2H_5$	 **30**	67[c]
5	**26a**	B	$ClCOOC_2H_5$	**30**	71[c]
6	**27a**	A	CD_3OD	 **29b**	76 (D=82%)
7	**27a**	A	CH_3CH_2CHO	 **29a**	59
8	**26b**	A	CH_3CH_2CHO	 $PhCH_2CH_2{-}CO\underset{CH(OH)C_2H_5}{CH}C_3H_7$	54[d]
9	**26b**	B	CH_3CH_2CHO	**28c**	83[d]

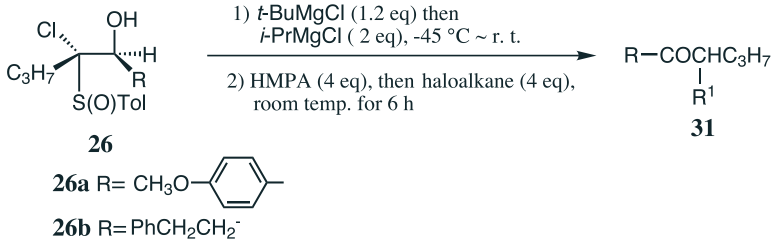
[a] Conditions: A: LDA (1.2 equiv), *t*-BuLi (4 equiv), −70 °C, then electrophile (4 equiv) stirred at −70 °C for 30 min. B: *t*-BuMgCl (1.2 equiv), *i*-PrMgCl (2 equiv), -45 °C∼ rt, then electrophile (4 equiv) stirred at room temperature for 30 min.

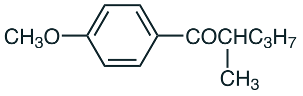
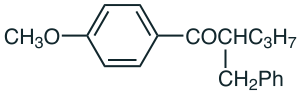
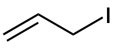
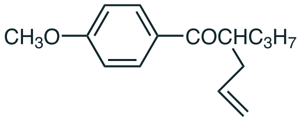
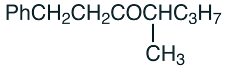
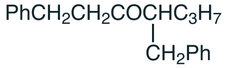
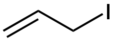
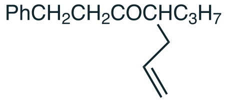
[b] A mixture of two diastereomers (2:1).

[c] A single isomer.

[d] About 1:1 mixture of two diastereomers.

Table 7. Synthesis of α-substituted ketones **31** from the adducts **26a** and **26b**

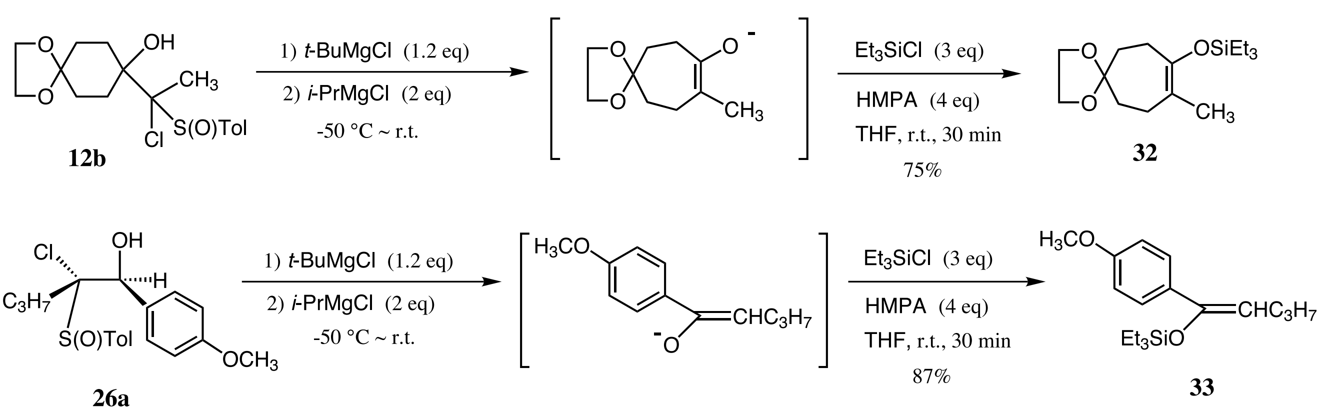


Entry	Adduct	Haloalkane	Product	Yield/%
1	**26a**	CH_3I	 **31a**	99
2	**26a**	$PhCH_2Br$[a]	 **31b**	85
3	**26a**		 **31c**	96
4	**26b**	CH_3I	 $PhCH_2CH_2COCH(CH_3)C_3H_7$ **31d**	85
5	**26b**	$PhCH_2Br$[a]	 $PhCH_2CH_2COCH(CH_2Ph)C_3H_7$ **31e**	73
6	**26b**		 **31f**	82

[a] The reaction mixture was stirred at room temperature for 19 h.

Me_3SiCl. This treatment gave the desired trimethylsilyl enol ether; however, as it was unstable on silica gel, we could not isolate it. After some investigation, the enolate intermediate was treated with Et_3SiCl in the presence of HMPA to give the desired triethylsilyl enol ether **32** in 75% yield. When this reaction was carried out without HMPA, the yield of **32** was reduced to 54%. As a whole, the silyl enol ether **32** was synthesized from cyclohexane-1,4-dione *mono*-ethylene ketal **18** in two steps in over 70% yield. From the adduct **26a** the silyl enol ether **33** was obtained in 87% yield as a single isomer.

In conclusion, we were able to trap the enolate intermediate of the β-oxido carbenoid rearrangement with several



Scheme 4.

electrophiles to give α,α-disubstituted ketones and triethylsilyl enol ethers in moderate to high yields. These reactions presented herein are a new and efficient method for the synthesis of α,α-disubstituted ketones from carbonyl compounds with one-carbon homologation in only two synthetic operations.

3. Experimental

3.1. General

All melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured in a $CDCl_3$ solution with JOEL JNM-LA 300, 500 and 600 spectrometer. Electron-impact mass spectra (MS) were obtained at 70 eV by direct insertion. Silica gel 60 (Merck) containing 0.5% fluorescence reagent 254 and a quartz column were used for column chromatography and the products having UV absorption were detected by UV irradiation. In experiments requiring a dry reagent and solvent, diisopropylamine was distilled from CaH_2 and THF was distilled from diphenylketyl. Acetone and HMPA were dried over $CaSO_4$ and distilled before use.

3.1.1. 1-[1-Chloro-1-(*p*-tolylsulfinyl)ethyl]-1-cyclodecanol (12d). To a solution of LDA (1.8 mmol) in 3 ml of dry THF in a flame-dried flask at −70 °C under argon atmosphere was added a solution of 1-chloroethyl *p*-tolyl sulfoxide (304 mg; 1.5 mmol) in 4 ml of dry THF dropwise with stirring and the solution was stirred at −70 °C for 10 min. To the reaction mixture was added cyclodecanone (278 mg; 1.8 mmol) dropwise with stirring. The reaction mixture was stirred at −70 °C for 30 min. The reaction was quenched with sat. aq. NH_4Cl. The whole was extracted with $CHCl_3$. The organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel column chromatography to afford **12d** (187 mg; 35%) as colorless crystals; mp 103–104 °C (AcOEt–hexane); IR (KBr) 3368 (OH), 2934, 1483, 1413, 1043 (SO), 1015, 809 cm^{-1}; ^{1}H NMR δ 1.46 (3H, s), 1.50–2.01 (16H, m), 2.16 (1H, dt, J=15.3, 6.7 Hz), 2.36 (1H, dt, J=15.3, 6.7 Hz), 2.44 (3H, s), 4.10 (1H, s), 7.34 (2H, d, J=8.3 Hz), 7.60 (2H, d, J=8.3 Hz). MS *m/z* (%) 356 (M$^+$, 0.1), 181 (11), 140 (100), 139 (32), 104 (40), 91 (73). Calcd for $C_{19}H_{29}ClO_2S$: M, 356.1577. Found: *m/z* 356.1569. Anal. Calcd for $C_{19}H_{29}ClO_2S$: C, 63.93; H, 8.19; Cl, 9.93; S, 8.98. Found: C, 63.95; H, 8.26; Cl, 9.89; S, 8.98.

3.1.2. 1-[1-Chloro-1-(*p*-tolylsulfinyl)ethyl]-1-cyclopentadecanol (12f). Colorless crystals; mp 113–114 °C ($CHCl_3$–hexane); IR (KBr) 3395 (OH), 2928, 2857, 1457, 1411, 1039 (SO), 815 cm^{-1}; ^{1}H NMR δ 1.25–1.50 (23H, m), 1.52 (3H, s), 1.63–1.69 (2H, m), 1.82–1.89 (1H, m), 2.12–2.18 (1H, m), 2.26–2.32 (1H, m), 2.44 (3H, s), 4.07 (1H, s), 7.34 (2H, d, J=7.9 Hz), 7.67 (2H, d, J=7.9 Hz). MS *m/z* (%) 426 (M$^+$, 0.1), 286 (68), 251 (47), 225 (33), 140 (100), 104 (39), 92 (84). Calcd for $C_{24}H_{39}ClO_2S$: M, 426.2359. Found: *m/z* 426.2345. Anal. Calcd for $C_{24}H_{39}ClO_2S$: C, 67.50; H, 9.20; Cl, 8.30; S, 7.51. Found: C, 67.48; H, 9.25; Cl, 8.30; S, 7.52.

The chloroalcohols **12a**,[10] **12b**,[10] **12c**,[5] and **12e**[5] have been reported before.

3.1.3. 3-Deuterio-3-methyl-2-tetralone (15a). To a solution of *t*-BuMgCl (0.36 mmol) in 3 ml of dry THF in a flame-dried flask at −45 °C under argon atmosphere was added a solution of the adduct **12a** (101 mg; 0.3 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min. To a solution of the magnesium alkoxide was added *i*-PrMgCl (0.6 mmol) dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min and slowly allowed to warm to room temperature for 2.5 h. The reaction was quenched with excess CD_3OD. The whole was extracted with $CHCl_3$ and the organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **15a** (33 mg; 70%) as a colorless oil; IR (neat) 2966, 2932, 1714 (CO), 1492, 1456, 1120, 747 cm^{-1}; ^{1}H NMR δ 1.19 (3H, s), 2.83 (1H, d, J=15.3 Hz), 3.08 (1H, d, J=15.3 Hz), 3.61 (2H, s), 7.12 (1H, m), 7.18–7.22 (3H, m). MS *m/z* (%) 161 (M$^+$, 47), 160 (67), 131 (24), 117 (61), 104 (100), 91 (17), 78 (13). Calcd for $C_{11}H_{11}DO$: M, 161.0949. Found: *m/z* 161.0948.

3.1.4. 9-Duterio-9-methyl-1,4-dioxaspiro[4.6]undecan-8-one (15b). Colorless oil; IR (neat) 2935, 2882, 1704 (CO), 1455, 1372, 1259, 1096, 1055, 1001, 948 cm^{-1}; ^{1}H NMR δ 1.09 (3H, s), 1.65–1.96 (6H, m), 2.41 (1H, ddd, J=15.0, 7.1, 3.0 Hz), 2.68 (1H, ddd, J=15.0, 11.6, 3.0 Hz), 3.92–3.98 (4H, m). MS *m/z* (%) 185 (M$^+$, 11), 157 (32), 128 (9), 99 (100), 86 (73). Calcd for $C_{10}H_{15}DO_3$: M, 185.1162. Found: *m/z* 185.1170.

3.1.5. 2-Deuterio-2-methylcyclononanone (15c). Colorless oil; IR (neat) 2929, 2872, 1702 (CO), 1467, 1445, 1374, 1067 cm^{-1}; ^{1}H NMR δ 1.04 (3H, s), 1.23–1.90 (12H, m), 2.40 (1H, ddd, J=13.1, 9.5, 3.1 Hz), 2.49 (1H, ddd, J=13.1, 9.5, 3.1 Hz). MS *m/z* (%) 155 (M$^+$, 39), 125 (9), 112 (49), 98 (100), 84 (36). Calcd for $C_{10}H_{17}DO$: M, 155.1419. Found: *m/z* 155.1419.

3.1.6. 2-Deuterio-2-methylcycloundecanone (15d). Colorless oil; IR (neat) 2930, 2868, 1706 (CO), 1471, 1461, 1374, 1056, 1029 cm^{-1}; ^{1}H NMR δ 1.05 (3H, s), 1.26–1.88 (16H, m), 2.36 (1H, ddd, J=15.7, 8.9, 2.8 Hz), 2.61–2.67 (1H, m). MS *m/z* (%) 183 (M$^+$, 43), 125 (39), 111 (40), 98 (71), 83 (50), 69 (61), 55 (100). Calcd for $C_{12}H_{21}DO$: M, 183.1733. Found: *m/z* 183.1734.

3.1.7. 2-Deuterio-2-methylcyclotridecanone (15e). Colorless oil; IR (neat) 2931, 2862, 1709 (CO), 1462, 1374, 729 cm^{-1}; ^{1}H NMR δ 1.04 (3H, s), 1.28–1.40 (17H, m), 1.50–1.56 (1H, m), 1.64–1.71 (1H, m), 1.76–1.82 (1H, m), 2.34 (1H, ddd, J=16.5, 7.7, 4.0 Hz), 2.58–2.67 (1H, m). MS *m/z* (%) 211 (M$^+$, 42), 181 (20), 153 (16), 139 (16), 125 (13), 111 (29), 98 (54), 83 (47), 69 (58), 55 (100). Calcd for $C_{14}H_{25}DO$: M, 211.2045. Found: *m/z* 211.2046.

3.1.8. 2-Deuterio-2-methylcyclohexadecanone (15f). Colorless oil; IR (neat) 2929, 2857, 1710 (CO), 1459, cm^{-1}; ^{1}H NMR δ 1.04 (3H, s), 1.22–1.36 (23H, m), 1.49–1.57 (1H, m), 1.66–1.74 (2H, m), 2.37 (1H, dt, J=16.8, 6.7 Hz), 2.47–2.53 (1H, m). MS *m/z* (%) 253 (M$^+$, 60), 223 (26), 139 (22), 125 (21), 111 (28), 98 (54), 73 (80), 69 (57), 55 (100). Calcd for $C_{17}H_{31}DO$: M, 253.2515. Found: *m/z* 253.2525.

3.1.9. 3-(1-Hydroxypropyl)-3-methyl-2-tetralone (16a). To a solution of *t*-BuMgCl (0.36 mmol) in 3 ml of dry THF in a flame-dried flask at -45 °C under argon atmosphere was added a solution of the adduct **12a** (101 mg; 0.3 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at -45 °C for 10 min. To a solution of the magnesium alkoxide was added *i*-PrMgCl (0.6 mmol) dropwise with stirring. The reaction mixture was stirred at -45 °C for 10 min. The reaction mixture was slowly allowed to warm to room temperature. To a solution of the enolate was added propionaldehyde (0.05 ml; 0.6 mmol) dropwise at room temperature with stirring. After 30 min, the reaction was quenched with sat. aq. NH_4Cl. The whole was extracted with $CHCl_3$ and the organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **16a** (25 mg; 38%) as a colorless oil; IR (neat) 3448 (OH), 2966, 2935, 1709 (CO), 1458, 1248, 1119, 1097, 976, 749 cm^{-1}; ^{1}H NMR δ 0.93 (3H, s), 1.04 (3H, t, $J=7.3$ Hz), 1.33–1.42 (1H, m), 1.58–1.64 (1H, m), 2.22 (1H, br s), 2.63 (1H, d, $J=15.3$ Hz), 3.38 (1H, d, $J=15.3$ Hz), 3.60 (2H, s), 3.80 (1H, br d, $J=11.3$ Hz), 7.08–7.12 (1H, m), 7.18–7.24 (3H, m). MS *m/z* (%) 218 (M$^+$, 5), 200 (9), 160 (92), 145 (100), 131 (22), 117 (33), 104 (39), 91 (19). Calcd for $C_{14}H_{18}O_2$: M, 218.1306. Found: *m/z* 218.1307.

3.1.10. Enol carbonate (17a). Colorless oil; IR (neat) 2984, 2917, 1752 (CO), 1276, 1243 (COC), 1037, 747 cm^{-1}; ^{1}H NMR δ 1.37 (3H, t, $J=7.0$ Hz), 1.74 (3H, s), 3.48–3.50 (2H, m), 3.55–3.60 (2H, m), 4.27 (2H, q, $J=7.0$ Hz), 7.10–7.18 (4H, m). ^{13}C NMR δ 153.2 (CO). MS *m/z* (%) 232 (M$^+$, 18), 188 (11), 173 (14), 160 (57), 145 (100), 131 (37), 115 (25), 91 (22). Calcd for $C_{14}H_{16}O_3$: M, 232.1098. Found: *m/z* 232.1096.

3.1.11. 9-(1-Hydroxypropyl)-9-methy-1,4-dioxaspiro-[4.6]undecan-8-one (16b). Colorless oil; IR (neat) 3480 (OH), 2970, 2879, 1699 (CO), 1464, 1200, 1111, 756 cm^{-1}; ^{1}H NMR δ 1.00 (3H, t, $J=7.3$ Hz), 1.08 (3H, s), 1.26–1.31 (1H, m), 1.47–1.55 (1H, m), 1.59–1.66 (2H, m), 1.70–1.80 (2H, m), 1.83–1.89 (1H, m), 1.91–1.97 (1H, m), 2.19 (1H, dd, $J=15.3$, 10.7 Hz), 2.51 (1H, ddd, $J=12.6$, 8.9, 2.2 Hz), 2.75 (1H, ddd, $J=12.6$, 11.6, 2.5 Hz), 3.54 (1H, d, $J=10.7$ Hz), 3.96 (4H, s). MS *m/z* (%) 242 (M$^+$, 1), 214 (16), 184 (62), 129 (29), 122 (41), 99 (100), 86 (70). Calcd for $C_{13}H_{22}O_4$: M, 242.1517. Found: *m/z* 242.1524.

3.1.12. 9-[Hydroxy(phenyl)methyl]-9-methyl-1,4-dioxaspiro[4.6]undecan-8-one (16c). Colorless crystals; mp 149–150 °C (AcOEt–hexane); IR (KBr) 3430 (OH), 2946, 1685 (CO), 1113, 1040, 705 cm^{-1}; ^{1}H NMR δ 1.02 (3H, s), 1.35 (1H, ddd, $J=15.3$, 9.2, 0.9 Hz), 1.64–1.75 (2H, m), 1.84–1.93 (2H, m), 2.30 (1H, dd, $J=15.0$, 11.0 Hz), 2.52 (1H, ddd, $J=12.2$, 9.2, 2.2 Hz), 2.67 (1H, d, $J=4.3$ Hz), 2.83 (1H, dt, $J=12.2$, 2.2 Hz), 3.90–3.96 (4H, m), 4.83 (1H, d, $J=4.0$ Hz), 7.27–7.34 (5H, m). MS *m/z* (%) 290 (M$^+$, trace), 184 (28), 156 (37), 99 (100), 86 (89), 77 (52). Calcd for $C_{17}H_{22}O_4$: M, 290.1519. Found: *m/z* 290.1526. Anal. Calcd for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64. Found: C, 70.23; H, 7.62.

3.1.13. Enol carbonate (17b). Colorless oil; IR (neat) 2937, 1751 (CO), 1371, 1267 (COC), 1234, 1113, 1098, 1050, 1035 cm^{-1}; ^{1}H NMR δ 1.33 (3H, t, $J=7.0$ Hz), 1.65 (3H, s), 1.76 (2H, m), 1.84 (2H, m), 2.15 (2H, m), 2.35 (2H, m), 3.94 (4H, s), 4.22 (2H, q, $J=7.0$ Hz). ^{13}C NMR δ 153.5 (CO). MS *m/z* (%) 256 (M$^+$, 54), 184 (31), 167 (35), 166 (31), 151 (27), 122 (100), 99 (55), 86 (96), 83 (20). Calcd for $C_{13}H_{20}O_5$: M, 256.1311. Found: *m/z* 256.1313.

3.1.14. 9-Benzoyl-9-methyl-1,4-dioxaspiro[4.6]undecan-8-one (16d). Colorless oil; IR (neat) 2938, 2885, 1709 (CO), 1672 (CO), 1447, 1249, 1113, 1056 cm^{-1}; ^{1}H NMR δ 1.54 (3H, s), 1.66 (1H, ddd, $J=15.0$, 10.1, 0.9 Hz), 1.73–1.89 (3H, m), 1.93 (1H, ddt, $J=15.0$, 10.1, 1.6 Hz), 2.37 (1H, ddd, $J=13.8$, 7.6, 3.7 Hz), 2.64 (1H, ddd, $J=13.8$, 10.7, 3.7 Hz), 2.74 (1H, ddd, $J=15.5$, 9.8, 1.5 Hz), 3.89–3.97 (4H, m), 7.41 (2H, t, $J=8.0$ Hz), 7.51 (1H, t, $J=8.0$ Hz), 7.78 (2H, dd, $J=8.0$, 1.3 Hz). ^{13}C NMR δ 211.8 (CO), 200.3 (CO). MS *m/z* (%) 288 (M$^+$, 14), 260 (8), 149 (15), 105 (100), 86 (27), 77 (30). Calcd for $C_{17}H_{20}O_4$: M, 288.1359. Found: *m/z* 288.1356.

3.1.15. 2-(1-Hydroxypropyl)-2-methylcyclohexadecanone (16e). Colorless oil; IR (neat) 3480 (OH), 2930, 2857, 1698 (CO), 1461, 1111, 973, 757 cm^{-1}; ^{1}H NMR δ 1.03 (3H, t, $J=7.3$ Hz), 1.10 (3H, s), 1.21–1.31 (23H, m), 1.43–1.55 (3H, m), 1.62–1.70 (2H, m), 2.20 (1H, d, $J=5.5$ Hz), 2.34–2.45 (1H, m), 2.59 (1H, dt, $J=17.9$, 7.5 Hz), 3.73–3.76 (1H, m). MS *m/z* (%) 310 (M$^+$, 8), 252 (100), 223 (17), 139 (16), 97 (28), 84 (47), 72 (40). Calcd for $C_{20}H_{38}O_2$: M, 310.2869. Found: *m/z* 310.2868.

3.1.16. 2-Methyl-2-ethoxycarbonylcyclohexadecanone (16f). Colorless oil; IR (neat) 2929, 2858, 1741 (CO for ester), 1713 (CO for ketone), 1241 (COC), 1099, 1023 cm^{-1}; ^{1}H NMR δ 1.25 (3H, t, $J=7.0$ Hz), 1.18–1.38 (25H, m), 1.32 (3H, s), 1.64–1.69 (1H, m), 1.80 (1H, dt, $J=13.3$, 3.4 Hz), 1.98 (1H, dt, $J=13.3$, 3.4 Hz), 2.33 (1H, ddd, $J=17.7$, 8.3, 6.4 Hz), 2.55 (1H, dt, $J=17.7$, 7.6 Hz), 4.17 (2H, q, $J=7.0$ Hz). ^{13}C NMR δ 207.9 (CO for ketone), 173.6 (CO for ester). MS *m/z* (%) 324 (M$^+$, 6), 126 (27), 112 (100), 98 (17). Calcd for $C_{20}H_{36}O_3$: M, 324.2663. Found: *m/z* 324.2665.

3.1.17. 2-Benzoyl-2-methylcyclohexadecanone (16 g). Colorless crystals; mp 76–77 °C (hexane); IR (KBr) 2931, 2856, 1709 (CO), 1670 (CO), 1468, 1245, 1224, 964, 707 cm^{-1}; ^{1}H NMR δ 1.16–1.46 (23H, m), 1.47 (3H, s), 1.66–1.71 (1H, m), 1.88 (1H, dt, $J=14.0$, 4.9 Hz), 2.01 (1H, ddd, $J=17.7$, 8.6, 5.5 Hz), 2.14 (1H, dt, $J=14.0$, 3.4 Hz), 2.60 (1H, ddd, $J=17.7$, 8.6, 6.7 Hz), 7.40 (2H, t, $J=7.3$ Hz), 7.51 (1H, t, $J=7.3$ Hz), 7.77 (2H, d, $J=7.3$ Hz). MS *m/z* (%) 356 (M$^+$, 17), 157 (22), 147 (25), 105 (100), 77 (17). Calcd for $C_{24}H_{36}O_2$: M, 356.2713. Found: *m/z* 356.2723.

3.1.18. 8-[1-Chloro-1-(*p*-tolylsulfinyl)propyl]-1,4-dioxaspiro[4.5]decan-8-ol (19). Colorless crystals; mp 127–129 °C ($CHCl_3$–hexane); IR (KBr) 3303 (OH), 2942, 2887, 1441, 1376, 1101, 1035 (SO), 998, 813 cm^{-1}; ^{1}H NMR δ

0.90 (3H, t, $J=7.3$ Hz), 1.69–2.32 (10H, m), 2.44 (3H, s), 3.92–3.99 (4H, m), 4.06 (1H, br s), 7.33 (2H, d, $J=8.0$ Hz), 7.65 (2H, d, $J=8.0$ Hz). Anal. Calcd for $C_{18}H_{25}ClO_4S$: C, 57.98; H, 6.76; Cl, 9.51; S, 8.60. Found: C, 57.97; H, 6.77; Cl, 9.47; S, 8.46.

3.1.19. Synthesis of 9-ethyl-1,4-dioxaspiro[4.6]undecan-8-one 20 and 8-(1-propenyl)-1,4-dioxaspiro[4,5]decan-8-ol 21 from the adduct 19. To a solution of *t*-BuMgCl (0.36 mmol) in 3 ml of dry THF in a flame-dried flask at −45 °C under argon atmosphere was added a solution of the adduct **19** (112 mg; 0.3 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min. To a solution of the magnesium alkoxide was added *i*-PrMgCl (0.6 mmol) dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min and slowly allowed to warm to room temperature for 2.5 h. The reaction was quenched with sat. aq. NH_4Cl. The whole was extracted with $CHCl_3$. The organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **20** (21 mg; 35%) and **21** (39 mg; 65%). **20**: Colorless oil; IR (neat) 2937, 2878, 1704 (CO), 1455, 1100, 1028 cm^{-1}; ^1H NMR δ 0.90 (3H, t, $J=7.3$ Hz), 1.43 (1H, quintet, $J=7.3$ Hz), 1.65–1.91 (7H, m), 2.31–2.37 (2H, m), 2.67–2.73 (1H, m), 3.95 (4H, s). MS *m/z* (%) 198 (M^+, 19), 170 (45), 155 (40), 141 (23), 99 (100), 86 (100). Calcd for $C_{11}H_{18}O_3$: M, 198.1256. Found: *m/z* 198.1253. **21**: Colorless oil; IR (neat) 3464 (OH), 2931, 2883, 1367, 1253, 1097, 972 cm^{-1}; ^1H NMR δ 1.26 (1H, br s, OH), 1.59–1.67 (4H, m), 1.70 (3H, dd, $J=6.4$, 1.5 Hz), 1.78 (2H, dt, $J=11.9$, 3.4 Hz), 1.92 (2H, dt, $J=11.9$, 3.4 Hz), 3.92–3.98 (4H, m), 5.60 (1H, dq, $J=15.6$, 1.5 Hz), 5.71 (1H, dq, $J=15.6$, 6.4 Hz). MS *m/z* (%) 198 (M^+, 0.4), 99 (100), 86 (40), 69 (13). Calcd for $C_{11}H_{18}O_3$: M, 198.1255. Found: *m/z* 198.1261.

3.2. Synthesis of 20 from 19 with LDA-*t*-BuLi

To a solution of LDA (0.36 mmol) in 3 ml of dry THF in a flame-dried flask at −70 °C under argon atmosphere was added a solution of the adduct **19** (112 mg; 0.3 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −70 °C for 10 min. To a solution of the lithium alkoxide was added *t*-BuLi (1.2 mmol) dropwise with stirring. The reaction mixture was stirred at −70 °C for 30 min. The reaction was quenched with sat. aq. NH_4Cl. The whole was extracted with $CHCl_3$. The organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **20** (38 mg, 64%).

3.2.1. 8-[1-Chloro-5-phenyl-1-(*p*-tolylsulfinyl)pentyl]-1,4-dioxaspiro[4.5]decan-8-ol (22). Colorless amorphous; IR (KBr) 3393 (OH), 2934, 1453, 1255, 1106, 1037 (SO), 701 cm^{-1}; ^1H NMR δ 1.43–2.50 (17H, m), 2.44 (3H, s), 3.92–3.99 (4H, m), 7.09–7.31 (7H, m), 7.59 (2H, d, $J=8.3$ Hz). MS (FAB) *m/z* (%) 477 ($[M+H]^+$, 48), 319 (57), 301 (30), 275 (22), 185 (24), 154 (39), 117 (50), 91 (100), 77 (23). Calcd for $C_{26}H_{34}ClO_4S$: M+H, 477.1866. Found: *m/z* 477.1869.

3.2.2. 9-Deuterio-9-ethyl-1,4-dioxaspiro[4.6]undecan-8-one (23a). To a solution of LDA (0.36 mmol) in 3 ml of dry THF in a flame-dried flask at −70 °C under argon atmosphere was added a solution of the adduct **19** (112 mg; 0.3 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −70 °C for 10 min. To a solution of the lithium alkoxide was added *t*-BuLi (1.2 mmol) dropwise with stirring. The reaction mixture was stirred at −70 °C for 30 min. The reaction was quenched with excess CD_3OD. The whole was extracted with $CHCl_3$. The organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **23a** (38 mg; 64%) as a colorless oil; IR (neat) 2938, 2877, 1699 (CO), 1455, 1111, 1066, 1048, 948 cm^{-1}; ^1H NMR δ 0.89 (3H, t, $J=7.3$ Hz), 1.43 (1H, dq, $J=14.1$, 7.3 Hz), 1.64–1.71(3H, m), 1.76–1.91 (4H, m), 2.28–2.36 (1H, m), 2.67–2.73 (1H, m), 3.95 (4H, s). MS *m/z* (%) 199 (M^+, 71), 157 (38), 143 (37), 137 (18), 115 (28), 99 (100), 86 (73). Calcd for $C_{11}H_{17}DO_3$: M, 199.1316. Found: *m/z* 199.1309.

3.2.3. 9-Ethyl-9-(1-hydroxypropyl)-1,4-dioxaspiro-[4.6]undecan-8-one (23b). Colorless oil (about 4:1 mixture of two diastereomers); IR (neat) 3479 (OH), 2934, 2881, 1695 (CO), 1108, 975 cm^{-1}; ^1H NMR δ 0.86 (2.4H, t, $J=7.6$ Hz), 0.90 (0.6H, t, $J=7.3$ Hz), 1.01 (3H, t, $J=7.3$ Hz), 1.32–1.38 (1H, m), 1.48–1.58 (2H, m), 1.74–1.96 (7H, m), 2.52 (1H, ddd, $J=12.1$, 8.9, 3.4 Hz), 2.66–2.72 (2H, m), 3.72 (1H, d, $J=10.7$ Hz), 3.96 (4H, s). MS *m/z* (%) 256 (M^+, 0.6), 198 (72), 170 (22), 155 (18), 136 (41), 99 (100), 86 (89). Calcd for $C_{14}H_{24}O_4$: M, 256.1674. Found: *m/z* 256.1676.

3.2.4. 9-Benzoyl-9-ethyl-1,4-dioxaspiro[4.6]undecan-8-one (23c). Colorless oil; IR (neat) 2963, 2881, 1721 (CO), 1669 (CO), 1447, 1226, 1113, 701 cm^{-1}; ^1H NMR δ 0.79 (3H, t, $J=7.6$ Hz), 1.60 (1H, dd, $J=14.1$, 10.1 Hz), 1.79–1.91 (5H, m), 2.31–2.41 (2H, m), 2.57 (1H, ddd, $J=13.1$, 10.1, 4.3 Hz), 2.72 (1H, dd, $J=14.7$, 9.8 Hz), 3.88–3.98 (4H, m), 7.40 (2H, t, $J=7.3$ Hz), 7.51 (1H, dt, $J=7.3$, 1.2 Hz), 7.81 (2H, dd, $J=7.3$, 1.2 Hz). MS *m/z* (%) 302 (M^+, 11), 274 (7), 197 (7), 180 (6), 149 (18), 105 (100), 77 (25). Calcd for $C_{18}H_{22}O_4$: M, 302.1518. Found: *m/z* 302.1518.

3.2.5. 9-Ethyl-9-ethoxycarbonyl-1,4-dioxaspiro[4.6]undecan-8-one (23d). Colorless oil; IR (neat) 2969, 2883, 1733 (CO), 1712 (CO), 1234 (COC), 1113, 1052, 1027 cm^{-1}; ^1H NMR δ 0.88 (3H, t, $J=7.3$ Hz), 1.26 (3H, t, $J=7.0$ Hz), 1.68–1.75 (2H, m), 1.79–1.85 (1H, m), 1.89–2.03 (4H, m), 2.21–2.25 (1H, m), 2.58–2.61 (2H, m), 3.92–3.97 (4H, m), 4.19 (2H, dq, $J=7.0$, 2.1 Hz). ^{13}C NMR δ 209.6 (CO for ketone), 172.1 (CO for ester); MS *m/z* (%) 270 (M^+, 14), 242 (44), 213 (45), 196 (21), 142 (26), 99 (100), 86 (74). Calcd for $C_{14}H_{22}O_5$: M, 270.1467. Found: *m/z* 270.1462.

3.2.6. 9-Deuterio-9-(4-phenylbutyl)-1,4-dioxaspiro-[4.6]undecan-8-one (23e). Colorless oil; IR (neat) 2934, 2859, 1700 (CO), 1453, 1113, 1069, 750, 700 cm^{-1}; ^1H NMR δ 1.23–1.41 (4H, m), 1.59–1.90 (8H, m), 2.29–2.34 (1H, m), 2.59 (2H, t, $J=7.6$ Hz), 2.66–2.72 (1H, m), 3.94 (4H, s), 7.14–7.19 (3H, m), 7.24–7.29 (2H, m). MS *m/z* (%) 304 (M^+, 22), 276 (18), 246 (15), 171 (22), 156 (25), 130 (15), 109 (18), 99 (96), 86 (100). Calcd for $C_{19}H_{25}DO_3$: M, 303.1942. Found: *m/z* 303.1938.

3.2.7. 9-(1-Hydroxypropyl)-9-(4-phenylbutyl)-1,4-dioxaspiro[4.6]undecan-8-one (23f). Colorless oil; IR (neat) 3494 (OH), 2933, 1694 (CO), 1454, 1112, 974, 749, 700 cm^{-1}; ^1H NMR δ 1.01 (3H, t, $J=7.3$ Hz), 1.16–2.04 (15H, m), 2.46–2.52 (1H, m), 2.56–2.62 (2H, m), 2.66–2.72 (1H, m), 3.63–3.65 (1H, m), 3.92–3.98 (4H, m), 7.12–7.19 (3H, m), 7.24–7.29 (2H, m). MS *m/z* (%) 360 (M^+, 3), 302 (52), 274 (22), 155 (27), 149 (24), 108 (18), 99 (100), 91 (71). Calcd for $C_{22}H_{32}O_4$: M, 360.2298. Found: *m/z* 360.2289.

3.2.8. 9-Ethoxycarbonyl-9-(4-phenylbutyl)-1,4-dioxaspiro[4.6]undecan-8-one (23 g). Colorless oil; IR (neat) 2934, 1733 (CO for ester), 1712 (CO for ketone), 1454, 1224 (COC), 1150, 1112, 749, 701 cm^{-1}; ^1H NMR δ 1.23 (3H, t, $J=7.2$ Hz), 1.28–1.37 (3H, m), 1.58–1.72 (4H, m), 1.79–1.84 (1H, m), 1.88–1.99 (4H, m), 2.17–2.26 (1H, m), 2.57–2.61 (3H, m), 3.90–3.98 (4H, m), 4.16 (2H, dq, $J=7.2$, 2.2 Hz), 7.15 (3H, m), 7.25 (2H, m). ^{13}C NMR δ 209.5 (CO for ketone), 172.2 (CO for ester). MS *m/z* (%) 374 (M^+, 43), 346 (17), 317 (21), 300 (11), 242 (30), 181 (14), 143 (14), 113 (17), 99 (100). Calcd for $C_{22}H_{30}O_5$: M, 374.2091. Found: *m/z* 374.2088.

3.2.9. 9,9-Dimethyl-1,4-dioxaspiro[4.6]undecan-8-one (24a). To a solution of *t*-BuMgCl (0.24 mmol) in 2 ml of dry THF in a flame-dried flask at −45 °C under argon atmosphere was added a solution of adduct **12b** (72 mg; 0.2 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min. To a solution of the magnesium alkoxide was added *i*-PrMgCl (0.4 mmol) dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min and slowly allowed to warm to room temperature for 2.5 h. To a solution of the enolate was added hexamethylphosphoramide (HMPA) (0.14 ml; 0.8 mmol) dropwise at room temperature. The reaction mixture was stirred for 10 min and iodomethane (0.05 ml; 0.8 mmol) was added dropwise with stirring. After being stirred for 19 h, the reaction was quenched with sat. aq. NH_4Cl. The whole was extracted with $CHCl_3$. The organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **24a** (29 mg, 73%) as colorless crystals; mp 61–62 °C (hexane); IR (KBr) 2958, 2876, 1699 (CO), 1471, 1112, 1043, 916 cm^{-1}; ^1H NMR δ 1.10 (6H, s), 1.67–1.70 (2H, m), 1.79–1.83 (4H, m), 2.56–2.59 (2H, m), 3.95 (4H, s). Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.63; H, 9.29.

3.2.10. 9-Benzyl-9-methyl-1,4-dioxaspiro[4.6]undecan-8-one (24b). Colorless crystals; mp 97–98 °C (AcOEt–hexane); IR (KBr) 2955, 2884, 1695 (CO), 1455, 1436, 1109, 1078, 1061, 703 cm^{-1}; ^1H NMR δ 1.06 (3H, s), 1.53–1.85 (5H, m), 2.06 (1H, dd, $J=15.0$, 10.1 Hz), 2.42 (1H, ddd, $J=11.8$, 8.9, 2.5 Hz), 2.59 (1H, dt, $J=11.8$, 2.5 Hz), 2.76 (1H, d, $J=13.4$ Hz), 2.81 (1H, d, $J=13.4$ Hz), 3.89–3.99 (4H, m), 7.08 (2H, d, $J=7.0$ Hz), 7.20–7.28 (3H, m). MS *m/z* (%) 274 (M^+, 38), 246 (50), 217 (21), 183 (16), 160 (20), 145 (19), 131 (22), 117 (32), 99 (100), 91 (73), 71 (14). Calcd for $C_{17}H_{22}O_3$: M, 274.1567. Found: *m/z* 274.1566. Anal. Calcd for $C_{17}H_{22}O_3$: C, 74.42; H, 8.08. Found: C, 74.24; H, 8.07.

3.2.11. 9-Allyl-9-methyl-1,4-dioxaspiro[4.6]undecan-8-one (24c). Colorless oil; IR (neat) 3076, 2938, 2879, 1702 (CO), 1638 (C=C), 1462, 1438, 1366, 1325, 1111, 1049, 1001, 911, 756 cm^{-1}; ^1H NMR δ 1.07 (3H, s), 1.62–1.87 (5H, m), 2.02 (1H, dd, $J=14.7$, 10.1 Hz), 2.23 (2H, d, $J=7.4$ Hz), 2.43 (1H, dt, $J=11.5$, 2.2 Hz), 2.70 (1H, dt, $J=11.5$, 2.2 Hz), 3.91–4.00 (4H, m), 5.04 (1H, d, $J=18.7$ Hz), 5.07 (1H, d, $J=11.3$ Hz), 5.67–5.75 (1H, m). MS *m/z* (%) 224 (M^+, 10), 196 (18), 167 (12), 142 (14), 113 (15), 99 (100), 86 (60). Calcd for $C_{13}H_{20}O_3$: M, 224.1411. Found: *m/z* 224.1405.

3.2.12. 9-Ethyl-9-methyl-1,4-dioxaspiro[4.6]undecan-8-one (24d). To a solution of LDA (0.36 mmol) in 3 ml of dry THF in a flame-dried flask at −70 °C under argon atmosphere was added a solution of the adduct **19** (75 mg; 0.2 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −70 °C for 10 min. To a solution of the lithium alkoxide was added *t*-BuLi (1.2 mmol) dropwise with stirring. The reaction mixture was stirred at −70 °C for 30 min. To a solution of the enolate was added HMPA (0.14 ml; 0.8 mmol) dropwise with stirring. After being stirred for 10 min, to the reaction mixture was added iodomethane (0.05 ml; 0.8 mmol) dropwise with stirring. After 30 min, the reaction was quenched with sat. aq. NH_4Cl. The whole was extracted with $CHCl_3$. The organic layer was washed with sat. aq. NH_4Cl and dried over $MgSO_4$. The products were separated by silica gel flash column chromatography to afford **24d** (16 mg; 38%) as a colorless oil; IR (neat) 2965, 2880, 1702 (CO), 1464, 1438, 1111, 1088, 1049 cm^{-1}; ^1H NMR δ 0.82 (3H, t, $J=7.6$ Hz), 1.04 (3H, s), 1.49–1.64 (4H, m), 1.71–1.76 (2H, m), 1.85–1.90 (1H, m), 2.03 (1H, dd, $J=15.1$, 11.3 Hz), 2.30 (1H, ddd, $J=11.6$, 8.6, 2.2 Hz), 2.79 (1H, dt, $J=11.6$, 2.2 Hz), 3.92–4.00 (4H, m). MS *m/z* (%) 212 (M^+, 7), 184 (52), 169 (37), 155 (15), 142 (15), 113 (21), 99 (100), 86 (96). Calcd for $C_{12}H_{20}O_3$: M, 212.1410. Found: *m/z* 212.1406.

3.2.13. 9-Allyl-9-ethyl-1,4-dioxaspiro[4.6]undecan-8-one (24e). Colorless oil; IR (neat) 3076, 2939, 2881, 1701 (CO), 1462, 1440, 1112, 1048, 994, 913 cm^{-1}; ^1H NMR δ 0.79 (3H, t, $J=7.2$ Hz), 1.48–1.87 (8H, m), 2.17 (1H, dd, $J=14.4$, 7.8 Hz), 2.39 (1H, t, $J=7.2$ Hz), 2.41 (1H, dd, $J=8.4$, 1.8 Hz), 2.69 (1H, dt, $J=11.4$, 2.4 Hz), 3.92–3.98 (4H, m), 5.04 (1H, d, $J=16.8$ Hz), 5.06 (1H, d, $J=7.8$ Hz), 5.65–5.71 (1H, m). MS *m/z* (%) 238 (M^+, 5), 210 (25), 195 (11), 181 (12), 142 (12), 113 (15), 99 (100), 86 (60). Calcd for $C_{14}H_{22}O_3$: M, 238.1568. Found: *m/z* 238.1570.

26a, **26b**, **27a**, **27b**, **28a**, **28b**, **29a**, and **29b** have been reported before.[5]

3.2.14. 2-Deuterio-1-(4-methoxyphenyl)-1-pentanone (28a). Colorless oil; IR (neat) 2959, 2934, 1679 (CO), 1602, 1510, 1258, 1171, 1031, 838 cm^{-1}; ^1H NMR δ 0.95 (3H, t, $J=7.5$ Hz), 1.41 (2H, sextet, $J=7.5$ Hz), 1.71 (2H, quintet, $J=7.5$ Hz), 2.91 (1H, t, $J=7.5$ Hz), 3.87 (3H, s), 6.93 (2H, d, $J=8.5$ Hz), 7.95 (2H, d, $J=8.5$ Hz). MS *m/z* (%) 193 (M^+, 3), 150 (27), 136 (9), 135 (100), 107 (7), 92 (7), 77 (12). Calcd for $C_{12}H_{15}DO_2$: M, 193.1211. Found: *m/z* 193.1207.

3.2.15. 3-Hydroxy-1-(4-methoxyphenyl)-2-propyl-1-pentanone (28b). Colorless oil (about 2:1 mixture of two diastereomers). Main product: IR (neat) 3445 (OH), 2960, 2934, 1661 (CO), 1600, 1260, 1172, 1031, 841 cm^{-1}; ^{1}H NMR δ 0.89 (3H, t, J=7.4 Hz), 0.96 (3H, t, J=7.3 Hz), 1.23–1.51 (4H, m), 1.70–1.82 (2H, m), 3.21 (1H, d, J=8.5 Hz), 3.49–3.53 (1H, m), 3.71–3.76 (1H, m), 3.88 (3H, s), 6.96 (2H, d, J=8.5 Hz), 7.95 (2H, d, J=8.5 Hz). MS *m/z* (%) 250 (M$^+$, trace), 208 (7), 192 (5), 150 (17), 135 (100), 92 (6), 77 (7). Calcd for $C_{15}H_{22}O_3$: M, 250.1568. Found: *m/z* 250.1569. Minor product: ^{1}H NMR δ 0.87 (3H, t, J=7.2 Hz), 0.98 (3H, t, J=7.5 Hz), 1.19–1.30 (3H, m), 1.45–1,56 (1H, m), 1.67–1.73 (1H, m), 1.81–1.89 (1H, m), 2.79 (1H, br s), 3.49 (1H, dt, J=9.8, 4.0 Hz), 3.75–3.80 (1H, m), 3.88 (3H, s), 6.96 (2H, d, J=8.9 Hz), 7.96 (2H, d, J=8.9 Hz).

3.2.16. Enol carbonate (30). Colorless oil; IR (neat) 2961, 2935, 1760 (CO), 1610, 1514, 1251 (COC), 1175, 1018, 837 cm^{-1}; ^{1}H NMR δ 0.90 (3H, t, J=7.3 Hz), 1.28 (3H, t, J=7.3 Hz), 1.44 (2H, sextet, J=7.3 Hz), 2.15 (2H, q, J=7.3 Hz), 3.82 (3H, s), 4.16 (2H, q, J=7.3 Hz), 5.50 (1H, t, J=7.3 Hz), 6.89 (2H, d, J=8.5 Hz), 7.35 (2H, d, J=8.5 Hz). ^{13}C NMR δ 159.5 (CO). MS *m/z* (%) 264 (M$^+$, 24), 191 (20), 174 (18), 163 (100), 136 (8), 135 (37), 121 (14), 92 (7), 77 (11). Calcd for $C_{15}H_{20}O_4$: M, 264.1360. Found: *m/z* 264.1357.

3.2.17. 2-Deuterio-2-(4-methoxyphenyl)pentanal (29b). Colorless oil; IR (neat) 2959, 2933, 2714 (CHO), 1722 (CO), 1513, 1250, 1180, 1034 cm^{-1}; ^{1}H NMR δ 0.91 (3H, t, J=7.3 Hz), 1.28 (2H, sextet, J=7.3 Hz), 1.68 (1H, dt, J=13.8, 7.3 Hz), 2.00 (1H, dt, J=13.8, 8.0 Hz), 3.80 (3H, s), 6.91 (2H, d, J=8.9 Hz), 7.11 (2H, d, J=8.9 Hz), 9.63 (1H, s). MS *m/z* (%) 193 (M$^+$, 15), 164 (58), 163 (20), 122 (100), 121 (88), 92 (10). Calcd for $C_{12}H_{15}DO_2$: M, 193.1211. Found: *m/z* 193.1211.

3.2.18. 5-Hydroxy-1-phenyl-4-propylheptan-3-one (28c). Colorless oil (about 1:1 mixture of inseparable two diastereomers); IR (neat) 3424 (OH), 2964, 2935, 1705 (CO), 1456, 1152, 1114, 1086, 974, 700 cm^{-1}; ^{1}H NMR δ 0.87 (1.5H, t, J=7.3 Hz), 0.88 (1.5H, t, J=7.3 Hz), 0.92 (1.5H, t, J=7.3 Hz), 0.95 (1.5H, t, J=7.3 Hz), 1.16–1.71 (6H, m), 2.31 (0.5H, m), 2.43 (0.5H, br d, J=7.3 Hz), 2.57–2.62 (1H, m), 2.74–2.92 (4H, m), 3.57–3.65 (1H, m), 7.18–7.21 (3H, m), 7.27–7.30 (2H, m). MS *m/z* (%) 248 (M$^+$, 2), 190 (38), 148 (28), 133 (37), 105 (81), 91 (100), 77 (15). Calcd for $C_{16}H_{24}O_2$: M, 248.1774. Found: *m/z* 248.1764.

3.2.19. 1-(4-Methoxyphenyl)-2-methylpentan-1-one (31a). Colorless oil; IR (neat) 2960, 2932, 1672 (CO), 1600, 1509, 1243, 1214, 1174, 1032, 973, 841 cm^{-1}; ^{1}H NMR δ 0.90 (3H, t, J=6.9 Hz), 1.18 (3H, d, J=6.9 Hz), 1.27–1.47 (3H, m), 1.68–1.83 (1H, m), 3.44 (1H, sextet, J=6.9 Hz), 3.87 (3H, s), 6.94 (2H, d, J=8.7 Hz), 7.95 (2H, d, J=8.7 Hz). MS *m/z* (%) 206 (M$^+$, 2), 164 (18), 136 (9), 135 (100), 92 (8), 77 (10). Calcd for $C_{13}H_{18}O_2$: M, 206.1307. Found: *m/z* 206.1307.

3.2.20. 2-Benzyl-1-(4-methoxyphenyl)pentan-1-one (31b). Colorless oil; IR (neat) 2958, 2932, 1671 (CO), 1600, 1509, 1244, 1170, 1031, 840, 700 cm^{-1}; ^{1}H NMR δ 0.84 (3H, t, J=7.3 Hz), 1.20–1.35 (2H, m), 1.47–1.54 (1H, m), 1.70–1.80 (1H, m), 2.75 (1H, dd, J=13.7, 7.6 Hz), 3.08 (1H, dd, J=13.7, 7.6 Hz), 3.68 (1H, quintet, J=7.6 Hz), 3.84 (3H, s), 6.88 (2H, d, J=9.2 Hz), 7.12–7.23 (5H, m), 7.85 (2H, d, J=9.2 Hz). MS *m/z* (%) 282 (M$^+$, 5), 240 (18), 136 (9), 135 (100), 91 (12), 77 (7). Calcd for $C_{19}H_{22}O_2$: M, 282.1618. Found: *m/z* 282.1611.

3.2.21. 1-(4-Methoxyphenyl)-2-propylpent-4-en-1-one (31c). Colorless oil; IR (neat) 3077, 2959, 2873, 1673 (CO), 1600, 1248, 1171, 1032, 841 cm^{-1}; ^{1}H NMR δ 0.87 (3H, t, J=7.3 Hz), 1.28 (2H, sextet, J=7.6 Hz), 1.47–1.54 (1H, m), 1.68–1.80 (1H, m), 2.24 (1H, quintet, J=7.0 Hz), 2.49 (1H, quintet, J=7.0 Hz), 3.47 (1H, quintet, J=7.3 Hz), 3.87 (3H, s), 4.95 (1H, d, J=10.1 Hz), 5.02 (1H, d, J=13.5 Hz), 5.74 (1H, m), 6.94 (2H, d, J=8.9 Hz), 7.95 (2H, d, J=8.9 Hz). MS *m/z* (%) 232 (M$^+$, 2), 190 (17), 136 (9), 135 (100), 92 (8), 77 (9). Calcd for $C_{15}H_{20}O_2$: M, 232.1462. Found: *m/z* 232.1468.

3.2.22. 4-Methyl-1-phenylheptan-3-one (31d). Colorless oil; IR (neat) 2961, 2932, 2873, 1712 (CO), 1496, 1455, 1377, 1082, 750, 700 cm^{-1}; ^{1}H NMR δ 0.87 (3H, t, J=7.4 Hz), 1.03 (3H, d, J=7.1 Hz), 1.18–1.32 (3H, m), 1.56–1.63 (1H, m), 2.50 (1H, sextet, J=7.1 Hz), 2.75 (2H, dt, J=7.6, 3.6 Hz), 2.89 (2H, t, J=7.6 Hz), 7.15–7.20 (3H, m), 7.27 (2H, t, J=7.6 Hz). MS *m/z* (%) 204 (M$^+$, 15), 162 (55), 133 (41), 119 (12), 105 (100), 91 (99), 71 (42). Calcd for $C_{14}H_{20}O$: M, 204.1514. Found: *m/z* 204.1521.

3.2.23. 4-Benzyl-1-phenylheptan-3-one (31e). Colorless oil; IR (neat) 3028, 2958, 2931, 1713 (CO), 1455, 1082, 748, 699 cm^{-1}; ^{1}H NMR δ 0.85 (3H, t, J=7.0 Hz), 1.18–1.27 (2H, m), 1.35–1.42 (1H, m), 1.57–1.64 (1H, m), 2.39 (1H, ddd, J=17.7, 9.5, 6.1 Hz), 2.57–2.85 (6H, m), 7.06 (2H, d, J=7.3 Hz), 7.09 (2H, d, J=7.3 Hz), 7.14–7.27 (6H, m). MS *m/z* (%) 280 (M$^+$, 15), 237 (31), 189 (7), 175 (14), 147 (9), 133 (20), 117 (7), 105 (39), 91 (100), 77 (7). Calcd for $C_{20}H_{24}O$: M, 280.1828. Found: *m/z* 280.1829.

3.2.24. 1-Phenyl-4-propylhept-6-en-3-one (31f). Colorless oil; IR (neat) 2959, 2932, 2873, 1712 (CO), 1641, 1454, 1083, 916, 700 cm^{-1}; ^{1}H NMR δ 0.86 (3H, t, J=7.3 Hz), 1.20 (2H, sextet, J=7.3 Hz), 1.33–1.40 (1H, m), 1.52–1.58 (1H, m), 2.14 (1H, quintet, J=7.1 Hz), 2.29 (1H, quintet, J=7.1 Hz), 2.51–2.57 (1H, m), 2.73 (2H, t, J=7.4 Hz), 2.87 (2H, t, J=7.4 Hz), 4.96–5.01 (2H, m), 5.62–5.71 (1H, m), 7.15–7.22 (3H, m), 7.26–7.29 (2H, m). MS *m/z* (%) 230 (M$^+$, 6), 188 (32), 133 (56), 105 (100), 91 (91), 77 (12). Calcd for $C_{16}H_{22}O$: M, 230.1671. Found: *m/z* 230.1673.

3.2.25. 9-Methyl-8-triethylsiloxy-1,4-dioxaspiro[4.6]undec-8-ene (32). To a solution of *t*-BuMgCl (0.24 mmol) in 2 ml of dry THF in a flame-dried flask at −45 °C under argon atmosphere was added a solution of the adduct **12b** (72 mg, 0.2 mmol) in 2 ml of dry THF dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min. To a solution of the magnesium alkoxide was added *i*-PrMgCl (0.4 mmol) dropwise with stirring. The reaction mixture was stirred at −45 °C for 10 min and slowly allowed to warm to room temperature. To a solution of the enolate was added HMPA (0.14 ml; 0.8 mmol) dropwise at room temperature with stirring. After being stirred for

10 min, to a solution of the enolate was added triethylsilylchloride (0.1 ml; 0.6 mmol) dropwise with stirring. After 30 min, the reaction was quenched with H_2O. The whole was extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over $MgSO_4$. The product was purified by silica gel flash column chromatography to afford **32** (45 mg, 75%) as a colorless oil; IR (neat) 2954, 2877, 1678, 1377, 1239, 1176, 1110, 1018, 860, 744 cm^{-1}; ^1H NMR δ 0.65 (6H, q, $J=7.9$ Hz), 0.98 (9H, t, $J=7.9$ Hz), 1.64 (3H, s), 1.70–1.73 (2H, m), 1.75–1.78 (2H, m), 2.02–2.06 (2H, m), 2.23–2.28 (2H, m), 3.93 (4H, s). MS *m*/*z* (%) 298 (M^+, 100), 253 (24), 237 (81), 207 (36), 197 (25), 183 (22), 123 (25), 115 (45), 99 (66), 87 (78). Calcd for $C_{16}H_{30}O_3Si$: M, 298.1963. Found: *m*/*z* 298.1963.

3.2.26. 1-(4-Methoxyphenyl)-1-triethylsiloxy-1-butene (33). Colorless oil; IR (neat) 2957, 2877, 1609, 1511, 1249, 1127, 846, 745 cm^{-1}; ^1H NMR δ 0.61 (6H, q, $J=8.0$ Hz), 0.88 (3H, t, $J=7.3$ Hz), 0.93 (9H, t. $J=8.0$ Hz), 1.39 (2H, sextet, $J=7.3$ Hz), 2.07 (2H, q, $J=7.3$ Hz), 3.82 (3H, s), 4.97 (1H, t, $J=7.3$ Hz), 6.86 (2H, d, $J=8.9$ Hz), 7.33 (2H, d, $J=8.9$ Hz). MS *m*/*z* (%) 306 (M^+, 30), 277 (100), 249 (49), 135 (24), 115 (23), 87 (28). Calcd for $C_{18}H_{30}O_2Si$: M, 306.2013. Found: *m*/*z* 306.2014.

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References and notes

1. For reviews: (a) William Lever, O., Jr. *Tetrahedron* **1976**, *32*, 1943. (b) Martin, S. F. *Synthesis* **1979**, 633. (c) Stowell, J. C. *Chem. Rev.* **1984**, *84*, 409. (d) Badham, N. F. *Tetrahedron* **2004**, *60*, 11.
2. (a) Krow, G. R. *Tetrahedron* **1987**, *43*, 3. (b) Hesse, M. In *Ring Enlargement in Organic Chemistry*; VHC: Weinheim, 1991. (c) Dowd, P.; Zhang, W. *Chem. Rev.* **1993**, *93*, 2091.
3. (a) Taguchi, H.; Yamamoto, H.; Nozaki, H. *J. Am. Chem. Soc.* **1974**, *96*, 6510. (b) Taguchi, H.; Yamamoto, H.; Nozaki, H. *Tetrahedron Lett.* **1976**, 2617. (c) Taguchi, H.; Yamamoto, H.; Nozaki, H. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1592. (d) Villieras, J.; Perriot, P.; Normant, J. F. *Synthesis* **1979**, 968. (e) Ward, H. D.; Teager, D. S.; Murray, Jr.; R, K. *J. Org. Chem.* **1992**, *57*, 1926.
4. Abraham, W. D.; Bhupathy, M.; Cohen, T. *Tetrahedron Lett.* **1987**, *28*, 2203.
5. (a) Satoh, T.; Itoh, N.; Gengyo, K.; Yamakawa, K. *Tetrahedron Lett.* **1992**, *33*, 7543. (b) Satoh, T.; Itoh, N.; Gengyo, K.; Takada, S.; Asakawa, N.; Yamani, Y.; Yamakawa, K. *Tetrahedron* **1994**, *50*, 11839.
6. (a) Satoh, T. *J. Syn. Org. Chem. Jpn.* **1996**, *54*, 481. (b) Satoh, T. *J. Syn. Org. Chem. Jpn.* **2003**, *61*, 98.
7. Preliminary results of this study were reported as a communication: Satoh, T.; Miyashita, K. *Tetrahedron Lett.* **2004**, *45*, 4859.
8. (a) Pirkle, W. H.; Adams, P. E. *J. Org. Chem.* **1980**, *45*, 4111. (b) Acherar, S.; Audran, G.; Cecchin, F.; Monti, H. *Tetrahedron* **2004**, *60*, 5907.
9. (a) Colvin, E. W. In *Silicon in Organic Synthesis*; Butterworths: London, 1981. (b) Weber, W. P. In *Silicon Reagents for Organic Synthesis*; Springer: Berlin, 1983. (c) Colvin, E. W. In *Silicon Reagents in Organic Synthesis*; Academic: London, 1988.
10. Satoh, T.; Onda, K.; Yamakawa, K. *J. Org. Chem.* **1991**, *56*, 4129.

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Efficient incorporation of 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-imidazole-4-carboxamide into DNA via a suitable convertible phosphoramidite derivative

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Abstract—The synthetic scheme of 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-imidazole-4-carboxamide (**3**) is based on the ring contraction of pyrimidine (5-BrdU) into imidazolin-2-one. The rearrangement leads to the unexpected mixture of the deoxynucleoside **4β** and its α anomer. The mechanism of the anomerisation under basic conditions is proposed. Further conversion of 4-carboxylic acid into amide affords the title compound **3**. The conversion of 4-carboxylic acid into ethyl ester is preferred for the preparation of the phosphoramidite derivative **11** suitable for chemical incorporation of the modified nucleobase into DNA. Thermal denaturation studies show that 2-oxoY within the sequence used pairs more favorably with the purines than the pyrimidines.

1. Introduction

There has been a tremendous interest in the design and synthesis of DNA nucleoside mimics for a variety of explorations and applications. For example, the search for universal or ambiguous base has been intensive in recent years.[1] Among the candidates a family of azole carboxamide nucleosides **1** (Fig. 1) has been studied.[2–5] Because of the rotation about the glycosidic bond (*syn*/*anti*) and the exocyclic carboxamide function, members of the family **1** may adopt four different conformations, each could pair with one of the four canonical bases. However, experimental data have shown that these molecules do not use this potential but manifest a preference for some particular nucleobases.[6–8] Other related five membered rings have been synthesized, such as the imidazolin-2-one (**2**), which has been proposed as structural isomer of thymidine,[9] and 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-imidazole-4-carboxamide (**3**) as a 'lure' at ambiguous positions.[10]

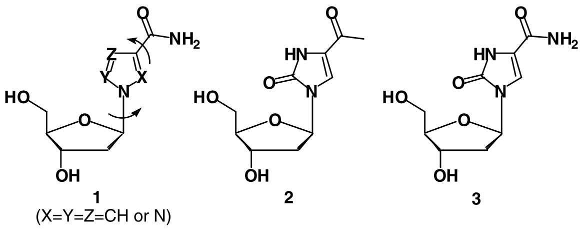

Figure 1. Structure of DNA nucleoside analogues.

We previously developed several artificial bases as mutagenic agents based on substituted five-membered ring.[6,11,12] Interestingly, nucleoside **3** (designed 2-oxodY) belonging to the imidazole carboxamide family **1**, features an added H-bond acceptor group at C-2 which might contribute to base pairing. Accordingly, 2-oxodY might have an enhanced potential for mutagenesis experiments. Although the synthesis of **3** and its DNA incorporation according to H-phosphonate chemistry have been previously reported,[10] a rapid access to oligonucleotides containing **3** via the phosphoramidite chemistry is still needed to study the ambiguous pairing potential of this artificial nucleobase.

Herein, we describe a new route for simple and efficient incorporation of the nucleobase 2-oxoY into DNA fragments via a convenient phosphoramidite derivative. During the synthesis of the nucleoside **3**, we observed the anomerisation of the intermediate 1-(2-deoxy-D-β-ribofuranosyl)-2-oxo-imidazole-4-carboxylic acid (**4**) and we propose a mechanism for this unexpected anomerisation.

Keywords: Nucleobase; Nucleoside; Base pairing; Anomerisaton; Conformation.

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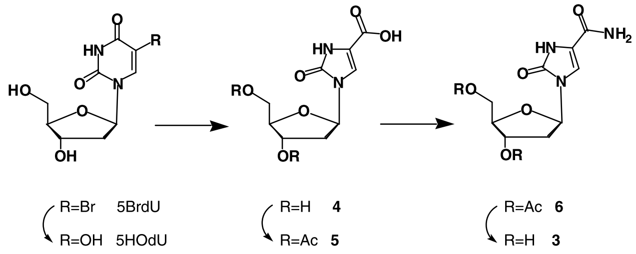
2. Results and discussion

2.1. Synthesis of 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-imidazole-4-carboxylic acid (4)

The synthetic scheme of **3** is based on the conversion of 5-halogeno-uridine into imidazoline nucleoside according to a known rearrangement.[13] Thus, mild alkaline treatment of 5-bromo-2′-deoxyuridine (5BrdU) results in the ring contraction of pyrimidine into imidazoline nucleoside **4** via the 5-hydroxy derivative (5OHdU) (Scheme 1).



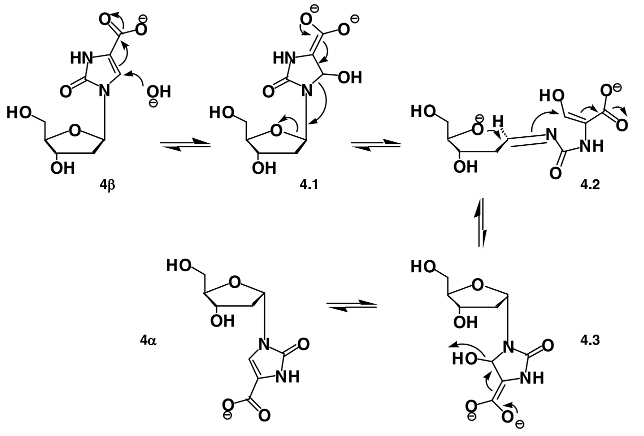
Scheme 1. Synthesis of 4-carboxamide-1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole (**3**).

In our hands, we observed that the yield of the reaction and the anomeric purity of the target nucleoside were dependent on the reaction time and basic conditions used. Treatment of 5BrdU in a buffered sodium bicarbonate solution for 25 h (pH maintained to ~8 by addition of CO_2), followed by further heating of the resulting solution for another 16 h (pH raised to ~10) resulted in the formation of the expected imidazoline nucleoside **4** in good yield (75–86%). However, examination of ^{1}H and ^{13}C NMR spectra of **4** revealed the presence of another deoxynucleoside in a proportion varying from 6 to 11% depending on the experiments. The H-5, H2′ and H2″ signals, as well as C5 and sugar carbon signals indicated a mixture of the desired **4β** and the corresponding α anomer. In particular, the chemical shifts of H2″ proton (ddd for the β anomer and apparent dt for the α anomer) and H2′ proton (apparent septuplet for the β anomer and apparent quintuplet for the α anomer) are separated from 0.52 ppm for the α anomer compared to 0.14 ppm for the β anomer. Chromatographic separation of anomers at this stage was tedious resulting in a reduced yield (40–50%) of pure 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxylic acid (**4β**); moreover it was not possible to isolate pure anomer **4α**.

In another attempts to synthesize compound **4**, 5BrdU was treated with a 0.1 N NaOH solution (pH 13) at 100 °C.[13] The reaction was stopped after 6 h while some starting material and the intermediate 5OHdU were still detected on TLC. NMR analysis of an aliquot of the reaction mixture before chromatography revealed the absence of detectable α anomer. After chromatographic purification, pure **4β** was isolated in 55% yield.

2.2. Base catalysed anomerisation of 4

Formation of such a by-product in the ribo or deoxy series was not discussed in the previous reports.[10,13] We explain this unusual anomerisation (Scheme 2) by a mechanism similar to that observed during treatment of 5-formyluridine with alkaline conditions at room temperature.[14] Attack of hydroxide ion on C-5 of **4β** and subsequent migration of the double bond give the intermediate **4.1**, which undergoes by opening both the deoxyribose and imidazolin-2-one rings into the acyclic ureide **4.2**. Ring closure leads to the formation of both **4.1** and **4.3**. From the hydroxy intermediate **4.3**, nucleoside **4α** can be formed. As this base-catalysed anomerisation does not occur on treatment of 5-bromo-(2-deoxy)ribouridine, this reactivity is explained by the presence of the electron withdrawing group, 5-formyl in the case of pyrimidine or 4-carboxylic acid in the case of imidazoline ring.



Scheme 2. Proposed mechanism for anomerisation of **4**.

2.3. Synthesis and properties of 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-imidazole-4-carboxamide (3)

The following steps for the preparation of **3** are the conversion of carboxylic acid into amide via activated ester. Acetylation of **4β** by acetic anhydride in pyridine afforded **5** in 84% yield (Scheme 1). Reaction of **5** in acetonitrile with pentafluorophenol in the presence of DCC, followed by treatment of the crude ester in 33% aqueous ammonia solution at 4 °C for 20 min gave **6** in 78% yield. The anomeric purity of **6** was confirmed by NMR analysis. When a mixture of α/β anomers was used in place of pure **4β**, the separation of **6α** and **6β** anomers is easier than of **4α** and **4β**, and pure acetylated **6β** was isolated in a good yield. Finally, ammonolysis of the acetyl group at room temperature (96% yield) gave the title nucleoside **3**, which was fully characterized by NMR, UV, HMRS and elemental analysis. These data are in agreement with those previously reported.[10] The route described here affords nucleoside **3** in a good overall yield (63% from **4**).

In order to assess the mutagenic potential of this nucleobase, we examined the possible occurrence of several tautomeric forms (acido–basic character) and of different base conformations about the deoxyribose.

The p*K*a value of nucleoside **3** was determined by pH-dependent NMR titration studies. ^{1}H NMR and HMBC NMR experiments were recorded at 400 MHz. The chemical shifts of aromatic proton (H-5) and carbons (C-2, C-4, C-5) were measured in the range of pH 6.5–12.6.

Table 1. Conformational parameters of **3** from PSEUROT

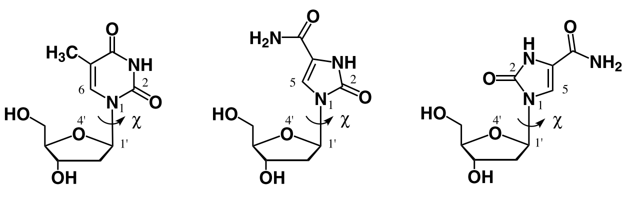
pH	P_N	P_S	Φ_N	Φ_S	p*N*	p*S*	r.m.s.
7.2	13.8	150.6	31.2	35.3	36%	64%	0.001
12.6	12.9	141.0	35.0	34.7	30%	70%	0.028

The marked shifts, noticed at C4 and C2, fitted to the equation of a single ionization equilibrium. The p*K*a value (N3) so obtained is 10.1 ± 0.2, while the p*K*a value (N3) for dT is 9.8.[15] This result indicates that the keto form predominates at physiological pH.

The conformational analysis of the furanose puckering of **3** was determined by ^{1}H NMR using vicinal spin-coupling constants of the sugar protons and PSEUROT program (version 6.1).[16] The values of the phase angle of pseudorotation (P), the amplitude of pucker (Φ) and the population of conformers are given Table 1. Calculations indicate that **3** preferentially adopts in solution a C2′-endo conformation (64% at pH 7.2 and 70% at pH 12.6).

The orientation of the aromatic base with respect to the sugar moiety is determined by the glycosidic torsion angle χ defined as the O-4′-C-1′-N-1-C-2 torsion in pyrimidines and the O-4′-C-1′-N-9-C-4 torsion in purines. Although quantitative evaluation of interproton distances by mean of NOE measurement is widely used, most often (and in absence of proton at C2 position) the values of χ are determined from the measurement of three-bond carbon–proton scalar couplings $^3J_{\mathrm{CH}}$ across the glycosidic bond and DFT analysis using Karplus equations.[17] Thus, the maximum magnitude of $^3J_{\mathrm{C2/4\text{-}H1'}}$ for purines and pyrimidines in the *anti* glycosidic torsion ($\chi \approx -120$ °C) is observed between 2.0 and 2.7 Hz, while in the *syn* orientation ($\chi \approx 60$ °C) the maximum is between 5.5 and 5.7 Hz for dA, dG, dC and 6.9 Hz for T. A significant difference between purine and pyrimidine bases is found for $^3J_{\mathrm{C6/8\text{-}H1'}}$; in the *anti* region, the maximal varied from 4.3 to 4.5 Hz for purines and from 3.9 to 4.0 Hz for pyrimidines, while in the *syn* region the maximum is 3.9 Hz for dA and dG, versus 5.7 Hz for dC and T.

The $^3J_{\mathrm{C2\text{-}H1'}}$ and $^3J_{\mathrm{C5\text{-}H1'}}$ values of nucleoside **3** were determined at 30 mM in D_2O (pH 7.2) by gradient selected ^3J-HMBC NMR experiments recorded at 600 MHz. The experimental values of $^3J_{\mathrm{CH}}$ were 2.05 Hz for $^3J_{\mathrm{C2\text{-}H1'}}$ and 4.20 Hz for $^3J_{\mathrm{C5\text{-}H1'}}$. Comparison of these values with the $^3J_{\mathrm{CH}}$ values reported for purines and pyrimidines[17] and the fact that $^3J_{\mathrm{C5\text{-}H1'}} > {}^3J_{\mathrm{C2\text{-}H1'}}$[18] led us to conclude that the preferred conformation of nucleoside **3** in solution is the *anti* conformation (Fig. 2).

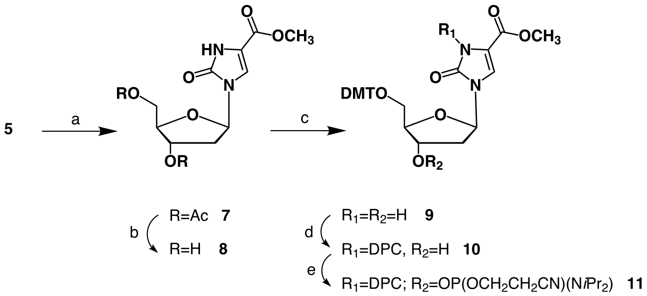


dT in *anti* orientation 2-oxodY in *anti* orientation 2-oxodY in *syn* orientation

Figure 2. *Anti* and *syn*-χ conformers of 2-oxodY compared to dT (*anti*-χ).

2.4. Synthesis of the new phosphoramidite building block (11) and oligonucleotides containing the nucleoside analogue 2-oxodY

The nucleoside was next converted into a phosphoramidite building block for its chemical incorporation into DNA fragments. In order to circumvent the reactivity of the carboxamide group under phosphitylation conditions,[5] we prepared a convertible phosphoramidite suitable for automatic DNA synthesis (Scheme 3). The methyl ester was synthesized from the 3′,5′-*O*-diacetylated acid derivative **5** by reaction with methyl chloroformate in anhydrous dichloromethane in the presence of triethylamine and DMAP.[19] The reaction occurred via the formation of mixed anhydride and compound **7** was isolated in 75% yield. Treatment of **7** with a 0.5 N sodium methylate in methanol afforded methyl 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-imidazole-4-carboxylate (**8**) in nearly quantitative yield.



Scheme 3. Synthesis of the phosphoramidite derivative **11**. Reagents and conditions: (a) CH_3OCOCl, CH_2Cl_2, Et_3N, 1 h, then DMAP, 8 h; (b) $NaOCH_3$, 4 °C, 30 min, 75% from **5**; (c) DMTCl chloride, pyridine, 1.5 h, 78%; (d) diphenylcarbamoyl chloride, DIPEA, pyridine, 2 h, 65%; (e) 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite, DIPEA, CH_2Cl_2, 30 min, 75%.

The expected conversion of the methyl ester into amide under oligonucleotide deprotection conditions (ammonia at 55 °C for 8 h) was checked at the nucleoside stage. Thus, complete amidation of **8** into **3** was confirmed by HPLC and NMR analyses. Moreover, no anomerisation was detected under these conditions.

The phosphoramidite **11** was obtained in three steps according to classical methodology.[20] The 5′-*O*-dimethoxytritylation of **8** (78% yield) followed by *N*-3 protection of resulting **9** by a diphenylcarbamoyl group gave compound **10** in 65% yield. When *N*-3 protection took place before 5′-*O*-dimethoxytritylation, **10** was isolated in a reduced global yield (29% in place of 51%). Finally, phosphitylation of **10** with 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite in the presence of di*iso*propylethylamine in CH_2Cl_2 afforded the phosphoramidite **11** in 75% yield.

Table 2. Synthesized oligonucleotides using **11**

Sequences	Calculated mass	Measured mass
5′-GCAT$\mathbf{Y}^{\mathbf{oxo}}$GTCATAGCTGTTTCCTG-3′	6704.1133	6704.1315
5′-TGAC$\mathbf{Y}^{\mathbf{oxo}}$GTCATAGCTGTTTCCTG-3′	6704.1133	6704.1395
5′-ACTTGGCC$\mathbf{Y}^{\mathbf{oxo}}$CCATTTTG-3′	5124.8586	5124.8692
5′-CAAAATGG$\mathbf{Y}^{\mathbf{oxo}}$GGCCAAGT-3′	5240.9171	5240.9126

Four oligonucleotides (17- and 22-mers, Table 2) containing the modified base 2-oxoY were synthesized on an automated DNA synthesizer according to standard β-cyanoethyl phosphoramidite chemistry. The coupling efficiency of **11** was not different from those of the normal phosphoramidites. Conversion of the ester into amide occurred during the final deprotection step. The crude oligomers were purified by reverse phase HPLC at two stages (DMT on, then after detritylation). Integrity of the modified base was assessed by high-resolution mass spectrometry (MALDI-TOF) and by reversed phase HPLC analysis of nucleosides after enzymatic digestion.

2.5. Thermal denaturation studies

In order to assess the thermal stability of heteroduplexes containing 2-oxodY, UV thermal-denaturation experiments were conducted using a system composed of two complementary heptadecamers. Placing the artificial base in the ninth position of each strand allowed evaluation of the pairing preferences of 2-oxodY and the effects of local nearest-neighbor bases (2-oxodY within a C- or G-rich region). The melting temperatures (T_m) derived from respective melting curves are listed in Table 3.

All duplexes containing **3** have lower T_m values (entries 3–11) than the corresponding natural bases (A:T, entry 1 and C:G, entry 2). However, these values are significantly different according to the central base pairs involved. The most favorable T_m values are found when **3** is located opposite A ($\Delta T_m = -3$ °C) and in a lesser extent opposite G ($\Delta T_m = -6$ °C). Duplexes involving C opposite **3** are significantly less stable, though in the range of mismatches (entries 12–14). No significant context effect can be inferred from the different T_ms involving 2-oxodY. On the basis of melting temperatures, the nucleoside **3** shows a preference for pairing to the natural bases in the order **3**:A > **3**:G > **3**:T > **3**:C,**3**:**3**.

3. Conclusion

In summary, we have described an efficient access to oligonucleotides containing 2-oxodY using a suitable convertible phosphoramidite derivative. During the first step of the synthesis of 2-oxodY, we have observed an unexpected anomerisation under the basic conditions of the ring contraction reaction and we proposed a mechanism for the formation of the α anomer. Using synthesized modified oligodeoxynucleotides, we have measured the thermal stability of different base pairs involving 2-oxoY and each of the four canonical bases. In the studied context, the nucleobase 2-oxoY shows a preference for pairing to purines. Because replication depends not only on the characteristic of the base pairs, but also on those of the DNA polymerase, further work is under way to determine the enzymatic recognition of this artificial base and its mutagenic potential through alternative pairing schemes.

4. Experimental

4.1. General

NMR spectra were recorded on a Bruker Avance 400 instrument at 400.13, 100.62 and 161.98 MHz, for ^{1}H, ^{13}C and ^{31}P, respectively. ^{1}H and ^{13}C chemical shifts are given in ppm (δ) relative to residual solvent peak in the case of DMSO-d_6 or relative to TMS in the case of D_2O and $CDCl_3$. Chemical shifts are reported in ppm (δ) relative to 85% phosphoric acid as external standard for ^{31}P NMR spectra. Mass spectra were recorded by the mass spectroscopy laboratory (CNRS-ICSN, Gif-sur-Yvette). TLC was carried out on analytical silica plates (Kieselgel 60 F_{254}/0.2 mm thickness) and spots were visualized by UV light, then revealed by sulfuric acid-anisaldehyde spray followed by heating. Silica gel column chromatography was performed using Merck silica gel 60 (230–400 mesh). HPLC was performed on a Perkin Elmer system equipped with a diode array detector. Preparative reverse phase HPLC were carried out with a C18 column using a flow rate of

Table 3. Melting temperatures (T_m)[a] of heteroduplexes containing canonical bases and 2-oxodY
5′-ACTTGGCC**X**CCATTTTG-3′
3′-TGAACCGG**Y**GGTAAAAC-5′

Entry	**X**:**Y**	T_m (°C)	Entry	**X**:**Y**	T_m (°C)
1	C:G	59	**2**	A:T	58
3	G:Y^{oxo}	53	**4**	A:Y^{oxo}	55
5	Y^{oxo}:G	52	**6**	Y^{oxo}:A	55
7	C:Y^{oxo}	46	**8**	T:Y^{oxo}	51
9	Y^{oxo}:C	46	**10**	Y^{oxo}:T	50
11	Y^{oxo}:Y^{oxo}	51	**12**	G:T[b]	55
13	C:T[b]	48	**14**	C:A[b]	49

[a] Conditions: 10 mM sodium cacodylate, 0.1 M NaCl at pH 7.2, 1 μM of each strand.
[b] Data from literature.[5]

5.5 mL/min and a linear gradient of CH_3CN (A) in 10 mM triethylammonium acetate buffer (B) at pH 7.5 over 20 min. Purity of all tested compounds was checked by analytical HPLC on a Perkin Elmer system with a reverse phase column (C18) using a flow rate of 1 mL/min and a linear gradient of A in B at pH 7.5 over 20 min and using a diode array detector. Solvents were spectroscopic or HPLC grade and reagents used without purification.

4.2. Synthesis of 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxamide (3)

4.2.1. 1-(2-Deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxylic acid (4β). *Method A*. A solution of 5-bromo-2′-deoxyuridine (3.34 g, 10.89 mmol) in 0.1 N NaOH (385 mL) was refluxed for 6 h while the pH was maintained at 13 by addition of 1 N NaOH (2×0.5 mL, after 2 and 4 h, respectively). The cooled reaction was neutralized by addition of 0.1 N HCl, concentrated and the resulting solution was passed through a column of cationic resin (Dowex H^+). The product was eluted with water and the eluate was concentrated under vacuo. The crude product was purified by silica gel column chromatography (0–20% MeOH in CH_2Cl_2, then 0–10% CH_3COOH in 80:20 CH_2Cl_2/MeOH); first fractions were collected to give **4β** as a pale yellow powder (1.46 g, 55%). R_f (*i*PrOH/NH_4OH/H_2O: 70:10:20): 0.34. ^1H NMR (DMSO-d_6) δ: 1.88 (ddd, 1H, H2′, $J=2.5$, 6.0, 13.0 Hz), 2.12 (m, 1H, H2″), 3.31–3.40 (m, 2H, H5′ and H5″), 3.60 (m, 1H, H4′), 4.12 (m, 1H, H3′), 5.72 (dd, 1H, H1′, $J=6.0$, 8.3 Hz), 6.97 (s, 1H, H5), 10.20 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 39.74 (C2′), 62.81 (C5′), 71.81 (C3′), 82.54 (C1′), 87.82 (C4′), 114.15 (C5), 119.28 (C4), 153.15 (C2), 162.63 (COOH). HRMS (MALDI-TOF) *m/z* calcd for $C_9H_{12}N_2O_6+Na$ 267.0593, found 267.0719.

Method B. A solution of 5-bromo-2′-deoxyuridine (5.0 g, 16.28 mmol) in 0.16 N $NaHCO_3$ (4.53 g in 0.33 mL) was refluxed for 25 h while the pH was maintained at 8.0 by addition of CO_2. The CO_2 arrival was suppressed and the pH raised rapidly to 10. After refluxing for 16 h, the cooled reaction was neutralized by addition of cationic resin (Dowex H^+), concentrated and the resulting solution was passed through a column of resin Dowex H^+. The product was eluted with water and the eluate was concentrated under vacuo. Purification by silica gel column chromatography afford compound **4** as a pale yellow powder (3.42 g, 86%) as a mixture of anomers *β/α* (95:5). ^1H NMR (D_2O) δ: 2.17 (dt, 0.05H, H2″α, $J=3.7$, 14.7 Hz), 2.27 (ddd, 0.95H, H2″β, $J=3.6$, 6.4, 14.0 Hz), 2.41 (ddd, 0.95H, H2′β, $J=6.4$, 7.4, 14.0 Hz), 2.69 (ddd, 0.05H, H2′α, $J=7.4$, 14.7 Hz), 3.59–3.70 (m, 2H, H5′ and H5″αβ), 3.92 (m, 0.95H, H4′β), 4.16 (m, 0.05H, H4′α), 4,36 (m, 0.05H, H3′α), 4.42 (m, 1H, 0.95H, H3′β), 5.94 (m, 1H, H1′αβ), 7.04 (s, 0.95H, H5β), 7.25 (s, 0.05H, H5α). ^{13}C NMR (D_2O) δ: 38.49 (C2′β), 39.02 (C2′α), 61.71 (C5′α), 62.07 (C5′β), 71.12 (C3′α), 71.47 (C3′β), 82.96 (C1′β), 83.57 (C1′α), 86.76 (C4′β), 87.40 (C4′α), 113.90 (C5β), 115.07 (C5α), 120.50 (C4), 153.51 (C2), 166.47 (COOH).

4.2.2. 1-(3,5-*O*-diacetyl-2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxylic acid (5). To compound **4** (1.46 g, 6.0 mmol) in dry pyridine (60 mL) was added acetic anhydride (2.26 mL, 24 mmol). After stirring for 3 h under argon, methanol (2 mL) was added. The reaction mixture was evaporated, the resulting residue adsorbed on silica gel and loaded onto a column (0–20% MeOH in CH_2Cl_2) to give **5** as a powder (1.65 g, 84%). Rf (*i*PrOH/NH_4OH/H_2O: 70:10:20): 0.56. ^1H NMR (DMSO-d_6) δ: 2.05 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.25 (m, 1H, H2″), 2.60 (m, 1H, H2′), 4.09 (m, 1H, H4′), 4.12 (m, 1H, H5′), 4.21 (m, 1H, H5″), 5.19 (m, 1H, H3′), 5.85 (dd, 1H, H1′, $J=6.0$, 8.7 Hz), 7.23 (s, 1H, H5), 10.51 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 21.40 (CH_3), 21.64 (CH_3), 35.86 (C2′), 64.63 (C5′), 75.20 (C3′), 81.76 (C4′), 82.81 (C1′), 115.64 (C4), 117.45 (C5), 153.14 (C2), 161.18 (4-COOH), 170.85 ($COOCH_3$), 170.98 ($COOCH_3$). MS (ESI-TOF) *m/z* 329.1 $(M+H)^+$, 351.1 $(M+Na)^+$, 367.0 $(M+K)^+$.

4.2.3. 1-(3,5-*O*-diacetyl-2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxamide (6). To compound **5** (1.57 g, 4.78 mmol) in anhydrous acetonitrile (50 mL) were added at 4 °C pentafluorophenol (1.32 g, 7.18 mmol) and DCC (1.48 g, 7.17 mmol). After stirring for 45 min at room temperature, the insolubles were filtered off and rinsed with acetonitrile. The filtrates were evaporated, taken up in acetonitrile (50 mL) and treated at 4 °C with 33% aqueous ammonia (8 mL). After 20 min, the solution was evaporated at low bath temperature and the resulting residue purified by silica gel column chromatography (0–15% MeOH in CH_2Cl_2) to give **6** as a white foam (1.22 g, 78%). R_f (CH_2Cl_2/MeOH 90:10): 0.28. ^1H NMR (DMSO-d_6) δ: 2.12 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.38 (oct, 1H, H2″, $J=2.7$, 6.1, 14.1 Hz), 2.51 (m, 1H, H2′), 4.13–4.20 (m, 2H, H4′ and H5′), 4.26 (m, 1H, H5″), 5.28 (m, 1H, H3′), 5.90 (dd, 1H, H1′, $J=6.2$, 8.1 Hz), 7.19 (bs, 1H, NH_2), 7.39 (d, 1H, H5, $J=1.9$ Hz), 7.43 (bs, 1H, NH_2), 10.58 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 21.43 (CH_3), 21.62 (CH_3), 36.23 (C2′), 64.63 (C5′), 79.92 (C3′), 81.54 (C4′), 82.63 (C1′), 113.04 (C5), 118.59 (C4), 152.93 (C2), 160.93 ($CONH_2$), 170.87 ($COOCH_3$), 171.05 ($COOCH_3$). MS (ESI-TOF) *m/z* 350.1 $(M+Na)^+$, 366.1 $(M+K)^+$.

4.2.4. 1-(2-Deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxamide (3). To compound **6** (1.22 g, 3.74 mmol) in MeOH (85 mL) was added 33% aqueous ammonia (85 mL). After stirring for 20 min at room temperature, solvents were removed and the residue purified by silica gel column chromatography (0–25% MeOH in CH_2Cl_2). Compound **3** was obtained as a white powder (0.90 g, 95%). R_f (CH_2Cl_2/MeOH 90:10): 0.38. Mp 160–162 °C. R_t (0–20% A in B): 9.21 min. ^1H NMR (DMSO-d_6) δ: 2.06 (ddd, 1H, H2″, $J=3.1$, 6.2, 13.2 Hz), 2.15 (ddd, 1H, H2′, $J=5.8$, 7.9, 13.2 Hz), 3.47 (m, 2H, H5′ and H5″), 3.72 (m, 1H, H4′), 4.24 (m, 1H, H3′), 4.85 (t, 1H, 5′OH, $J=5.6$ Hz), 5.21 (d, 1H, 3′OH, $J=4.3$ Hz), 5.83 (dd, 1H, H1′, $J=6.2$, 7.8 Hz), 7.20 (bs, 1H, NH_2), 7.31 (s, 1H, H5), 7.35 (bs, 1H, NH_2), 10.43 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 39.22 (C2′), 62.45 (C5′), 71.17 (C3′), 81.87 (C1′), 87.31 (C4′), 112.89 (C5), 117.66 (C4), 152.50 (C2), 160.52 ($CONH_2$). MS (ESI-TOF) *m/z* 244.2 $(M+H)^+$, 266.1 (M+Na), 282.1 $M+K)^+$. HRMS (ESI-TOF) *m/z* calcd for $C_9H_{13}N_3O_5+Na$ 266.0753, found 266.0738. Anal. calcd for $C_9H_{13}N_3O_5+3/4H_2O$: C, 42.11; H, 5.31; N, 16.37, found C, 42.46; H, 5.34; N, 16.24. UV (H_2O/pH 6.2) λ_{max} 263 nm (ε

8920); (H_2O–NaOH/pH 12.6) λ_{max} 283 nm (ε 7340). UV (H_2O/pH 7.0) λ 260 nm (ε 8720).

4.3. Synthesis of the phosphoramidite unit (11)

4.3.1. Methyl 1-(3,5-*O*-diacetyl-2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-*1H*-imidazole-4-carboxylate (7). To a stirred solution of compound **5** (1.80 g, 5.48 mmol) in anhydrous CH_2Cl_2 (20 mL) and Et_3N (1.46 mL, 8.22 mmol) was added slowly at 0 °C methyl chloroformate (0.64 mL, 8.23 mmol). After 1 h at room temperature, the anhydride intermediate was totally formed (as judged by TLC). DMAP (0.80 g, 6.58 mmol) was added and the stirring was maintained for 8 h. The reaction was quenched with MeOH (5 mL), evaporated and purified by silica gel column chromatography (0–10% MeOH in CH_2Cl_2) to give **7** as a white powder (1.41 g, 75%). R_f (CH_2Cl_2/MeOH 90:10): 0.60. ^1H NMR (DMSO-d_6) δ: 2.05 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.29 (m, 1H, H2′), 2.65 (m, 1H, H2″), 3.75 (s, 3H, OCH_3), 4.10–4.18 (m, 2H, H4′ and H5′), 4.22 (m, 1H, H5″), 5.20 (m, 1H, H3′), 5.87 (dd, 1H, H1′, $J=6.0$, 8.6 Hz), 7.60 (d, 1H, H5, $J=1.7$ Hz), 11.00 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 21.38 (CH_3), 21.63 (CH_3), 35.85 (C2′), 52.41 (OCH_3), 64.61 (C5′), 75.15 (C3′), 81.81 (C4′), 82.87 (C1′), 114.57 (C4), 118.27 (C5), 153.06 (C2), 160.15 (4-$COOCH_3$), 170.84 ($COOCH_3$), 170.98 ($COOCH_3$). MS (ESI-TOF) *m/z* 343.0 $(M+H)^+$, 365.1 $(M+Na)^+$, 381.1 $(M+K)^+$.

4.3.2. Methyl 1-(2-deoxy-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-1*H*-imidazole-4-carboxylate (8). To compound **7** (1.40 g, 4.09 mmol) in MeOH (40 mL) was added at 0 °C a 0.5 N solution of MeONa in MeOH (20 mL). After 30 min, the solution was neutralized by addition of Dowex H^+. The resin was filtered off, rinsed with MeOH and the filtrates evaporated. Compound **8** was isolated by chromatography on a silica gel column as a white foam (1.06 g, 98%). R_f (CH_2Cl_2/MeOH: 90:10): 0.18. ^1H NMR (DMSO-d_6) δ: 2.03 (m, 1H, H2′), 2.27 (m, 1H, H2″), 3.43–3.55 (m, 2H, H5′ and H5″), 3.74–3.76 (m, 4H, OCH_3 and H4′), 4.24 (m, 1H, H3′), 4.91 (t, 1H, 5′OH, $J=5.6$ Hz), 5.19 (d, 1H, 3′OH, $J=4.1$ Hz), 5.85 (dd, 1H, H1′, $J=6.0$, 8.2 Hz), 7.57 (s, 1H, H5), 10.91 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 39.95 (C2′), 52.32 (OCH_3), 62.61 (C5′), 71.73 (C3′), 82.81 (C1′), 88.10 (C4′), 114.12 (C4), 118.52 (C5), 153.04 (C2), 160.21 (4-$COOCH_3$). MS (ESI-TOF) *m/z* 259.1 $(M+H)^+$, 281.2 $(M+Na)^+$, 297.2 $(M+K)^+$. HRMS (ESI-TOF) *m/z* calcd for $C_{10}H_{14}N_2O_6+Na$ 281.0750, found 281.0748.

4.3.3. Methyl 1-(2-deoxy-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-2-oxo-2,3-dihydro-*1H*-imidazole-4-carboxylate (9). Compound **8** (0.42 g, 1.63 mmol) was coevaporated with pyridine (3×5 mL) and resuspended in anhydrous pyridine (10 mL/mmol). To this was added DMTCl (1.15 equiv) and the resulting solution was allowed to stir at room temperature until the complete disappearance of the starting material as judged by TLC (less than 4 h). The reaction mixture was extracted with CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$, water, dried over Na_2SO_4, and evaporated. The crude product was purified by chromatography on silica gel to give **9** (0.71 g, 78%) as a pale yellow foam. R_f (dichloromethane/MeOH 90:10): 0.50. ^1H NMR (DMSO-d_6) δ: 2.10 (m, 1H, H2′), 2.36 (m, 1H, H2″), 3.05 (dd, 1H, H5′, $J=3.5$, 10.1 Hz), 3.16 (dd, 1H, H5″, $J=6.0$, 10.1 Hz), 3.68 (s, 3H, $COOCH_3$), 3.74 (2 s, 6H, OCH_3), 3.86 (m, 1H, H4′), 4.25 (m, 1H, H3′), 5.27 (d, 1H, 3′OH, $J=4.5$ Hz), 5.87 (t, 1H, H1′, $J=6.9$ Hz), 6.87 (m, 4H, H arom. DMT), 7.20–7.32 (m, 7H, H arom. DMT), 7.39 (m, 2H, H arom. DMT), 7.40 (d, 1H, H5), 10.95 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 37.30 (C2′), 49.85 (OCH_3), 53.35 (OCH_3–DMT), 53.45 (OCH_3–DMT), 62.67 (C5′), 69.13 (C3′), 80.16 (C1′), 83.76 (C4′), 83.97 (Cq DMT), 111.61 (CH DMT), 111.86 (C4), 115.78 (C5), 125.07 (Cq DMT), 126.13, 126.24, 128.12, 128.16 (CH DMT), 133.98, 134.07 and 143.36 (Cq DMT), 150.59 (C2), 156.49 and 156.51 (Cq DMT), 157.74 ($COOCH_3$). MS (ESI-TOF) *m/z* 259.1 $(M+H)^+$, 281.2 $(M+Na)^+$, 297.2 $(M+K)^+$.

4.3.4. Methyl 1-(2-deoxy-5-*O*-dimethoxytrityl-β-D-ribofuranosyl)-N^3-diphenylcarbamoyl-2-oxo-2,3-dihydro-*1H*-imidazole-4-carboxylate (10). Compound **9** (0.70 g, 1.25 mmol) was coevaporated with pyridine (3×5 mL) and resuspended in anhydrous pyridine (10 mL/mmol). To this solution wad added diphenylcarbamoyl chloride (1.2 equiv) and the resulting solution was allowed to stir at room temperature until the complete disappearance of the starting material as judged by TLC (less than 4 h). The reaction mixture was extracted with CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$, water, dried over Na_2SO_4, and evaporated. The crude product was purified by chromatography on silica gel to afford the title compound **10** as a foam (0.61 g, 65% yield). ^1H NMR (DMSO-d_6) δ: 2.30 (m, 1H, H2′), 2.41 (m, 1H, H2″), 3.10 (m, 1H, H5′), 3.16 (m, 1H, H5″), 3.68 (s, 3H, $COOCH_3$), 3.72 (s, 6H, OCH_3), 3.97 (m, 1H, H4′), 4.33 (m, 1H, H3′), 5.41 (d, 1H, 3′-OH, $J=4.6$ Hz), 5.99 (t, 1H, H1′, $J=6.3$ Hz), 6.84 (m, 4H, H arom. DMT), 7.17–7.29 (m, 7H, H arom. DMT), 7.36 (m, 4H, H arom. DMT and DPC), 7.38–7.55 (m, 8H, H arom. DPC), 7.86 (s, 1H, H5). ^{13}C NMR (DMSO-d_6) δ: 40.69 (C2′), 51.49 ($COOCH_3$), 55.34 (OCH_3), 64.02 (C5′), 70.39 (C3′), 84.35 (C1′), 85.93 (Cq DMT), 86.37 (C4′), 113.50 (CH DMT), 122.20 (C5), 126.96 (Cq DMT), 128.00 and 128.15 (CH DMT), 128.26 (C4), 129.77 (CH DPC), 130.00 and 130.07 (CH DMT), 135.80 and 135.82 (Cq DMT), 143.57 (C2), 145.11 (Cq DMT), 150.39 (CO DPC), 158.37 and 158.40 (Cq DMT), 162.28 ($COOCH_3$). HRMS (MALDI-TOF) *m/z* calcd for $C_{44}H_{41}N_3O_9+Na$ 778.2740, found 778.2761.

4.3.5. Phosphoramidite building block (11). To a solution of compound **10** (0.29 g, 0.39 mmol) and DIEA (0.27 mL, 1.56 mmol) in CH_2Cl_2 (2 mL) was added 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (0.1 mL, 0.43 mmol). After stirring for 1 h at room temperature, the reaction mixture was diluted in CH_2Cl_2 (3 mL) and washed in turn with 10% sodium carbonate (2×2 mL). The organic layer was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (AcOEt/Et_3N/CH_2Cl_2 2:1:17). Compound **11** was isolated after precipitation by CH_2Cl_2/hexane (2:30 mL) at −78 °C as a powder (0.28 g, 75%). ^1H NMR ($CDCl_3$) δ: 1.03–1.13 (7s, 12H, CH–CH_3), 2.24–2.32 (m, 1H, H2′), 2.36 (m, 1H, CH_2CN), 2.40–2.56 (m, 2H, H2′ and CH_2CN), 3.16–3.26 (m, 2H, H5′ and H5″), 3.48–3.67 (m, 4H, *CH*–CH_3 and OCH_2), 3.71 (2s, 9H, $COOCH_3$ and OCH_3), 4.16 (m, 1H, H4′), 4.51 (m, 1H, H3′), 5.84 (m, 1H, H1′), 6.72–6.78 (m, 4H, H arom. DMT), 7.13–7.35 (m, 19H, H arom. DMT and DPC), 7.57 and 7.59 (each s, 1H, H5). ^{31}P NMR (CDCl$_3$) δ: 150.17 and 150.58. MS (ESI-TOF) *m/z* 957.4 $(M+H)^+$, 978.6 $(M+Na)^+$, 994.8 $(M+K)^+$. HRMS (MALDI-TOF) *m/z* calcd for $C_{53}H_{58}N_5O_{10}P+Na$ 978.3819, found 978.3800.

4.4. Oligonucleotide syntheses

Oligonucleotides were synthesized on an Expedite Millipore DNA synthesizer according to standard β-cyanoethyl phosphoramidite chemistry on 1 μmol scale trityl-on mode using a 0.15 M solution of **11** in CH_3CN with a lengthened coupling time (10 min). Conversion of ester into amide was achieved during the standard deprotection conditions used (33% aqueous ammonia solution at 55 °C for 8 h). The synthesized oligonucleotides were separated from failure sequences at DMT stage by preparative HPLC on a Perkin Elmer system with a reverse phase column (C18) using a flow rate of 5.5 mL/min and a linear gradient of CH_3CN (A) in 10 mM triethylammonium acetate buffer (B) at pH 7.5 over 20 min. The corresponding fractions (detection at 254 nm) were concentrated and treated with 80% acetic acid for 15 min at room temperature. The detritylated oligonucleotides were further purified by reverse phase HPLC. Retention times after purification were determined on a C18 column using a 5–25% linear gradient of CH_3CN (A) in 10 mM triethylammonium acetate buffer (B) at pH 7.5 over 20 min. Oligomers were quantified by UV absorption at 260 nm. The molar extinction coefficient of oligonucleotides containing 2-oxodY were approximated by calculating the molar extinction coefficients of oligomers according to the nearest neighbor method where the analog was replaced by thymidine, since their ε were very similar: $\varepsilon_{260}=8400$ (pH 7.0) for **3** and $\varepsilon_{260}=8700$ (pH 7.0) for d*T*. Intact sequences of the four synthesized oligomers were confirmed by HRMS (MALDI-TOF) analysis. 5′-ACTTGGCC$\mathbf{Y}^{\mathbf{oxo}}$C CATTTTG-3′ (44% yield) $R_t=8.64$ min, *m/z* calcd for $C_{164}H_{211}N_{55}O_{105}P_{16}+Na$ 5124.8586, found 5124.8692. 5′-CAAAATGG$\mathbf{Y}^{\mathbf{oxo}}$GGCCAAGT-3′ (40% yield) $R_t=7.85$ min, *m/z* calcd for $C_{166}H_{207}N_{71}O_{97}P_{16}+Na$ 5240.9171, found 5240.9126. 5′-GCAT$\mathbf{Y}^{\mathbf{oxo}}$GTCA TAGCTGTTTCCTG-3′ (39% yield) $R_t=8.29$ min, *m/z* calcd for $C_{214}H_{273}N_{74}O_{136}P_{21}+Na$ 6704.1133, found 6704.1315. 5′-TGAC$\mathbf{Y}^{\mathbf{oxo}}$GTCATAGCTGTTTCCTG-3′ (39% yield) $R_t=8.09$ min, *m/z* calcd for $C_{214}H_{273}N_{74}O_{136}P_{21}+Na$ 6704.1133, found 6704.1395.

4.5. p*K*a Determination

Solutions of nucleoside **3** completely exchanged by deuterium were prepared in D_2O at 10 mM concentration. The pD of the solution has been adjusted by addition of microliter volumes of NaOD solutions (0.5, 0.1 and 0.01 M) and the pH value measured with a pH meter calibrated with standard buffers in H_2O. ^{1}H and ^{13}C spectra at different pH were recorded at 298 K using 64 scans on a Bruker Avance 400 spectrometer. pH-titration study consists of 12–15 data points over the range $6.5<\mathrm{pH}<12.6$. The p*K* value so obtained in D_2O was converted to value in H_2O by subtracting 0.2 unit to allow the isotopic effect.

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References and notes

1. Loakes, D. *Nucleic Acids Res.* **2001**, *29*, 2437.
2. Pochet, S.; Dugué, L.; Meier, A.; Marlière, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1679.
3. Bergstrom, D. E.; Zhang, P. M.; Johnson, W. T. *Nucleosides Nucleotides* **1996**, *15*, 59.
4. Johnson, W. T.; Zhang, P. M.; Bergstrom, D. E. *Nucleic Acids Res.* **1997**, *25*, 559.
5. Pochet, S.; Dugué, L. *Nucleosides Nucleotides Nucleic Acids* **1998**, *17*, 2003.
6. Sala, M.; Pezo, V.; Pochet, S.; WainHobson, S. *Nucleic Acids Res.* **1996**, *24*, 3302.
7. Zhang, P. M.; Johnson, W. T.; Klewer, D.; Paul, N.; Hoops, G.; Davisson, V. J.; Bergstrom, D. E. *Nucleic Acids Res.* **1998**, *26*, 2208.
8. Paul, N.; Nashine, V. C.; Hoops, G.; Zhang, P. M.; Zhou, J.; Bergstrom, D. E.; Davisson, V. J. *Chem. Biol.* **2003**, *10*, 815.
9. Jiang, X. J.; Kalman, T. I. *Nucleosides Nucleotides* **1994**, *13*, 379.
10. Fukuda, T.; Hamana, T.; Kikuchi, K.; Marumoto, R. *Z. Nat. J. Chem. Sci.* **1986**, *41*, 1571.
11. Strobel, H.; Dugué, L.; Marlière, P.; Pochet, S. *Nucleic Acids Res.* **2002**, *30*, 1869.
12. Strobel, H.; Dugué, L.; Marlière, P.; Pochet, S. *ChemBioChem* **2002**, *3*, 1251.
13. Otter, B. A.; Falco, E. A.; Fox, J. J. *J. Org. Chem.* **1969**, *34*, 2636.
14. Armstrong, V. W.; Dattagupta, J. K.; Eckstein, F.; Saenger, W. *Nucleic Acids Res.* **1976**, *3*, 1791.
15. Hall, R. H. In *The Modified Nucleosides in Nucleic Acids*; Columbia University Press: New York, 1971.
16. Altona, C.; Sundaral, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333.
17. Munzarova, M. L.; Sklenar, V. *J. Am. Chem. Soc.* **2002**, *124*, 10666.
18. Davies, D. B. *Studia Biophysica* **1976**, *55*, 29.
19. Kim, S.; Lee, J. I.; Kim, Y. C. *J. Org. Chem.* **1985**, *50*, 560.
20. Gait, M. J. In *Oligonucleotide Synthesis: The Practical Approach*; IRL/Oxford University Press: Cambridge, 1985.

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Molecular recognition of biotin, barbital and tolbutamide with new synthetic receptors

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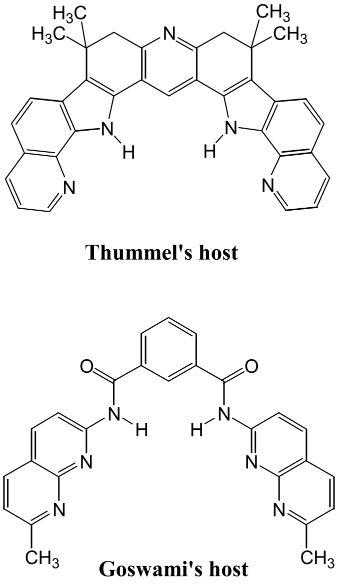
Dedicated to our friend Dr. Carmela Ochoa de Ocáriz on her 65th anniversary

Abstract—We have measured, by means of NMR titrations, the binding constants for the complexes between hosts *N*,*N*′-bis(6-methylpyridin-2-yl)-1,3-benzenedicarboxamide (**7**) and 4-chloro-*N*,*N*′-bis(6-methylpyridin-2-yl)-2,6-pyridinedicarboxamide (**8**, hydrated) with biotin methyl ester (**1**), *N*,*N*′-dimethylurea (**2**), 2-imidazolidone (**3**), *N*,*N*′-trimethylenurea (**4**), barbital (**5**) and tolbutamide (**6**) as guests. Molecular Mechanics calculations (Monte Carlo Conformational Search, AMBER and OPLS force fields, MacroModel v.8.1) on the complexes formed between the foregoing guests and hosts **7** and **8**, comparatively with 4-oxo-*N*,*N*′-bis(6-methylpyridin-2-yl)-1,4-dihydro-2,6-pyridinedicarboxamide (**9a**) have been carried out in order to determine the correlation between experimental and theoretical results and to understand the behaviour of the designed new hosts. Finally we have performed single point DFT [B3LYP/6-31G(d,p)] calculations on the optimised Molecular Mechanics geometries for the complexes between hosts **7**–**9** and water.

1. Introduction

In a preceding paper we have started the systematic study of host–guest complexes using guests of biological interest, with the final purpose to mimic the function of natural receptors by means of an iterative optimisation approach.[1] In that work we used two known hosts, those of Thummel[2] and Goswami[3] (Scheme 1) and five urea derivatives (the first five ones of the present work, biotin methyl ester (**1**), *N*,*N*′-dimethylurea (**2**), 2-imidazolidone (**3**), *N*,*N*′-trimethylenurea (**4**) and barbital (**5**) (Scheme 2).

Now we present our results on the interaction of two new hosts, *N*,*N*′-bis(6-methylpyridin-2-yl)-1,3-benzenedicarboxamide (**7**) and 4-chloro-*N*,*N*′-bis(6-methylpyridin-2-yl)-2,6-pyridinedicarboxamide (**8**), with six guests **1**–**6** (Scheme 2) adding to the previous list,[1] a sulfonyl urea, the anti-diabetic oral hypoglycaemic agent tolbutamide (**6**).[4] Even if our attempts to prepare host 4-oxo-*N*,*N*′-bis-



Scheme 1. Thummel's and Goswami's hosts.

Keywords: Molecular recognition; Binding constants; Molecular modelling; NMR titrations.

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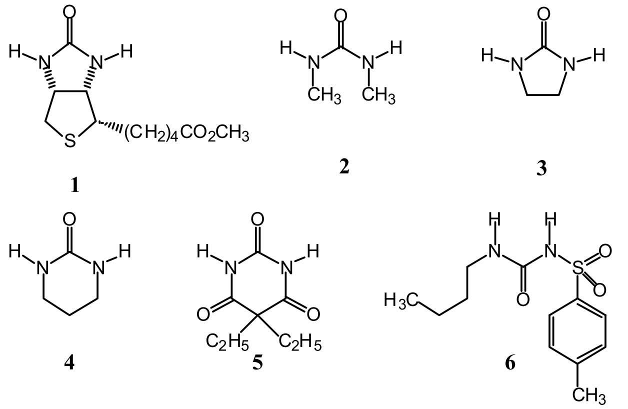
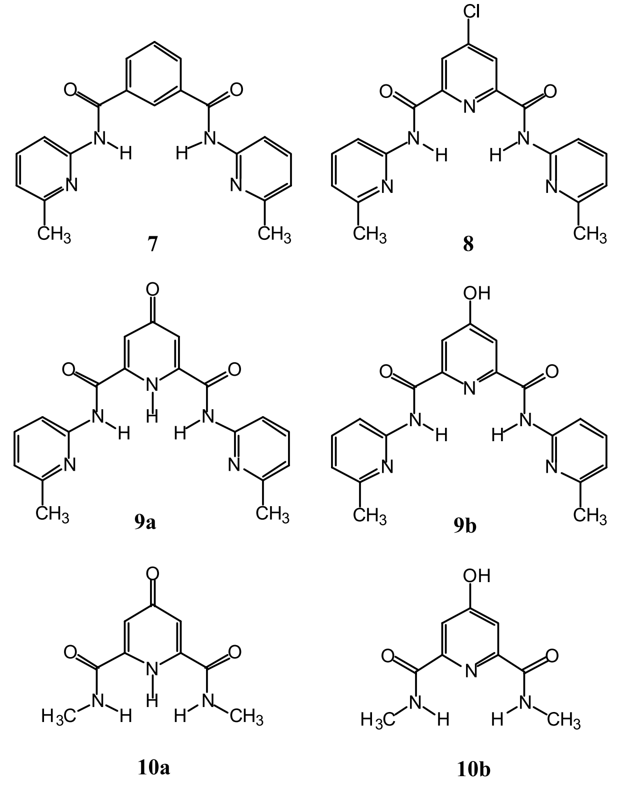


Scheme 2. The six guests (*N*,*N*′-dimethylurea is represented in the *E*,*E* conformation).

(6-methylpyridin-2-yl)-1,4-dihydro-2,6-pyridinedicarboxamide (**9a**) have been unsuccessful, we have studied its properties theoretically in comparison with hosts **7** and **8**.

^{1}H NMR titrations have been performed to measure and analyse the binding constants (K_b) of all guests with host **7** by a direct method,[1] using the Chemical Induced Shifts (CIS) on the 2-CH benzenic proton and the NHs of the 1,3-dicarboxamide groups. The same method was employed for complexes **8**:water and **8**:**1**, where the CIS on the NHs of the 2,6-pyridinedicarboxamides were quantified. The competitive method was needed to determine K_b in complexes of **8** and the remaining guests **2**–**6** measuring the NH-CIS of the urea moieties and the H_2O-CIS.[5]



Scheme 3. Hosts **7**, **8** and **9** and model compound **10**.

All complexes have been modelled at different theoretical levels using the Monte Carlo conformational search with both AMBER and OPLS Force Fields (MacroModel v.8.1). We have carried out single point calculations at B3LYP/ 6-31G(d,p) level on the optimised Molecular Mechanics geometries (AMBER Force Field) for the complexes between hosts **7**, **8** and the two tautomers **9a** and **9b** with water (Scheme 3). The pyridone/hydroxypyridine tautomerism in **9** has also been approached with two models **10a** and **10b** at the B3LYP/ 6-31G(d,p) level but with complete optimisation of the geometry.

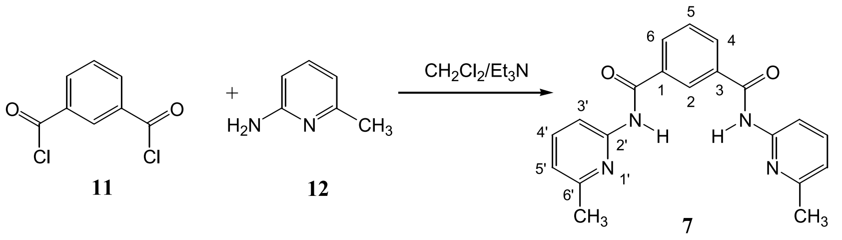
2. Results and discussion

2.1. Chemistry

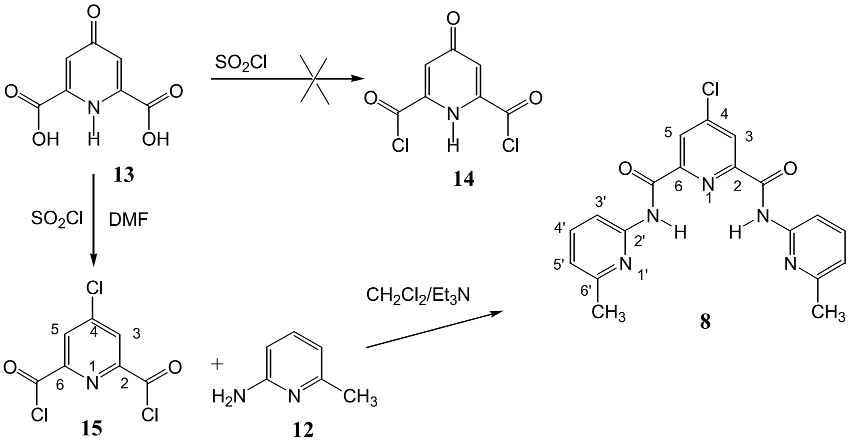
N,*N*′-Bis(6-methylpyridin-2-yl)-1,3-benzenedicarboxamide (**7**) was prepared according to Scheme 4 by condensation reaction of isophthaloyl chloride (**11**) with 2-amino-6-methylpyridine (**12**).[3]

The various attempts to obtain host **9** resulted in the synthesis of **8** (Scheme 5). 4-Oxo-1,4-dihydro-2,6-pyridinedicarboxylic acid or chelidamic acid (**13**) was treated with thionyl chloride to yield only 4-chloro-2,6-pyridinedicarbonyl dichloride (**15**),[6] being unable to reproduce the literature results where a mixture of **14** with **15** was formed and used without isolation to obtain several dicarboxamides.[7]

We also prepared the diethyl ester **16** from chelidamic acid **13** and ethyl orthoformate in acetic acid which would be further reacted with **12**, but the condensation between the ester and the amine did not occur. Other assays were the reaction of chelidamic acid **13** with 2-amino-6-methylpyridine (**12**) in the presence of several dehydrating agents (EDC/DMAP, DCC/DMAP) in different conditions but no signals attributable to **9** were apparent in the ^{1}H NMR spectra.



Scheme 4. Synthesis of host **7** from isophthaloyl chloride (**11**) and 2-amino-6-methylpyridine (**12**).



Scheme 5. Synthesis of host **8** from chelidamic acid (**13**) and 2-amino-6-methylpyridine (**12**).

Table 1. Experimental binding constants (M^{-1}) for complexes of host **7**

Guest	K_b (20–80%) 2-CH CIS	K_b (20–80%) NH CIS	Average K_b	ΔG (kJ mol^{-1})
1	950±86	1000±62	975	−17.2
2	≤10 [a]	≤10[a]	≤10	−4.0[b]
3	1400±85	1500±90	1450	−18.1
4	2250±353	2350±412	2300	−19.3
5	2442±384	2308±294	2375	−19.4
6	[c]	600±125	600	−15.9

[a] No CIS were observed.

[b] Calculated from $K_b=5$.

[c] No CIS was observed on the 2-CH proton.

Table 2. Experimental binding constants (M^{-1}) for complexes of host **8**

Guest	K_b (20–80%)	Round K_b	ΔG (kJ mol^{-1})
Water	93±10[a]	95	−11.4
1	3600±640[b]	3600	−20.4
2[c,d]	≤10	≤10	−4.0[c]
3	141±25[d]	140	−12.3
4	100±18[d]	100	−11.5
5	274±74[d]	275	−14.0
6	735±207[d]	735	−16.5

[a] Direct titration measuring the NH-CIS of the host **8**.

[b] Direct titration measuring the NH-CIS of the host **8**, the biotin NH chemical shifts do not change on complexation.

[c] Calculated from $K_b=5$. No CIS was observed.

[d] Competitive titration measuring the NH-CIS of the urea derivative and H_2O protons.

Table 3. Interaction energy values ($-E_{min}$ in kJ mol^{-1}) obtained with AMBER

Guest	**7**	**8**	**8**[a]	**9a**
1	60.7	69.3	71.6	58.6
(*E*,*E*)-**2**	29.0	31.5	23.0	43.0
(*Z*,*E*)-**2**	47.8	51.3	44.4	37.2
(*Z*,*Z*)-**2**	47.8	48.9	44.2	39.0
3	51.7	49.2	38.5	43.1
4	53.0	51.3	41.1	44.6
5	65.0	75.4	62.8	68.3
6	63.4[b]	107.8	84.2	72.6

[a] With the GB/SA model for water.

[b] This value has been calculated taking into account the real interactions in the **7**/**6** complex where only the SO_2–NH intervenes, on the basis of NOESY experiments. There is a minimum energy value for a theoretical complex that considers both urea NHs with a $-E_{min}$ in kJ mol^{-1} of 73.4.

2.2. Binding constants

The experimental binding constants (M^{-1}) measured in $CDCl_3$ at 300 K for complexes of the six guests **1–6** with host **7** are gathered in Table 1, and in Table 2 that with host **8** plus the measured binding constant **8**:water. The interaction energies of the process ($-E_{min}$ in kJ mol^{-1}) evaluated by Molecular Mechanics calculations for hosts **7**, **8** and **9a** with AMBER force field are shown in Table 3 and with OPLS force field in Table 4. As usual with this kind of studies, entropy changes have been assumed to be the same or rather close for all series.[1]

Special mention deserves the case of *N*,*N'*-dimethylurea **2**. For both hosts **7** and **8** we have failed to measure CIS with this guest. To determine values of K_b lower than 10 M^{-1} it would be necessary to use very concentrated solutions, 0.2 M or larger, thus preventing to attain the 20–80% saturation range in the titration procedure. Therefore, we

Table 4. Interaction energy values ($-E_{min}$ in kJ mol^{-1}) obtained with OPLS

Guest	**7**	**8**	**9a**
1	92.4	98.3	84.4
(*E*,*E*)-**2**	56.0	58.0	43.1
(*Z*,*E*)-**2**	70.4	68.0	59.4
(*Z*,*Z*)-**2**	57.5	56.3	42.6
3	80.3	77.0	53.2
4	81.7	81.0	55.6
5	92.5	101.5	73.2
6[a]	—	—	—

[a] Lack of parameters in OPLS force field for this compound.

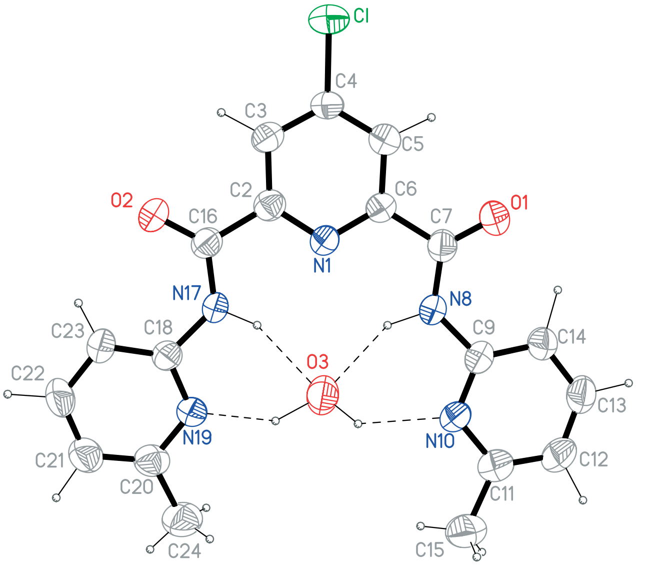


Figure 1. The X-ray structure of host **8** including a water molecule as a guest.

have indicated in Tables 1 and 2 a value of ≤10. We do not know the actual value (that can be different for **7** and **8**) of K_b, somewhere between near 0 and 10, so we have decided to adopt $K_b=5$ in both cases as a working hypothesis. Using other values such as $K_b=1$ or $K_b=0.1$, the conclusions do not change very much only the correlations slightly worsened.

We have represented in Fig. 1 the X-ray structure of **8**:H_2O. The crystal consists of molecules bonded through hydrogen bonds to water molecules. The **8**:H_2O entity is almost coplanar, the maximum dihedral angle between the pyridine rings on the 2,6-dicarboxamide nitrogens is 12.1 (1)°, and the oxygen atom O3 is located 0.543(3) Å out the least square plane formed by N10–N8–N17–N19 atoms. In addition, these entities (**8**:H_2O) are bonded with the centrosymmetric ones through C3–H3···O2 ($-x+1$, $-y+2$, $-z+2$) forming dimers, which are arranged in chains via C21–H21···O1 ($x-1$, $y-1$, z). These hydrogen bonds give rise to a ribbon of 19.22 Å width as depicted in Fig. 2.

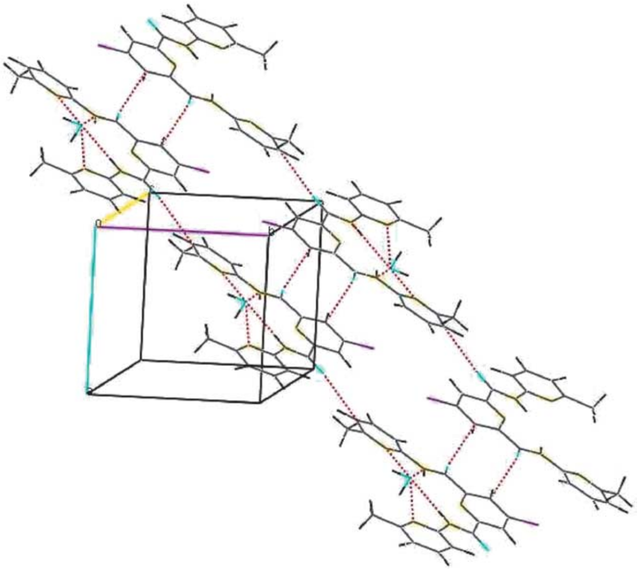


Figure 2. Dimers of Host **8** including a water molecule as a guest forming a ribbon. Dashed lines show hydrogen bonds.

A comparison of the molecular structure determined by X-ray crystallography with the structures obtained by molecular mechanics for the **8**:H_2O complex is reported in Table 5.

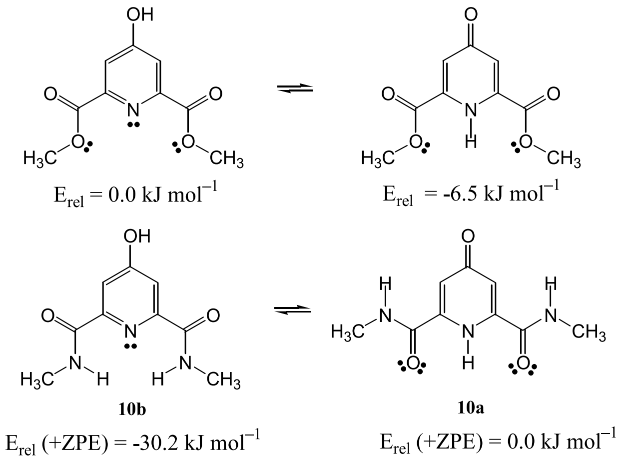
Table 5. Experimental and calculated geometries for **8**:H_2O

D (Å)/<(°)	X-Ray	AMBER	OPLS
N1···H17	2.26	2.18	2.14
N1···H8	2.20	2.24	2.13
N8–H8	1.00	1.02	1.02
O3···H8	2.13	1.87	1.86
N17–H17	0.95	1.02	1.02
O3···H17	2.15	1.88	1.85
O3–H3A	1.09	0.97	0.97
N10···H3A	2.04	2.12	2.13
O3–H3B	1.20	0.97	0.97
N19···H3B	1.76	2.08	2.14
N8–H8···O3	144.1	152.1	152.2
N17–H17···O3	154.9	154.3	152.5
O3–H3A···N10	129.2	143.6	138.8
O3–H3B···N19	140.5	137.7	138.2

Taking into account the difficulty to reproduce hydrogen bond interactions with molecular mechanics, the agreement is best than acceptable and gives confidence in both AMBER and OPLS optimised geometries.

2.3. Tautomerism pyridone/hydroxypyridine: compounds 9a/9b and 10a/10b

Before discussing the host–guest properties of the compounds under study, we will examine the pyridone (**a**)/hydroxypyridine (**b**) tautomerism of compounds **9** and **10** based on B3LYP/6-31G(d,p) calculations as well as the influence of a water molecule on these equilibria. Some of us have reported a theoretical study of the unsubstituted pyridone/hydroxypyridine equilibrium [B3LYP/6-31G(d)].[8] In the absence of any perturbation, 4-hydroxypyridine is more stable then 4-pyridone by 6.3 kJ mol^{-1}; the presence of two methyl ester substituents at positions 2,6 (the conformation of the arms was fixed to simulate the beginning of a crown ether), produces an inversion of the stability in favour of the pyridone by 6.5 kJ mol^{-1}. In the case of model compound **10** (fully optimisation, B3LYP/ 6-31G(d,p) and ZPE correction) the hydroxypyridine derivative **10b** is 30.2 kJ mol^{-1} more stable than **10a** (Scheme 6). This effect can be interpreted as due to the amide N–H groups that interact attractively with the lone pair on $N-1$ in the minimum conformation of **10b**. When the complexes formed by **10a** and **10b** with water are calculated, the energy difference becomes larger in favour of the hydroxy tautomer: **10b** $E_{rel}(+ZPE)=-61.2$ kJ mol^{-1} and **10a**, $E_{rel}(+ZPE)=0.0$ kJ mol^{-1}, as the conformation of the N,N'-dimethyl-2,6-dicarboxamides



Scheme 6. The hydroxy/oxo tautomerism of pyridone derivatives taken from Ref. 8 (diesters) and from this work (diamides).

changes on formation of the complex **10a**:H_2O to include the water molecule into the cavity.

Although for compound **9** the calculations correspond to B3LYP/6-31G(d,p)//MM ones, tautomer **9b** is 167.3 kJ mol^{-1} more stable than **9a**, possibly due to the increased acidity of the N–H protons at position 2 of the pyridine ring. The 167.3 kJ mol^{-1} difference decreases to 111.3 kJ mol^{-1} if a water molecule is placed in the cavity of both **9a** and **9b** because the less stable tautomer **9a** is a better host for water than **9b**.

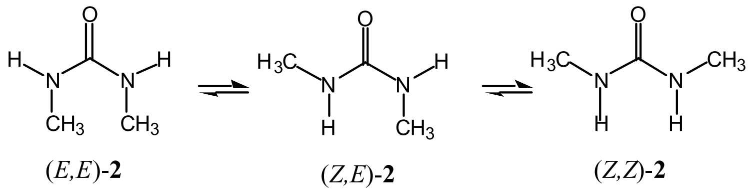
According to the B3LYP6-31G(d,p)//Monte Carlo Conformational Search, AMBER force field level calculations, the interaction energies of the four hosts with water (H_2O was always situated in the concave part) defined as $E_{\text{complex}} - (E_{\text{host}} + E_{\text{water}})$, are: $+19.9$ kJ mol^{-1} for **7** (destabilisation), -51.4 kJ mol^{-1} for **8** (stabilisation), -88.7 kJ mol^{-1} for **9a** (stabilisation), and -32.6 kJ mol^{-1} for **9b** (stabilisation). Note that **8**:H_2O fits the experimental X-ray structure (Fig. 1).

2.4. Experimental versus calculated binding constants

The interactions with guest **6** cannot be calculated with OPLS (Table 4), but it is possible to compare the AMBER and OPLS results for the remaining compounds. There is a rough proportionality between both series of values (Eq. (1)):

$$-E_{\min}(\text{OPLS}) = (1.38 \pm 0.04) \times -E_{\min}(\text{AMBER}), \tag{1}$$

$$n = 21, \quad r^2 = 0.983$$



Scheme 7. The different conformations of *N*,*N*′-dimethylurea **2**.

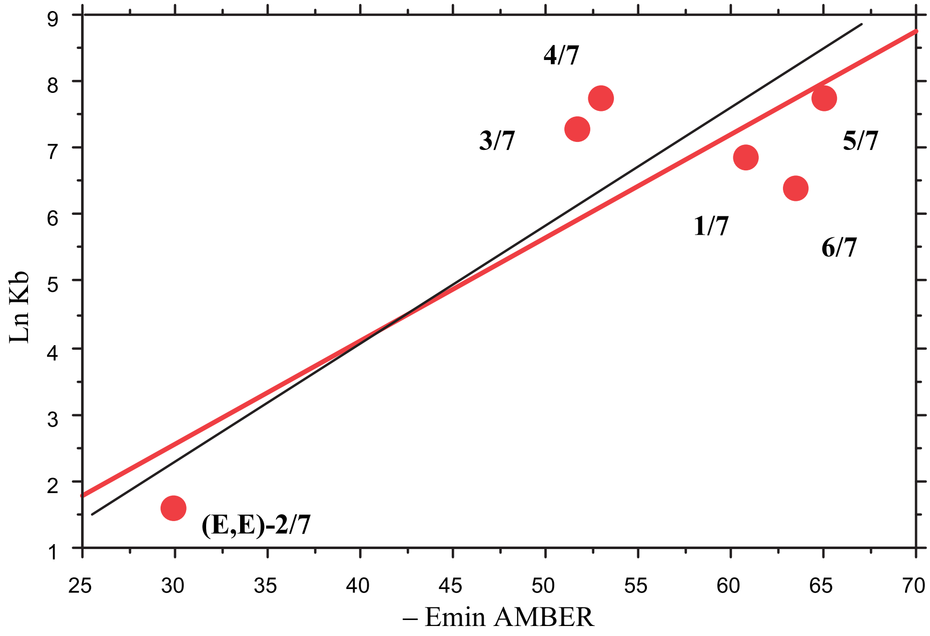


Figure 3. A plot the experimental results (Table 1) vs. the calculated ones (Table 3) for host **7**.

Comparison with the experimental results in the case of **7** is not good enough to discriminate between both kinds of calculations. Therefore, we will use the more complete AMBER values (Table 3).

When comparing the average K_b values for complexes of host **7** in Table 1 (after transforming them into Ln K_b) with $-E_{min}$ AMBER in Table 3, the first problem that arises is the isomerism of *N*,*N'*-dimethylurea **2** (Scheme 7). What of the three conformations of this compound is the most consistent with the experimental value?

Since the CIS are too small we have assumed an experimental value of $K_b=5$, and from the variation of ln K_b against $-E_{min}$ AMBER it appears that the (*E*,*E*)-**2** isomer is the one interacting with the host. For (*Z*,*E*)-**2** and (*Z*,*Z*)-**2** the residuals are much larger, so we have excluded these points in Fig. 3.

The red line corresponds to:

$$\text{Ln } K_b = -(2.1 \pm 2.6) + (0.155 \pm 0.047) \times -E_{min}(\text{AMBER}), \quad n = 6, \quad r^2 = 0.73 \tag{2}$$

Removing tolbutamide (**6**) one obtains the black line:

$$\text{Ln } K_b = -(2.9 \pm 2.5) + (0.176 \pm 0.047) \times -E_{min}(\text{AMBER}), \quad n = 5, \quad r^2 = 0.82 \tag{3}$$

We turn now to the data of host **8**. For homogeneity reasons we have based our discussion on the AMBER calculations of Table 3. Like in the preceding case, the best agreement in the case of guest **2** is found for the *E*,*E* conformation with an experimental K_b value of 5. Of the two columns for **8**, the results are better using the GB/SA model for water as shown in Fig. 4.

The blue line corresponds to:

$$\text{Ln } K_b = (0.8 \pm 1.4) + (0.083 \pm 0.024) \times -E_{min} \text{ AMBER (Water} - \text{GB/SA)}, \quad n = 6, \quad r^2 = 0.76 \tag{4}$$

Here again, tolbutamide (**6**) is the worse point. Removing it one obtains as indicated in the black line:

$$\text{Ln } K_b = -(0.3 \pm 1.3) + (0.112 \pm 0.025) \times -E_{min} \text{ AMBER (Water} - \text{GB/SA)}, \quad n = 5, \quad r^2 = 0.87 \tag{5}$$

It is possible to treat together hosts **7** and **8** using AMBER ($CHCl_3$-GB/SA) for **7** and AMBER (Water-GB/SA) for **8**:

Three regression lines can be calculated for the points of Fig. 5:

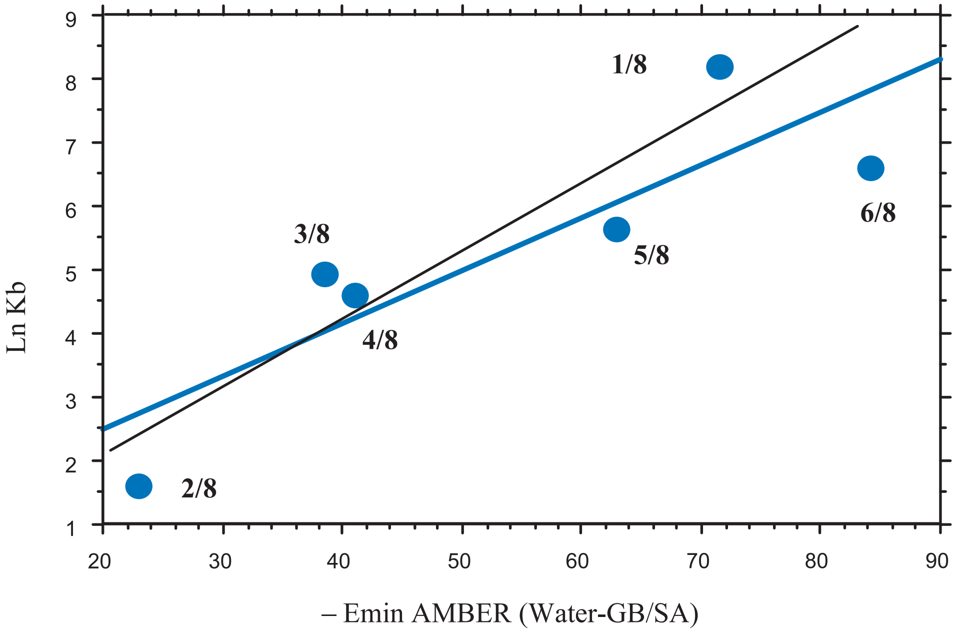


Figure 4. A plot the experimental results (Table 2) vs. the calculated ones (Table 3) for host **8**.

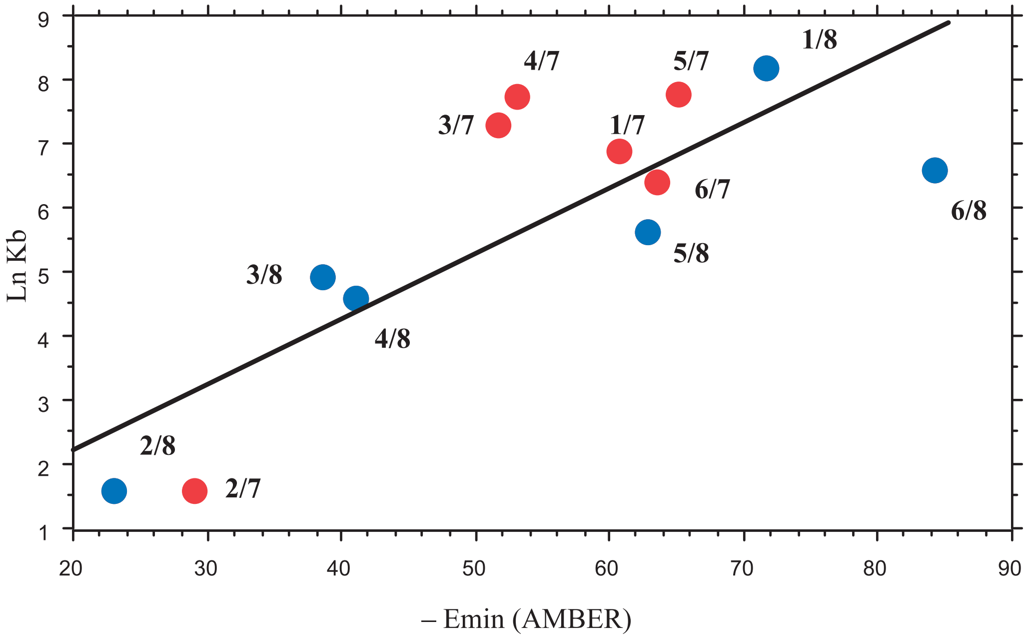


Figure 5. A plot the experimental results (Tables 1 and 2) vs. the calculated ones (Table 3) for hosts **7** and **8**.

$$\text{All points : Ln } K_b = (0.4 \pm 1.3) + (0.101 \pm 0.023) \times -E_{\min} \text{ AMBER}, \quad n = 12, \quad r^2 = 0.65 \qquad (6)$$

$$\text{Without 6 : Ln } K_b = -(1.1 \pm 1.2) + (0.135 \pm 0.023) \times -E_{\min} \text{ AMBER}, \quad n = 10, \quad r^2 = 0.81 \qquad (7)$$

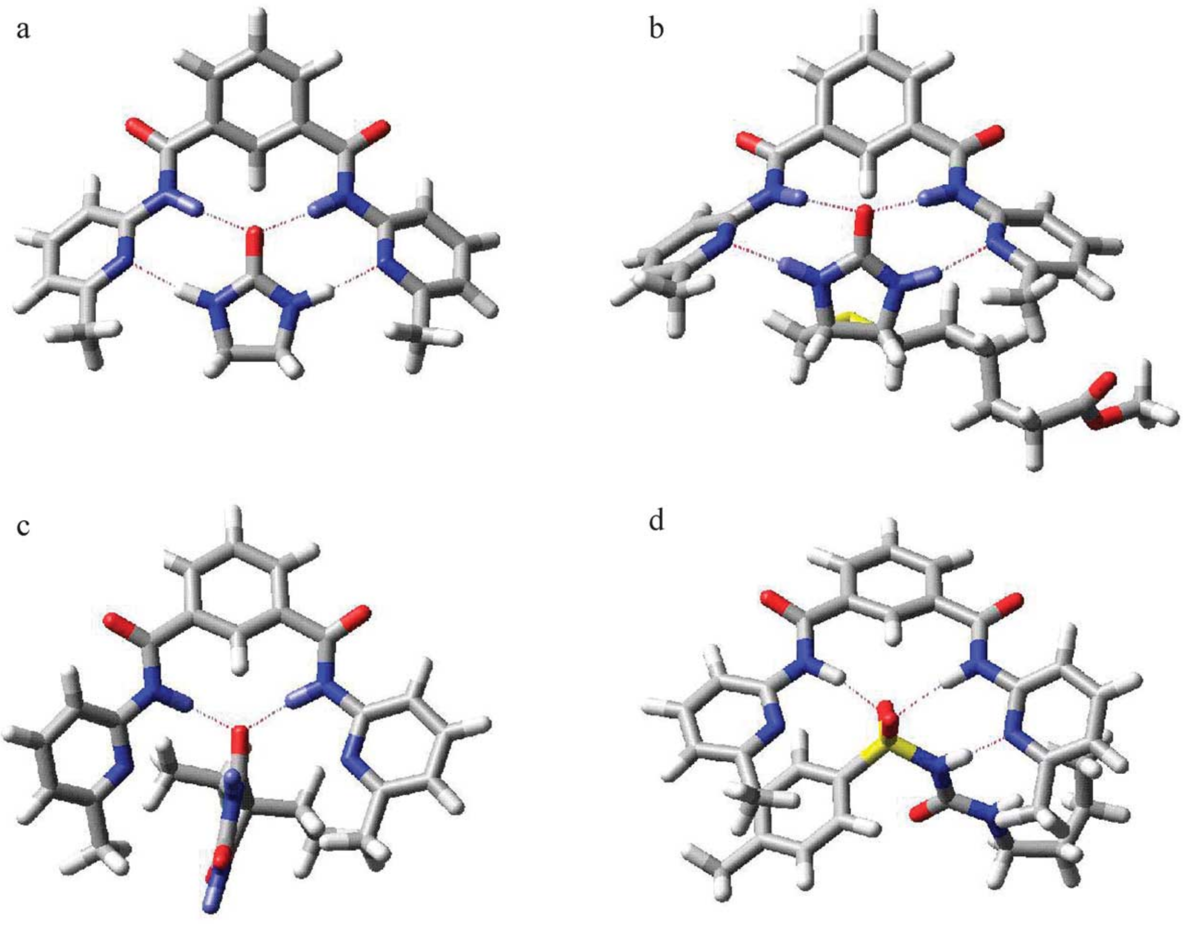


Figure 6. Structures of complexes: a. **7**:**3**; b. **7**:**1**; c. **7**:**5**; d. **7**:**6**.

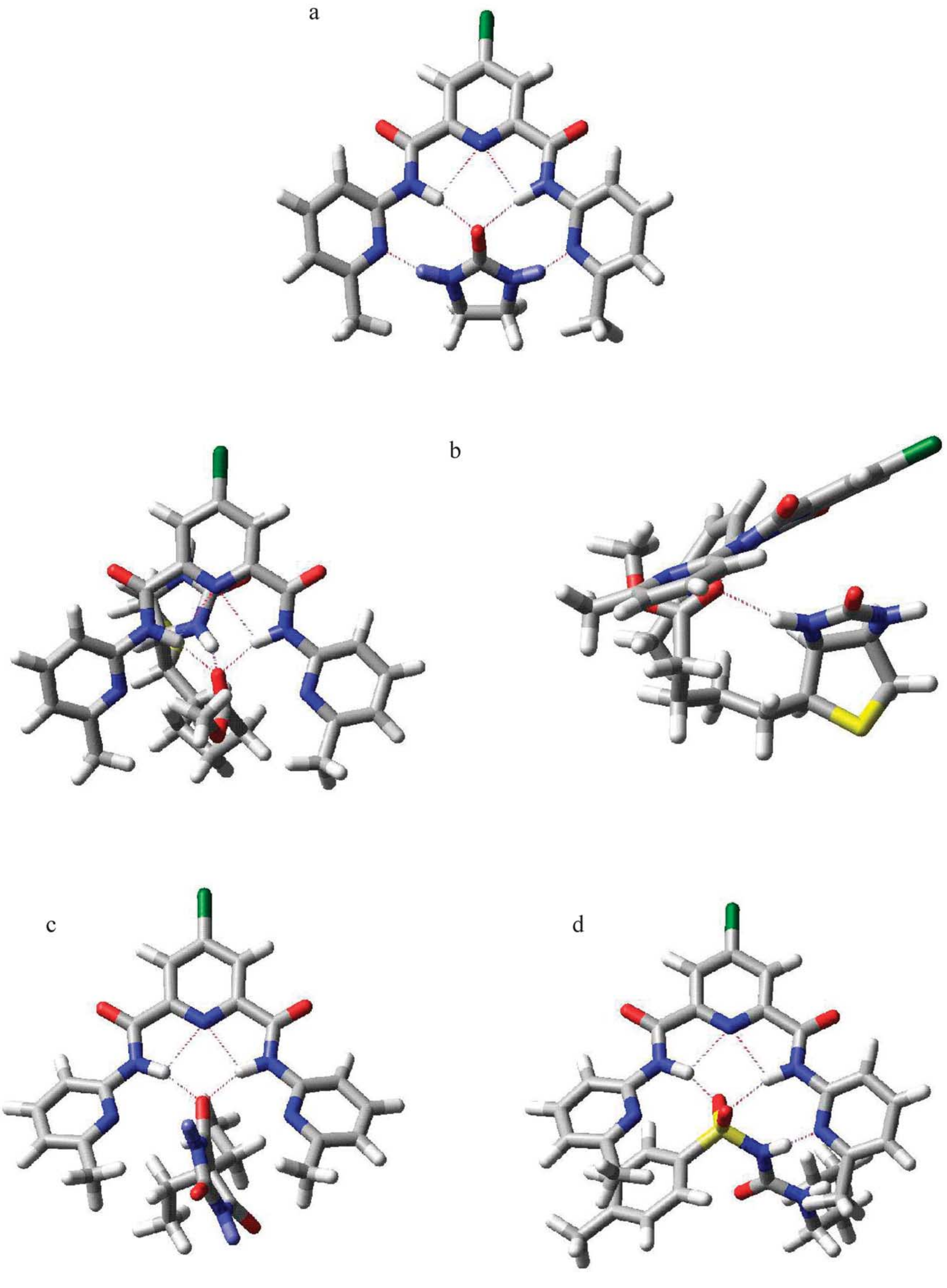


Figure 7. Structures of complexes: a. **8**:**3**; b.**8**:**1**; c. **8**:**5**; d. **8**:**6**.

$$\text{No intercept : Ln } K_b = (0.115 \pm 0.007) \times -E_{\min} \text{ AMBER}, \quad n = 10, \quad r^2 = 0.97 \tag{8}$$

To explain why tolbutamide (**6**) deviates it is necessary to examine the structure of the complexes as calculated by AMBER for hosts **7** and **8**. If we consider the complex between **7** and 2-imidazolidone (**3**) (Fig. 6a) as representative of the studied complexes, we observe that those of biotin methyl ester (**1**) (Fig. 6b), *N*,*N*′-dimethylurea (**2**) in its *E*,*E*-conformation and trimethyleneurea (**4**) are very similar. That of barbital (**5**) uses only the carbonyl group (Fig. 6c) and tolbutamide (**6**) only the SO_2–NH moiety (Fig. 6d).

Similar features are found for host **8** (Fig. 7a–c), save in the case of biotin methyl ester (**1**) where no CIS were observed on the NH chemical shifts (Table 2) proving that there is no interaction through the urea moiety. The binding takes place involving the amide NHs of the host and the carbonyl group of the biotin side chain. In the two views of the structure of the complex **8**:**1** shown in Fig. 7b, that corresponds to the energy minimum, the two internal hydrogen bonds remain intact and there are no changes in the original conformations nor in the host neither in the guest.

The binding modes were confirmed in the complex of tolbutamide (**6**) with host **7** by means of NOESY NMR experiments that proved the closeness of the *p*-tolyl protons of **6** to the methyl group of the host (Fig. 8a) and the guest aliphatic chain protons to host pyridine ones (Fig. 8b). This information allowed us to calculate the new energy minimum of -63.4 kJ mol^{-1} depicted in Table 3, that fits the experimental binding constant K_b.

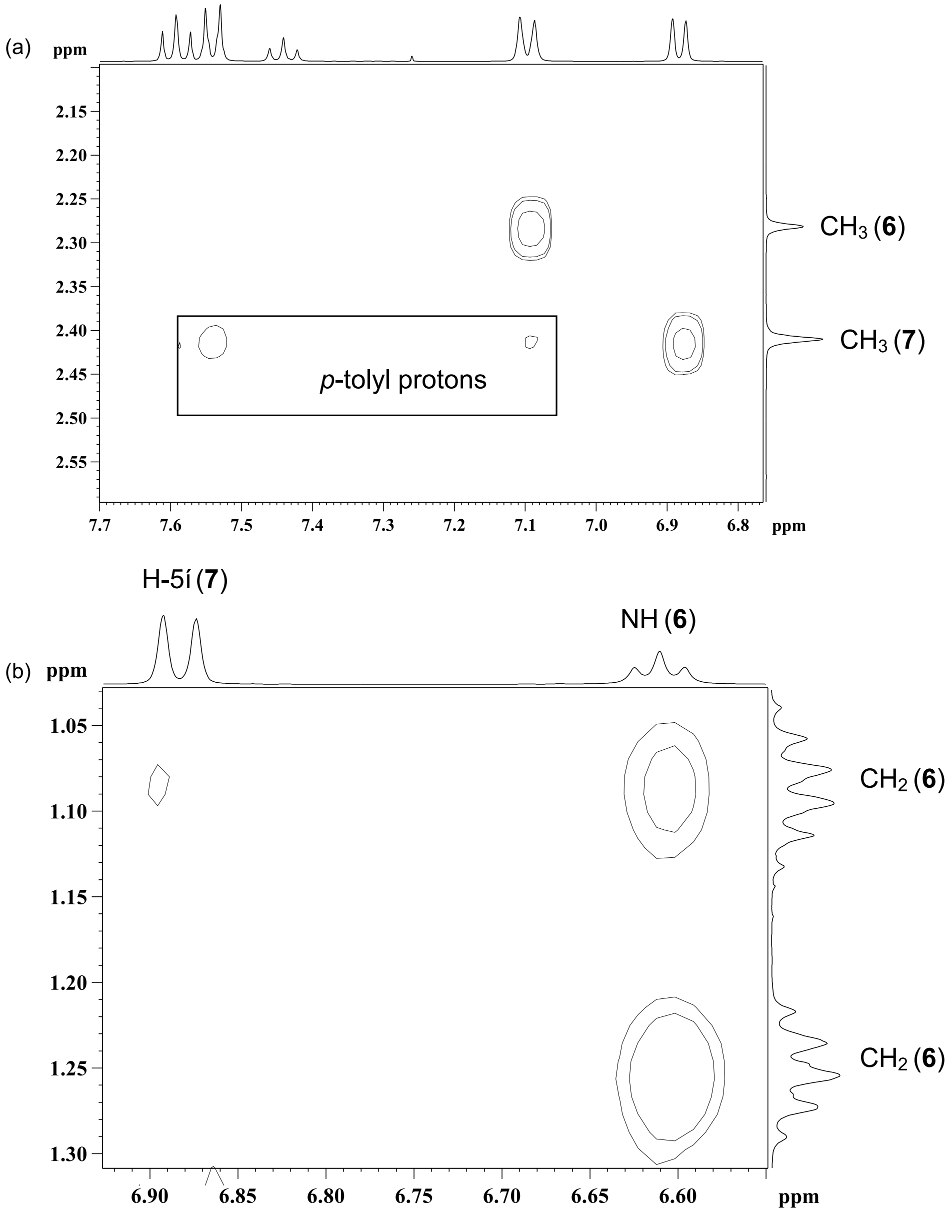


Figure 8. Enlarged regions of the NMR NOESY spectra.

Moreover, no changes in the ^{13}C NMR chemical shift of the urea carbonyl group of tolbutamide (**6**) are induced when complexes **7**:**6** and **8**:**6** are formed.

3. Conclusions

As a result of our studies, two different conformations in the complexation mode of biotin methyl ester (**1**) with **7** and **8** have been found. The conformation with **7** is similar to the normal one shown by the ureas, but with **8** is completely different and takes place through the carbonyl group of the biotin side chain. Concerning barbital (**5**), the hosts are only able to accommodate one part of the molecule but the K_b values are high (the highest with **7**), a similar observation was made with the hosts of our precedent paper.[1]

Tolbutamide (**6**) is rather different from the other five ureas because the sulfonyl group increases considerably the acidity of the contiguous NH[11] but also modifies the conformation. Based on its X-ray structure,[12] we have represented it in Scheme 2 with both NHs opposite to the C=O (like a *Z*,*Z*-dimethylurea). Although this is the conformation found in the complexes, tolbutamide is too different from classical ureas to fit well in the same series of calculations.

4. Experimental

4.1. General

The six guests are commercially available: biotin methyl ester (**1**) (>99%, dried under vacuum), *N*,*N*′-dimethylurea (**2**) (99%, recrystallized from ethyl acetate), 2-imidazolidone (**3**) (96%, recrystallized from ethyl acetate), *N*,*N*′-trimethyleneurea (**4**) (>98%, recrystallized from ethyl acetate), barbital (**5**) (>99%) and tolbutamide (**6**) (>99%). Melting points were determined in a Thermo-Galen hot stage microscope and are uncorrected. Elemental analyses for carbon, hydrogen, and nitrogen were carried out by the Microanalytical Service of the Complutense University on a Perkin-Elmer 240 analyser.

4.2. NMR spectroscopy

NMR spectra were recorded on a Bruker DRX 400 (9.4 T, 400.13 MHz for ^{1}H, 100.62 MHz for ^{13}C and 40.56 MHz for ^{15}N) spectrometer at 300 K. Chemical shifts (δ in ppm) are given from internal solvent CDCl$_3$ 7.26 for ^{1}H and 77.0 for ^{13}C, DMSO-d_6 2.49 for ^{1}H and 39.5 for ^{13}C and for ^{15}N NMR nitromethane was used as external standard. Coupling constants (J in Hz) are accurate to $J=\pm 0.2$ Hz for ^{1}H and ^{13}C and $J=\pm 0.6$ Hz for ^{15}N. 2D-Inverse proton detected homonuclear shift correlation spectra gs-COSY (^{1}H–^{1}H), NOESY and 2D inverse proton detected heteronuclear shift correlation spectra, gs-HMQC (^{1}H–^{13}C), gs-HMBC (^{1}H–^{13}C) and gs-HMBC (^{1}H–^{15}N), were carried out with the standard pulse sequences to assign the ^{1}H, ^{13}C and ^{15}N signals.

4.3. Synthesis of *N*,*N*′-bis(6-methylpyridin-2-yl)-1,3-benzenedicarboxamide (7)

See Scheme 4. Isophthaloyl chloride (**11**, 1 g, 4.9 mmol) was dissolved, under Ar, in 100 mL of dry CH$_2$Cl$_2$, then a solution of 2-amino-6-methylpyridine (**12**, 1.1 g, 9.9 mmol) and Et$_3$N freshly distilled (4 mL) in 90 mL of dry CH$_2$Cl$_2$ was added slowly from a pressure-equalising addition funnel. The resulting solution was stirred for 4 h and then washed with saturated solution of NaHCO$_3$ and water, dried (Na$_2$SO$_4$) and concentrated to yield a yellow-pale solid which is recrystallized from MeOH to obtain 0.74 g (44%) of **7**, mp 230 °C. ^{1}H NMR (CDCl$_3$): δ (ppm) 8.81 (broad s, 2H, NH), 8.48 (dd, 1H, H-2, $J_{2,4}=J_{2,6}=1.8$ Hz), 8.16 (d, 2H, H-3′, $J_{3',4}=8.2$ Hz), 8.10 (dd, 2H, H-4/H-6, $J_{4,5/6,5}=7.8$ Hz), 7.63 (dd, 2H, H-4′, $J_{4',5'}=7.7$ Hz), 7.58 (dd, 1H, H-5), 6.92 (d, 2H, H-5′), 2.42 (s, 6H, CH$_3$). ^{13}C NMR (CDCl$_3$): δ (ppm) 164.5 (CO), 156.9 (C6′), 150.5 (C2′, $^{3}J=9.2$ Hz), 138.9 (C4′, $^{1}J=161.0$ Hz), 135.0 (C1/C3, $^{3}J=7.7$ Hz), 130.8 (C4/C6, $^{1}J=161.8$ Hz, $^{3}J=6.1$ Hz), 129.4 (C5, $^{1}J=162.6$ Hz), 125.9 (C2, $^{1}J=161.0$ Hz, $^{3}J=6.1$ Hz), 119.7 (C5′, $^{1}J=162.6$ Hz, $^{3}J=6.2$ Hz), 111.0 (C3′, $^{1}J=171.8$ Hz, $^{3}J=6.1$ Hz), 23.9 (CH$_3$, $^{1}J=127.3$ Hz, $^{3}J=3.1$ Hz). ^{15}N NMR (CDCl$_3$): δ (ppm) −242.9 (NH), −98.9 (N1′). Anal. Calcd for $C_{20}H_{18}N_4O_2$: C, 69.35; H, 5.24; N, 16.17%. Found: C, 69.09; H, 5.29; N, 16.09%.

4.4. Synthesis of 4-chloro-*N*,*N*′-bis(6-methylpyridin-2-yl)-2,6-pyridindicarboxamide (8)

See Scheme 5. Chelidamic acid (**13**, 0.5 g, 2.73 mmol) is dissolved in thionyl chloride (10 mL, 137.4 mmol) with the minimum quantity of DMF and the solution is heated at 110 °C for 3 h. After that, DMF and thionyl chloride are evaporated at reduce pressure until a white solid of 4-chlorochelidamic acid dichloride (**15**) is obtained, 0.45 g (74%), mp 200 °C. ^{1}H NMR (DMSO-d_6): δ (ppm) 8.46 (s, 2H, H3/H5).

4-Chlorochelidamic acid dichloride (**15**, 0.5 g, 2.10 mmol) was dissolved, under Ar, in 20 mL of dry CH$_2$Cl$_2$, then a solution of 2-amino-6-methylpyridine (**12**, 0.68 g, 4.55 mmol) and Et$_3$N freshly distilled (3 mL) in 30 mL of dry CH$_2$Cl$_2$ was added slowly from a pressure-equalising addition funnel. The resulting solution was stirred for 4 h and then washed with saturated solution of NaHCO$_3$ and water, dried (Na$_2$SO$_4$) and concentrated to yield a white solid which is recrystallized from MeOH to obtain 0.35 g (40%) of **8**, mp 244 °C. ^{1}H NMR (CDCl$_3$): δ (ppm) 10.23 (broad s, 2H, NH), 8.46 (s, 2H, H-3/H-5), 8.20 (d, 2H, H-3′, $J_{3',4'}=8.2$ Hz), 7.67 (dd, 2H, H-4′, $J_{4',5}=7.5$ Hz), 6.98 (d, 2H, H-5′), 2.52 (s, 6H, CH$_3$). ^{13}C NMR (CDCl$_3$): δ (ppm) 160.5 (CO), 157.2 (C6′), 150.2 (C2/C6), 150.0 (C2′, $^{3}J=9.2$ Hz), 148.3 (C4, $^{2}J=3.1$ Hz), 138.8 (C4′, $^{1}J=161.0$ Hz), 126.1 (C3/C5, $^{1}J=174.6$ Hz, $^{3}J=4.5$ Hz), 119.9 (C5′, $^{1}J=162.9$ Hz, $^{3}J=6.1$ Hz), 111.3 (C3′, $^{1}J=171.6$ Hz, $^{3}J=6.1$ Hz), 24.0 (CH$_3$, $^{1}J=127.3$ Hz). ^{15}N NMR (CDCl$_3$): δ (ppm) −247.1 (NH), −97.9 (N1′), −97.2 (N1). Anal. Calcd for $C_{19}H_{16}ClN_5O_2 \cdot H_2O$: C, 57.07; H, 4.54; N, 17.52%. Found: C, 57.17; H, 4.67; N, 17.49%.

4.5. NMR titrations

Each NMR titration was carried out at least three times at 300 K in $CDCl_3$ as a solvent (Merck S33657, deuterium content $>99.8\%$, water content $<0.01\%$). The syringes are from Hamilton–Bonaduz, 5 μL (divisions 0.05 μL), 10 μL (divisions 0.1 μL), 250 μL (divisions 5 μL) and the balance for weighting the host and the guest a Metler AE260-Delta Range (error ± 0.00005 g). ^1H NMR titrations are used in order to quantify K_b values, these titrations are carried out following the Chemical Induced Shift (CIS) in one or several protons of host or guest while the concentration of the complex formed is changed by the addition of one of the components. For host **7** we performed a double independent quantification following the CIS for amide protons and the H-2 in the central benzene ring, while guest solution aliquots are added. There are a large number of ways to fit the data from a titration,[9] but that consisting in non-linear curve fitting is generally accepted as the method with the lowest error in the determination of K_b values, in comparison to others that employ approximations to reach a linear relationship between δ and K_b. To fit the experimental data the Sigmaplot 8.1 program from SPSS Science Software Gmbh was employed. The basic equation used in this kind of titrations is represented by [Eq. (9)], showing the relationship between chemical shifts (δ), concentrations of host H, guest G and complex C, and the binding constant $K_b=[C]/([H][G])$, this equation is valid only for 1:1 stoichiometry as is our case.[10]

$$\delta_{OBS} = (\delta_C - \delta_H)(\{(1 + [G]/[H] + 1/Kb[H])/2\} - \{(1 + [G]/[H] + 1/K_b[H])^2/4 - [G]/[H]\}^{1/2}) + \delta_H \tag{9}$$

In order to obtain K_b values with the lowest error the titrations are carried out in the 20–80% saturation range for the compound which CIS is being followed. This condition determines the concentrations to be used in the titrations for both host and guest and a calculation has to be done to find those concentrations that best cover the whole range of p in order to get the maximum information from the titration curve. The accuracy in the concentration range to be used in titrations is usually disregarded in most publications of the host–guest field, affording K_b values totally different from those obtained following this procedure. The error determined by this magnitude is intrinsic to the measurement method and it is not reflected by the standard deviation (S_d) which is a measure of the fit goodness of the data employed.

The titrations for the complex between host **8** and water are carried out on the same way (20–80% saturation range), water concentration is determined by the integration of its NMR signal. The host sample for the titration is prepared with freshly distilled $CDCl_3$ and molecular sieve 4 Å is added to keep water at the minimum concentration, on this way all the samples had an initial saturation range between 20–30%, so the titration was carried out in the right saturation range. The results were reported in Table 2. Titrations between host **8** and biotin methyl ester (**1**) are carried out by the same method but keeping the concentration of water under 1 mM in order to avoid a competitive behaviour of the water.[5]

For guests **3**, **4**, **5** and **6** a competitive titration is used, the CIS of the two guests are measured while aliquots of the host are added. The fitting of the data to the (Eq. (10)) allows to obtain a relative K_b for the guest we are studying, as the K_b for the complex **8**:water was previously measured we can calculate the value for the complex **8**:guest.

$$K_{b(water)}/K_{b(guest)} = [(1/F_{guest}) - 1]/[(1/F_{water}) - 1] \tag{10}$$

F_{water} and F_{guest} are the molar fractions of water and guest that are bound to the host, if no another equilibria arise (which it has been proved with titrations of the guest versus water), then $F_i=(\delta_{i,Free}-\delta_{iObserved})/(\delta_{i,Free}-\delta_{i,Complexed})$.

4.6. MM calculations

MacroModel v.8.1, with the GB/SA model for chloroform[13] was used in order to perform the molecular simulations of the complexes in all cases, save as indicated in Table 3. All calculations were achieved with Monte Carlo (MC) conformational analyses.[14] Minimisation is carried out using Polak-Ribiere conjugate gradient optimiser.[15] In a typical MC run a MCMM is performed with never less than 8000 steps, to carry out the search both torsional rotations in host and guest and translation/rotation (10 Å/360°) of the guest is performed, for all the MC a cutoff is applied to van der Waals, electrostatic and H-bond interactions with 7, 12 and 4 Å, respectively. These calculations were carried out with two different force fields, AMBER*,[16] and OPLS*,[17] as implemented in the version of the program.

4.7. DFT calculations

Single point calculations were carried out at the B3LYP/6-31G(d,p) level of theory[18,19] with the Gaussian ’03 program on the optimised Molecular Mechanics geometries.[20] At this level we calculated the energies for the complexes between hosts **7**, **8** and **9a/9b** with water, analysing the energy differences between the two possible tautomers in host **9** (**a**, 4-pyridone/**b**, 4-hydroxypyridine). BSSE were determined for all the complexes computed by DFT with the counterpoise correction.[21]

Model compound **10a/10b** and its water complexes were fully optimised (no imaginary frequencies).

4.8. Crystallographic data collection and structure determination of 8a:H_2O

Suitable crystal for X-ray diffraction experiments was obtained by crystallization from acetone/hexane. Data collection was carried out at room temperature on a Bruker Smart CCD diffractometer using graphite-monochromated Mo K(radiation ($\lambda=0.71073$ Å) operating at 50 Kv and 30 mA. Data were collected over a hemisphere of the reciprocal space by combination of three exposure sets. Each exposure of 30 s covered 0.3 in ω. Structure was

Table 6. Crystal data and structure refinement for **8**:H_2O

Empirical formula	$C_{19}H_{18}ClN_5O_3$	
Formula weight	399.83	
Temperature	293(2) K	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	$a=8.274(2)$ Å	$\alpha=114.003(5)°$
	$b=10.560(3)$ Å	$\beta=91.997(5)°$
	$c=11.942(3)$ Å	$\gamma=94.132(6)°$
Volume	948.4(4) $Å^3$	
Z	2	
Density (calculated)	1.400 Mg/m^3	
Absorption coefficient	0.233 mm^{-1}	
$F(000)$	416	
θ (°)	1.87–25.00°	
Index ranges	$-9\leq h\leq 9$, $-10\leq k\leq 12$, $-14\leq l\leq 13$	
Reflections collected	5030	
Independent reflections	3315 [R(int)=0.0494]	
Data/restraints/ parameters	3315/0/255	
G.o.f. (F^2)	0.815	
R_1 [$I>2\text{sigma}(I)$][a]	0.0489 (ref. obs. 1342)	
R_2 (all data)[b]	0.1228	

[a] $\Sigma[|F_o|-|F_c|]/\Sigma|F_o|$.

[b] $\{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.

solved by direct methods and refined by full-matrix least-square procedures on F^2 (SHELX-97).[22]

All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were located in a difference Fourier map, included and fixed. Crystal data and other structure determination details are presented in Table 6.

CCDC-256026 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data centre, 12 union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336033; or www.deposit@ccdc.cam.uk.

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References and notes

1. Claramunt, R. M.; Herranz, F.; Santa María, M. D.; Jaime, C.; de Federico, M.; Elguero, J. *Biosensors Bioelectron.* **2004**, *20*, 1242–1249.
2. Hedge, V.; Hung, C.-Y.; Madhukar, P.; Cunningham, R.; Höpfner, T.; Thummel, R. P. *J. Am. Chem. Soc.* **1993**, *115*, 872–878.
3. Goswami, S.; Mukherjee, R. *Tetrahedron Lett.* **1997**, *38*, 1619–1622.
4. Kecskemeti, V.; Bagi, Z.; Pacher, P.; Posa, I.; Kocsis, E.; Koltai, M. Z. *Curr. Med. Chem.* **2002**, *9*, 53–71.
5. Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678–680.
6. Hirao, T.; Moriuchi, T.; Ishikawa, T.; Nishimura, K.; Mikami, S.; Ohshiro, Y.; Ikeda, I. *J. Mol. Catal. A* **1996**, *113*, 117–130.
7. Jouaiti, A.; Hosseini, M. W.; Kyritsakas, N. *Chem. Commun.* **2003**, 1898–1899.
8. Alkorta, I.; Elguero *J. Heterocycl. Chem.* **2001**, *38*, 1387–1391.
9. Fielding, L. *Tetrahedron* **2000**, *56*, 6151–6170.
10. Hirose, K. *J. Incl. Phenom. Macrocycl. Chem.* **2001**, *39*, 193–209.
11. NH-acidity (pK_a): tolbutamide: 5.3, barbital: 7.7: www.medchem.ku.edu/MDCM625.
12. Nirmala, K. A.; Gowda, D. S. S. *Acta Crystallogr. Sect. B* **1981**, *37B*, 1597–1599. Donaldson, J. D.; Leary, J. R.; Ross, S. D.; Thomas, M. J. K.; Smith, C. H. *Acta Crystallogr. Sect. B* **1981**, *37B*, 2245–2248.
13. MacroModel, Schrödinger LLC, 2004. http://www.schrodinger.com/Products/macromodel.html.
14. Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1999**, *111*, 4379–4385.
15. Polak, E. Computational Methods in Optimization; Academic Press: New York, 1971. Brodlie, K. W. In *The State of the Art in Numerical Analysis*; Jacobs, D. A. H., Ed.; Academic Press: London, 1977; Chapter III. 1.7.
16. Weiner, P. K.; Kollmann, P. A. *J. Comput. Chem.* **1981**, *2*, 287–303.
17. Jorgensen, W. L.; Tirado-Rives *J. Am. Chem. Soc.* **1998**, *110*, 1657–1664.
18. Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652. Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789. Miehlich, B.; Savin, A.; Stoll, H.; Preuss, H. *Chem. Phys. Lett.* **1989**, *157*, 200–206.
19. Ditchfield, R.; Hehre, W. J.; Pople, J. A. *J. Chem. Phys.* **1971**, *54*, 724–728.
20. Gaussian 03, Revision B.04, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A. Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P.M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian, Inc., Pittsburgh PA, 2003.
21. Boys, S. B.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553–558.
22. Sheldrick, G.M. SHELX97, Program for Refinement of Crystal Structure, University of Göttingen, Göttingen, Germany, 1997.

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Synthesis of lipid A analogues containing glucose instead of glucosamine and their LPS-antagonistic activities

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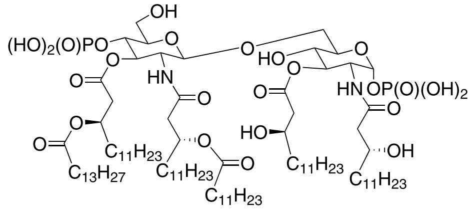
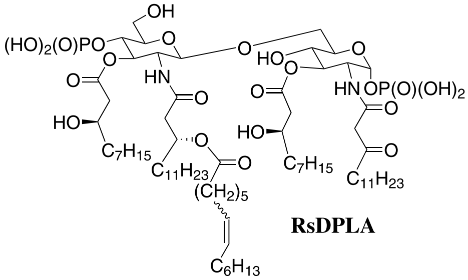
Abstract—Lipid A analogues containing glucose in substitution for glucosamine on the reducing end were synthesized, and the inhibitory activities on LPS-induced TNFα production (LPS-antagonistic activity) in vitro using human whole blood cells were measured. The IC_{50} values (nM) of these ten compounds, **8**, **14**, **21**, **31**, **40**, **51**, **57**, **62**, **67** and **72**, were 11.2, 15.4, 2.7, 0.1, 0.4, 1.3, 3.2, 3.2, 1.4 and 14.4, respectively. And also inhibitory activities (ID_{50}) on TNFα production toward galactosamine loaded C3H/HeN mice in vivo of compounds **21**, **31**, **57**, **62** and **67** were measured. The values of these compounds were 0.29, 0.50, 0.61, not dose-dependent and 0.33 mg/kg, respectively.

1. Introduction

The study of endotoxin has proceeded extensively[1] since Shiba and Kusumoto's[2] total synthesis of lipid A, a toxic component of endotoxin (lipopolysaccharide, LPS) existing in the outer surface membrane of Gram-negative bacteria. On the contrary, a nontoxic natural lipid A-related compound (RsDPLA)[3] was isolated from *Rhodobacter sphaeroides* by an Eisai group. This compound unlike lipid A has a unique structural feature, that is, it contains two amides composed of an unsaturated fatty acid ((*R*)-3-(7-tetradecenoyloxy)tetradecanoic acid) and a 3-oxotetradecanoic acid in its long fatty acid chains, and shows LPS-agonistic activity toward neither human nor mouse macrophages.[4] Furthermore, the Eisai group found that many RsDPLA-related compounds having an olefinic double bond in their molecules behave as LPS antagonists toward human and murine macrophages,[4] and E5564,[5] a compound related to RsDPLA, has been developed as a highly potent anti-septicemia drug (Fig. 1).


Lipid A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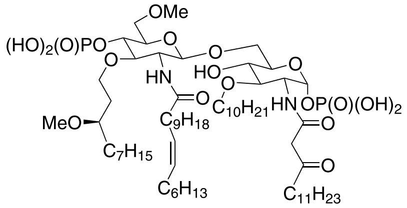
E5564

Figure 1. Structures of Lipid A, RsDPLA, and E5564.

The active structures of all natural lipid A- and also RsDPLA-related compounds are constructed with an β(1-6)-linked glucosamine–glucosamine disaccharide moiety, and the configuration of the anomeric phosphate

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Keywords: LPS-antagonist; RsDPLA.

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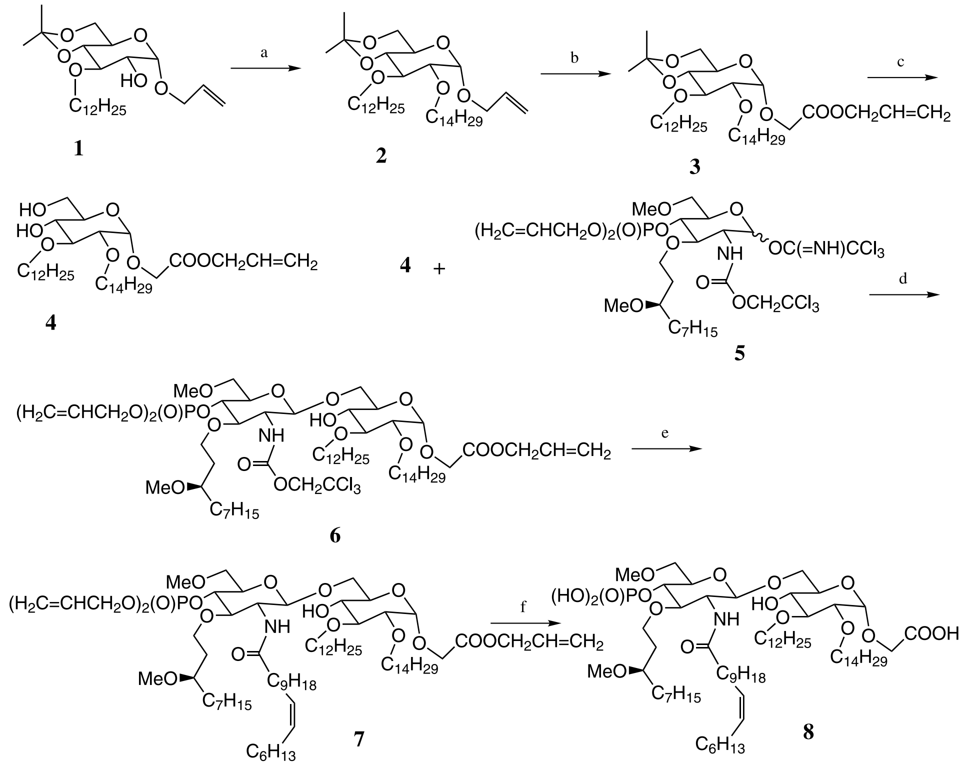
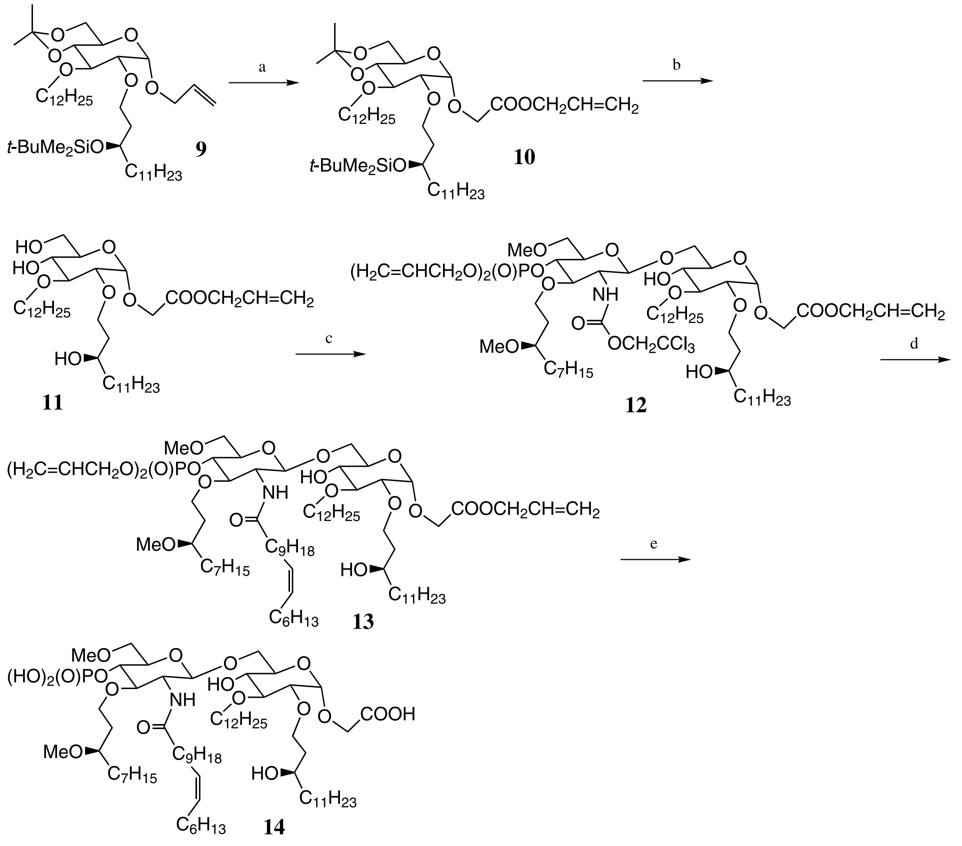
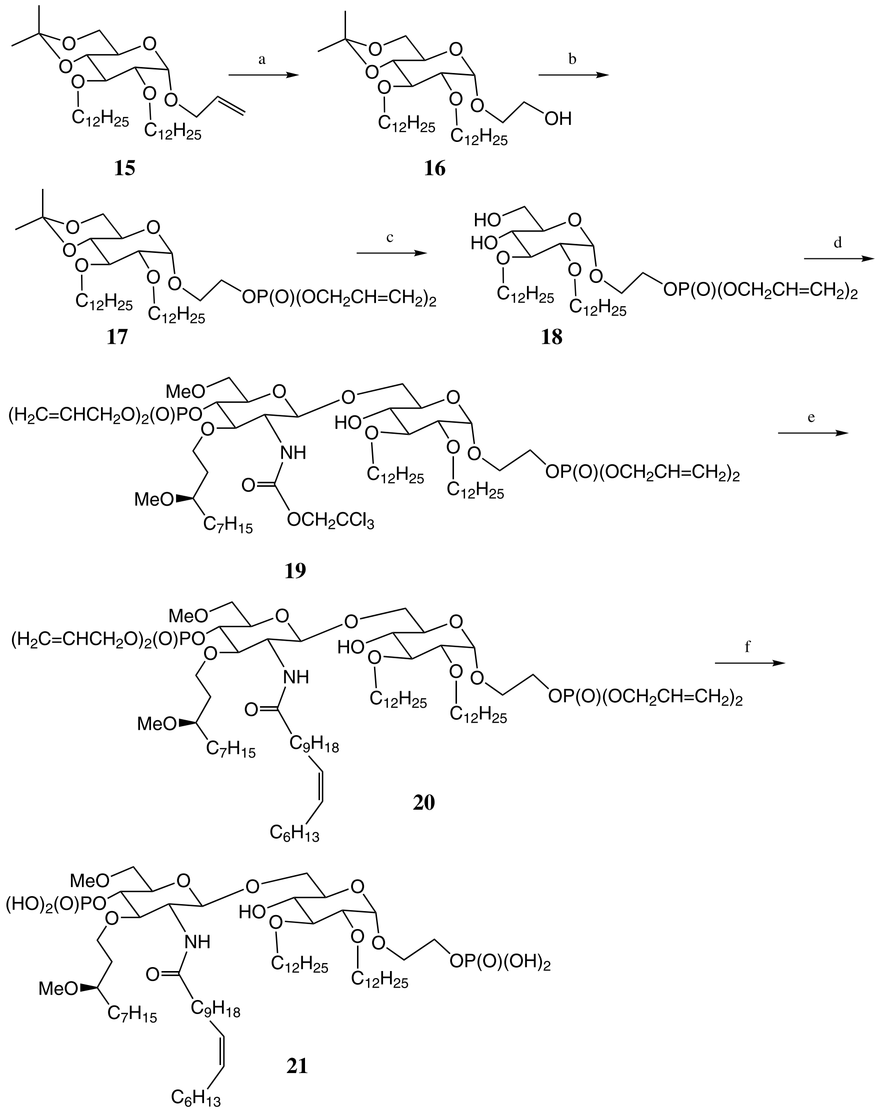


Scheme 1. Reagents and conditions: (a) tetradecyl methanesulfonate, NaH, DMF, rt, 16 h, 75%; (b) $RuO_2.xH_2O$, $NaIO_4$, $MeCN–CCl_4–H_2O$ (2:2:3), rt, 3 h, then allyl bromide, Et_3N, DMF, rt, 16 h, 72%; (c) $AcOH–H_2O$ (4:1), 65 °C, 2 h, 71%; (d) TMSOTf, MS 4A, CH_2Cl_2, −40 °C, 1 h, 43%; (e) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, $THF–H_2O$ (5:1), rt, 30 min, two steps 77%; (f) $(PPh_3)_4Pd$, PPh_3, Et_3N, HCOOH, THF, under N_2, 55 °C, 4 h, 95%.



Scheme 2. Reagents and conditions: (a) RuO_2 hydrate, $NaIO_4$, $MeCN–CCl_4–H_2O$ (2:2:3), rt, 3 h, then allyl bromide, Et_3N, DMF, rt, 16 h, 69%; (b) $AcOH–H_2O$ (4:1), 65 °C, 2 h, 60%; (c) **5**, TMSOTf, MS 4A, CH_2Cl_2, −40 °C, 1 h, 45%; (d) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, $THF–H_2O$ (5:1), rt, 30 min, 67%; (e) $(PPh_3)_4Pd$, PPh_3, $Et_3N–HCOOH$, THF, under N_2, 55 °C, 4 h, 55%.



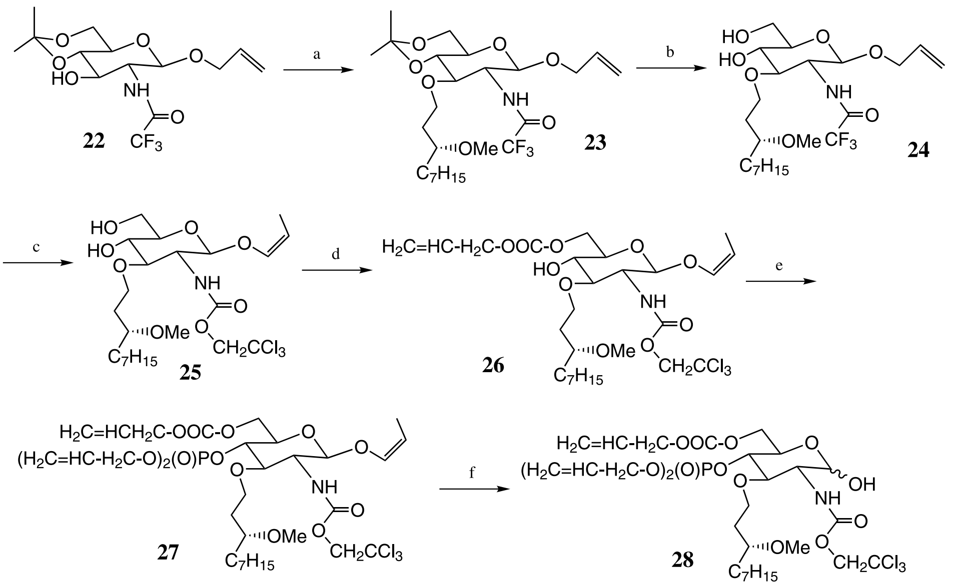
Scheme 3. Reagents and conditions: (a) OsO_4, $NaIO_4$, THF–H_2O (7:2), rt, 3 h, then $NaBH_4$, EtOH, rt, 20 min, quenched with AcOH, 73%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 30 min, then, aq 30% H_2O_2, THF–CH_2Cl_2, rt, 15 min, 99%; (c) *p*-TsOH, MeOH, rt, 2 h, 96%; (d) **5**, AgOTf, TMSOTf, MS 4A, under N_2, CH_2Cl_2, rt, 16 h, 60%; (e) (1) Zn, AcOH–THF (1:1), rt, 3.5 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 2 h, two steps 75%; (f) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 16 h, 45%.

of the reducing glucosamine part is α without exception. We were interested in the structures of RsDPLA and E5564, and synthesized some E5564-related compounds, which were replaced with glucose analogues instead of the glucosamine at the reducing end, to examine the LPS-antagonistic activity. At this time, we fixed the nonreducing glucosamine end to that of E5564 except for compound **31**, and also we restricted the anomeric substituents of the reducing glucose end to five structural groups as the α-carboxymethyl, α-2-(phosphonooxy)ethyl, α-2-(phosphono)ethyl, α-3-(phosphonooxy)propyl and α-3-(phosphono)propyl groups such as compounds **8** and **14**, and **21**, **31**, **40**, **51** and **57**, and **62**, and **67**, and **72**, respectively.

2. Results and discussion

2.1. Synthesis

Firstly, we synthesized compounds **8** and **14** having an α-carboxymethyl group at the anomeric position. For synthesis of **8**, known alcohol **1**[6] was used as a starting material. Compound **1** was treated with tetradecyl methanesulfonate using sodium hydride as a base to give **2**. Oxidative cleavage of the allyl group of **2** with ruthenium oxide hydrate and sodium periodate gave carboxylic acid, which was esterified with allyl bromide using triethyl amine in *N*,*N*-dimethylformamide (DMF) to yield allyl ester **3**. The 4,6-*O*-isopropylidene group of **3** was deprotected with aqueous 80% acetic acid at 65 °C to yield diol **4**, which was coupled with already reported trichloromethylimidoyl 2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(trichloroethoxycarbonylamino)-D-glucopyranoside **5**[5] at −40°C using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst to afford β(1-6)-linked[5] disaccharide **6**. The 2,2,2-trichloroethoxycarbonyl group of **6** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **7**. The three allyl groups of **7** were deprotected with

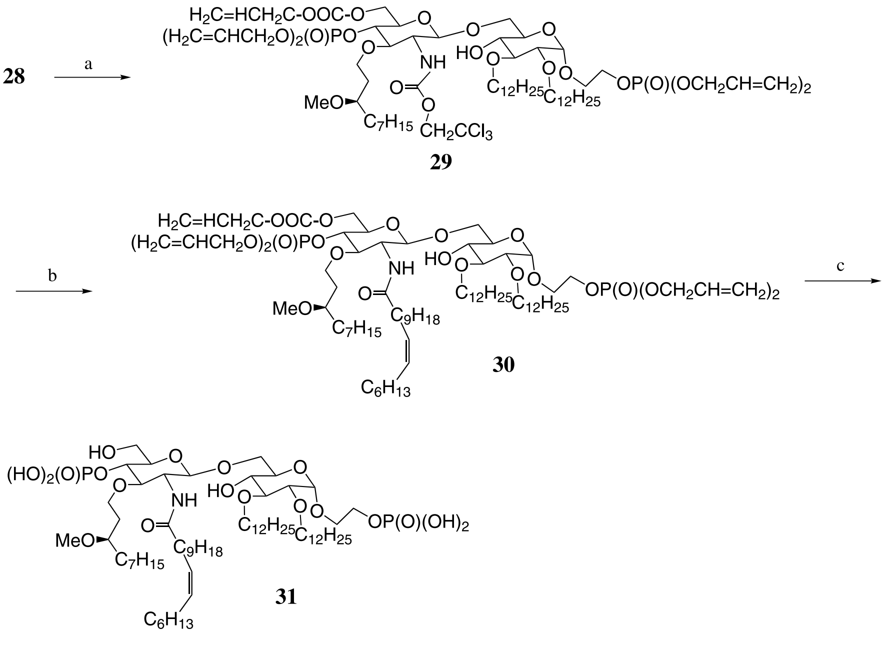


Scheme 4. Reagents and conditions: (a) (*R*)-3-methoxydecyl *p*-tolouenesulfonate, NaH, DMF, rt, 5 h, 77%; (b) *p*-TsOH, MeOH, rt, 2 h, 92%; (c) (1) *t*-BuOK, DMSO, 85 °C, N_2, 2 h, then H_2O, 85 °C, 2 h, (2) $ClCOOCH_2CCl_3$, aq satd $NaHCO_3$, THF, 0 °C, 30 min, 76%; (d) $ClCOOCH_2CH{=}CH_2$, pyridine, 0 °C, 30 min, 94%; (e) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 20 min, then aq. 30% H_2O_2, THF–CH_2Cl_2, 0 °C, 30 min, 86%; (f) I_2, THF–H_2O (5:1), rt, 30 min, 94%.

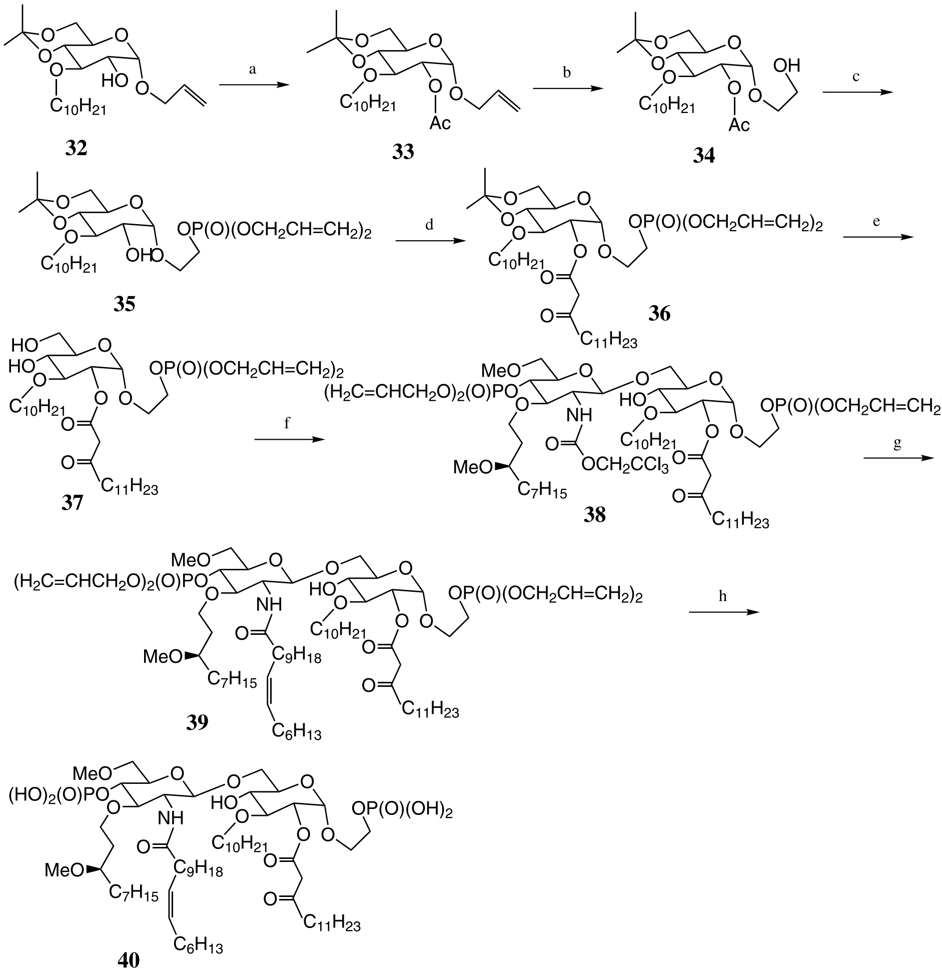
tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$], triphenylphosphine (PPh_3) and triethylamine-formic acid (Et_3N–HCOOH) in THF to yield **8** (Scheme 1).

For the synthesis of **14**, known alcohol **9**[6] was used as a starting material under almost the same conditions as for the synthesis of **8** from **1**. Oxidative cleavage of the allyl group of **9** with ruthenium oxide hydrate and sodium periodate gave carboxylic acid, which was esterified with allyl bromide using triethyl amine in DMF to yield allyl ester **10**, and the successive cleavage of 4,6-*O*-isopropylidene and *tert*-butyldimethylsilyl groups from **10** was performed with aqueous 80% acetic acid at 65 °C to yield triol **11**. Compound **11** was coupled with imidate **5** at −40 °C using TMSOTf to afford β(1-6)-linked disaccharide **12**. The 2,2,2-trichloroethoxycarbonyl group of **12** was deprotected with zinc powder in AcOH-THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **13**. The three allyl groups of **13** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **14** as mentioned above (Scheme 2).

Secondly, we synthesized compounds **21**, **31**, **40**, **51** and **57** having an α-2-(phosphonooxy)ethyl group at the anomeric position. Known compound **15**[6] was used as a starting



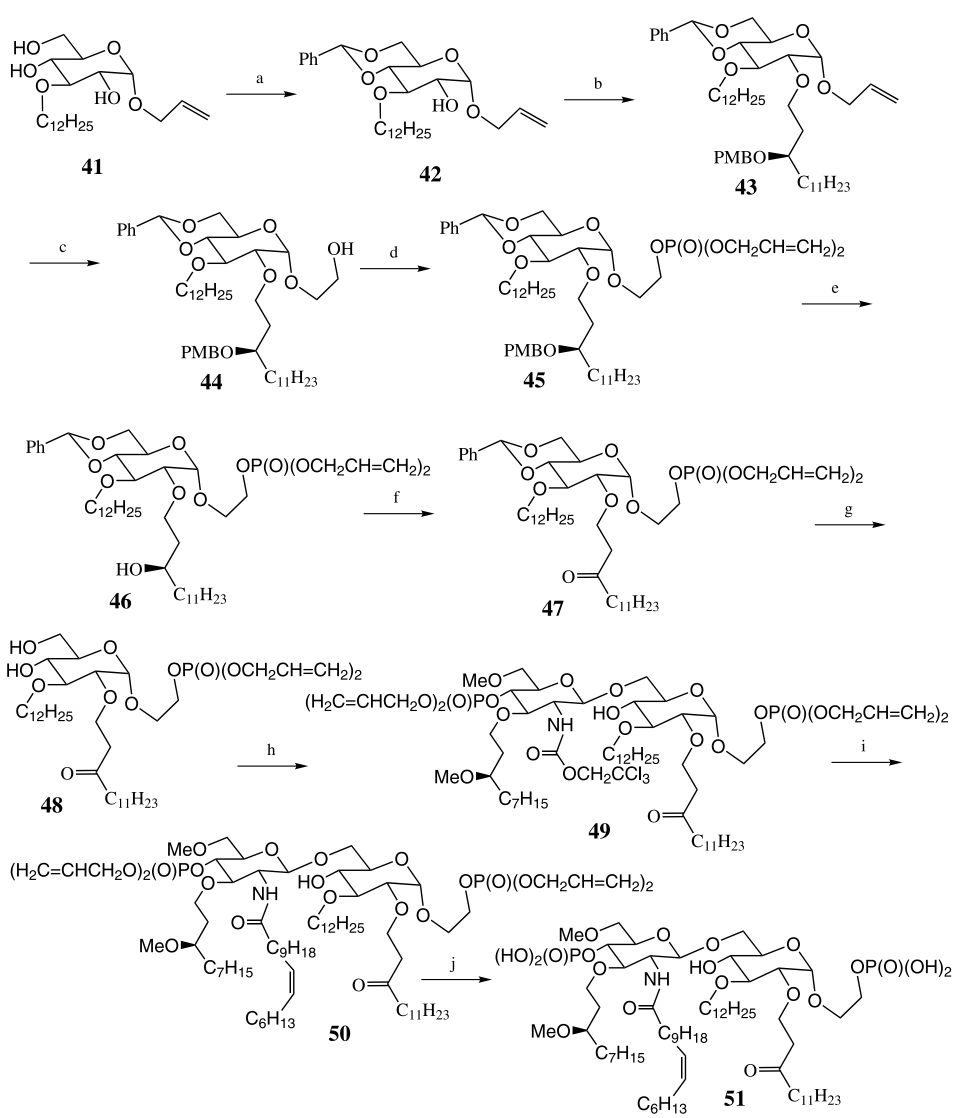
Scheme 5. Reagents and conditions: (a) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 1 h, then **18**, TMSOTf, MS 4A, CH_2Cl_2, 0 °C, then rt, 16 h, 52%; (b) (1) Zn, AcOH–THF (1:9), 24 °C, 3.5 h; (2) (*Z*)-11-octadecenoic acid, WSC.HCl, CH_2Cl_2, rt, 10 h, two steps 47%; (c) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 16 h, 76%.



Scheme 6. Reagents and conditions: (a) Ac_2O, pyridine, rt, 5 h, 99%; (b) (1) OsO_4, $NaIO_4$, THF–H_2O (3:1), rt, 2 h; (2) $NaBH_4$, EtOH, rt, 10 min, 71%; (c) (1) KOH, EtOH, rt, 1.5 h; (2) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 20 min, then aq 30% H_2O_2, CH_2Cl_2–THF (1:1), rt, 10 min, 80%; (d) 3-oxotetradecanoic acid, WSC HCl, CH_2Cl_2, rt, 40 min, 75%; (e) AcOH–H_2O (v/v, 4:1), 85 °C, 1.5 h, 86%; (f) **5**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, under N_2, rt, 16 h, 68%; (g) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 30 min, two steps 81%; (h) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 16 h, 84%.

material for the synthesis of **21**. The allyl group of **15** was converted to aldehyde by OsO_4–$NaIO_4$ oxidation in THF–H_2O (7:2), and the aldehyde was successively reduced to alcohol **16** using $NaBH_4$ in EtOH. Treatment of **16** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole in THF yielded phosphite, which was contiguously oxidized to phosphate **17** by use of H_2O_2. The 4,6-*O*-isopropylidene protecting group of **17** was cleaved using *p*-toluenesulfonic acid monohydrate in MeOH to give diol **18**. Compound **18** was coupled with imidate **5** at room temperature using AgOTf and TMSOTf to afford β(1-6)-linked disaccharide **19**. In this coupling, we found that the use of one equivalent of AgOTf and catalytic amount of TMSOTf at room temperature improved the yield comparing with the known method of single use of TMSOTf at −78 ~ −40 °C. When TMSOTf reagent was not used as a catalyst, excess AgOTf (about 2 equiv) was needed in the formation of **60** from **5** and **59** as described later. The 2,2,2-trichloroethoxycarbonyl group of **19** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **20** according to almost the same procedure as for the synthesis of **7** from **6**. The three allyl groups of **20** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **21** as mentioned above (Scheme 3).

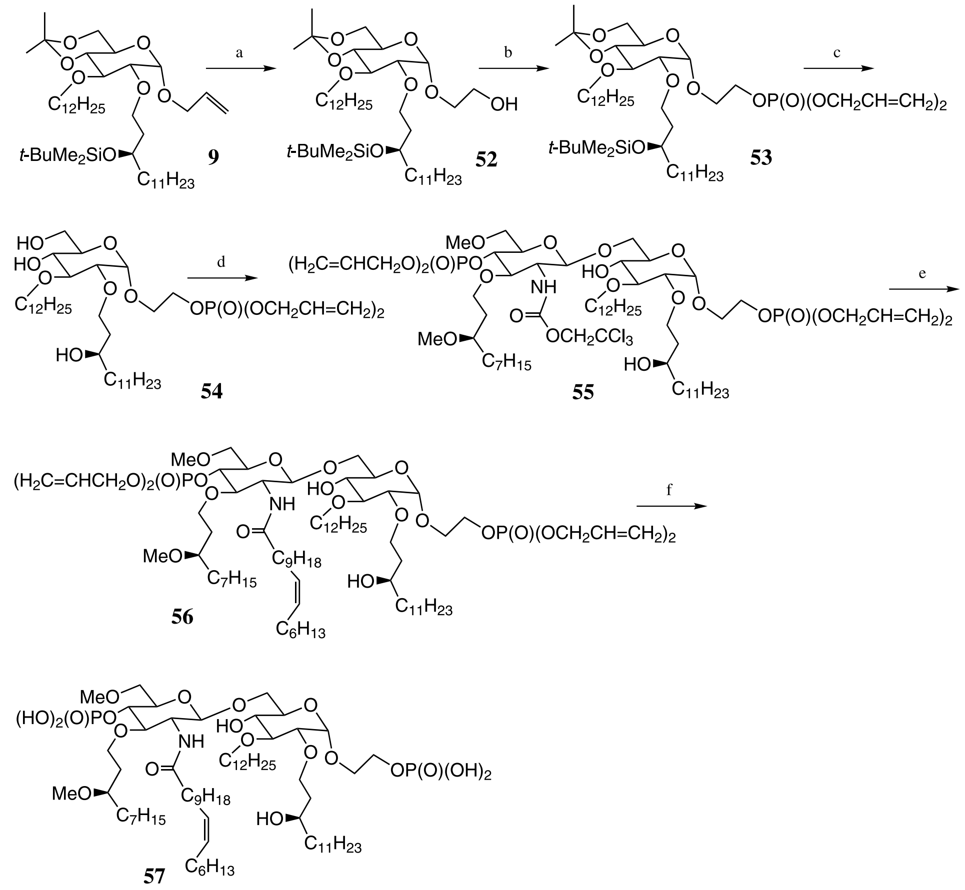
Compound **22**[7] was used as a starting material for the synthesis of **31**. Alcohol **22** was reacted with (*R*)-3-methoxydecyl *p*-toluenesulfonate in DMF using NaH to yield **23**, which was treated with *p*-toluenesulfonic acid in methanol to afford diol **24**. Double bond isomerization[5] of **24** was performed with potassium *tert*-butoxide in dimethylsulfoxide (DMSO) at 85 °C for 2 h, and hydrolysis of C2 trifluoroacetamide to yield amino-free (*Z*)-vinyl ether, which was successively protected with 2,2,2-trichloroethyl chloroformate in aqueous THF using $NaHCO_3$ to give **25**. Treatment of diol **25** with allyl chloroformate using pyridine gave **26** being protected by the 6-*O*-allyloxycarbonyl group. The remaining C5 secondary alcohol of **26** was treated with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and subsequent oxidation of the generated phosphite with aq 30% H_2O_2 yielded phosphate **27**. Oxidative cleavage of the vinyl ether group of **27** was accomplished by iodine in THF–H_2O to yield **28**. After the activation of the anomeric alcohol of **28** with trichloroacetonitrile using cesium carbonate the generated imidoyl compound was coupled with diol **18** using TMSOTf as a condensing catalyst to



Scheme 7. Reagents and conditions: PMB = 4-methoxybenzyl; (a) $PhCH(OMe)_2$, cat. *p*-TsOH, DMF, rt, 16 h, 69%; (b) (*R*)-3-(4-methoxybenzyloxy)tetradecyl methanesulfonate, NaH, DMF, rt, 6 h, 67%; (c) (1) OsO_4, $NaIO_4$, acetone–H_2O (4:1), rt, 4 h, (2) $NaBH_4$, EtOH, rt, 1 h, 64%; (d) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, THF, rt, 4 h, then aq 30% H_2O_2, 0 °C, 1 h, 96%; (e) DDQ, CH_2Cl_2–H_2O (10:1), rt, 1.5 h, 90%; (f) PCC, CH_2Cl_2, rt, 5 h, 93%; (g) aq 80% AcOH, 65 °C, 2 h, 92%; (h) **5**, cat. TMSOTf, MS 4A, CH_2Cl_2, N_2, 0 °C, 1 h, 59%; (i) Zn, AcOH–THF (1:9), rt, 4 h, then (*Z*)-11-octadecenoic acid, WSC.HCl, CH_2Cl_2, rt, 10 h, 56%; (j) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 55 °C, 4 h, 90%.

afford disaccharide **29**. The 2,2,2-trichloroethoxycarbonyl group of **29** was deprotected with zinc powder in AcOH–THF (1:9) to give amine, which was treated with (*Z*)-11-octadecenoic acid in CH_2Cl_2 using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC·HCl) as a dehydrating agent to afford amide **30** in accordance with the same procedure as that to convert **49** to **50** as described later. The five allyl groups of **30** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **31** as mentioned above (Schemes 4 and 5).

Compound **32**[6] was used as a starting material for the synthesis of **40**. The C2 alcohol of **32** was acetylated with acetic anhydride–pyridine to give **33**. This protection was necessary for OsO_4 oxidation, because alcohol **32** did not react with OsO_4 under the same conditions as for the synthesis of **34** from **33**. The allyl group of **33** was treated with OsO_4–$NaIO_4$ in THF–H_2O, and subsequent reduction with $NaBH_4$ in EtOH to give alcohol **34**, which was deacetylated by a catalytic amount of KOH in EtOH and the primary alcohol was selectively reacted with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and continuous oxidation of generated phosphite with aq 30% H_2O_2 to yield phosphate **35**. Esterification of C2 alcohol **35** with 3-oxotetradecanoic using WSC·HCl as a dehydrating agent gave **36**. Treatment of **36** with aq 80% AcOH at 85 °C for 1.5 h yielded diol **37**, which was coupled with imidate **5** using silver trifluoromethanesulfonate (AgOTf) and TMSOTf as condensing catalysts to afford disaccharide **38**. The 2,2,2-trichloroethoxycarbonyl group of **38** was replaced with (*Z*)-11-octadecenoyl group using zinc powder in AcOH–THF (1:1) and then (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **39** in accordance with the same procedure as that to convert **6** to **7**. Five allyl groups of **39** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **40** as mentioned above (Scheme 6).

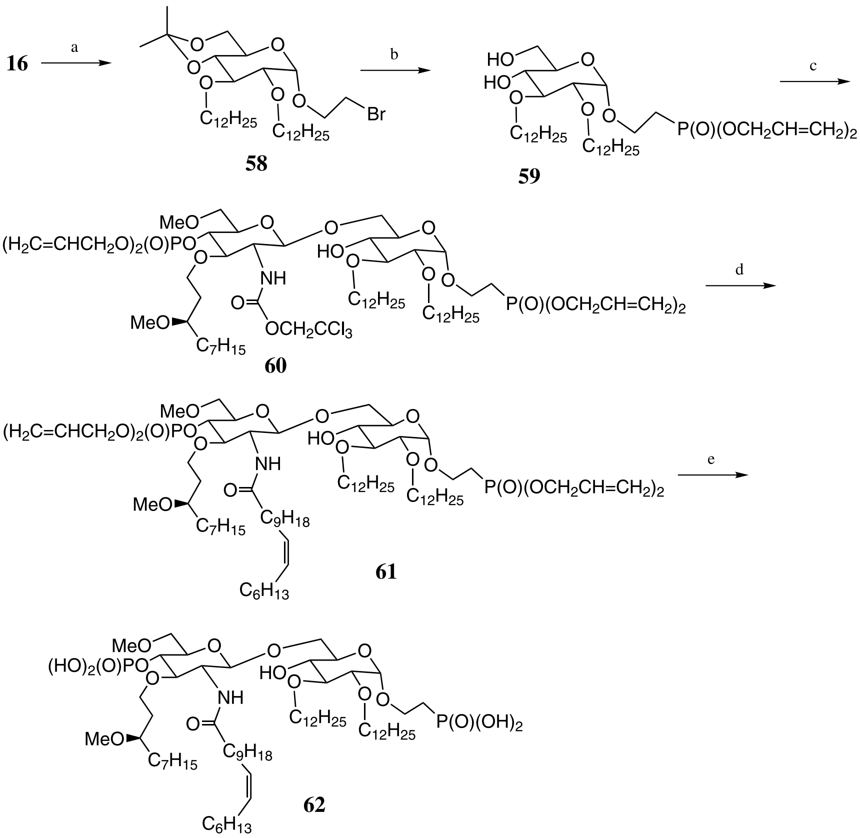


Scheme 8. Reagents and conditions: (a) OsO_4, $NaIO_4$, THF–H_2O (7:2), rt, 2 h, and then $NaBH_4$, EtOH, 0 °C, 20 min, 41%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 30 min, then aq 31% H_2O_2, rt, 15 min, 97%; (c) *p*-TsOH.HCl, MeOH, rt, 2 h, 84%; (d) **5**, AgOTf, TMSOTf, MS 4A, N_2, rt, 16 h, 67%; (e) Zn, AcOH–THF (1:1), rt, 4 h, and then (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 2 h, 82%; (f) $Pd(PPh_3)_4$, PPh_3, Et_3N–HCOOH, N_2, 55 °C, 16 h, 67%.

Alcohol **41**[6] was used as a starting material for the synthesis of **51**. Treatment of **41** with benzaldehyde dimethyl acetal using *p*-TsOH monohydrate gave benzylidene **42**, which was treated with (*R*)-3-(4-methoxybenzyloxy)tetradecyl methanesulfonate using sodium hydride to give **43**. Oxidative cleavage of the allyl group of **43** with OsO_4–$NaIO_4$ in acetone–H_2O, and subsequent reduction of the generated aldehyde with $NaBH_4$ gave alcohol **44**, which reacted with diallyl diisopropylphosphoramidite using 1*H*-tetrazole as an acid catalyst, and continuous oxidation of generated phosphite with aq 30% H_2O_2 to yield phosphate **45**. Oxidative cleavage of the *p*-methoxybenzyl group of the alcohol protective group of **45** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_2Cl_2–H_2O gave alcohol **46**. The alcohol on the branched chain of **46** was oxidized with pyridinium chlorochromate (PCC) to afford ketone **47**. The 4,6-*O*-benzylidene group of **47** was deprotected with aq 80% AcOH at 60 °C to yield diol **48**. Treatment of **48** and imidate **5** at 0 °C using TMSOTf afforded β(1-6)-linked disaccharide **49**. The 2,2,2-trichloroethoxycarbonyl group of **49** was deprotected with zinc powder in AcOH–THF (1:9) to give amine, which was treated with (*Z*)-11-octadecenoic acid using WSC·HCl as a dehydrating agent to afford amide **50**. The four allyl groups of phosphate **50** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **51** as mentioned in the formation of **8** from **7** (Scheme 7).

Compound **9**[6] was used as a starting material for the synthesis of **57**. The allyl group of **9** was converted to aldehyde by OsO_4–$NaIO_4$ oxidation in THF–H_2O (7:2), and the aldehyde was continuously reduced to alcohol **52** using $NaBH_4$ in EtOH. Treatment of **52** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole in THF yielded phosphite, which was immediately oxidized to phosphate **53** using aq 31% H_2O_2. The 4,6-*O*-isopropylidene and *tert*-butyldimethylsilyl protecting groups of **53** were cleaved by using *p*-TsOH monohydrate in MeOH to give triol **54**. Compound **54** was coupled with imidate **5** at room temperature using AgOTf and catalytic amount of TMSOTf to afford β(1-6)-linked disaccharide **55**. The 2,2,2-trichloroethoxycarbonyl group of **55** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **56**. Three allyl groups of **56** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **57** according to almost the same procedure as for the synthesis of **21** from **20** (Scheme 8).

Thirdly, we synthesized compound **62** having an α-2-(phosphono)ethyl group at the anomeric position.



Scheme 9. Reagents and conditions: (a) (1) CBr_4, PPh_3, CH_2Cl_2, rt, 2 h, 99%; (b) (1) $P(OCH_2CH{=}CH_2)_3$, 180 °C, 3 h, (2) aq. 80% AcOH, 60 °C, 1 h, two steps 60%; (c) **5**, AgOTf, CH_2Cl_2, rt, 1 h, 74%; (d) (1) Zn, AcOH–THF (1:8), rt, 3 h; (2) (*Z*)-11-octadecenoic acid, WSC.HCl , rt, 20 h, two steps 52%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 4 h, 82%.

Compound **16** was used as a starting material for the synthesis of **62**. The alcohol of **16** was brominated with carbon tetrabromide and triphenylphosphine to give **58**. Treatment of **58** with triallylphosphite at 180 °C for 3 h, and then aq 80% AcOH at 60 °C for 1 h gave diol **59**, which was coupled with imidate **5** at room temperature using two equivalents of AgOTf to afford β(1-6)-linked disaccharide **60**. The 2,2,2-trichloroethoxycarbonyl group of **60** was deprotected with zinc powder in AcOH–THF (1:8) to give amine, which was treated with (*Z*)-11-octadecenoic acid in CH_2Cl_2 using WSC·HCl as a dehydrating agent to afford amide **61** as mentioned in the formation of **50** from **49**. The four allyl groups of **61** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **62** (Scheme 9).

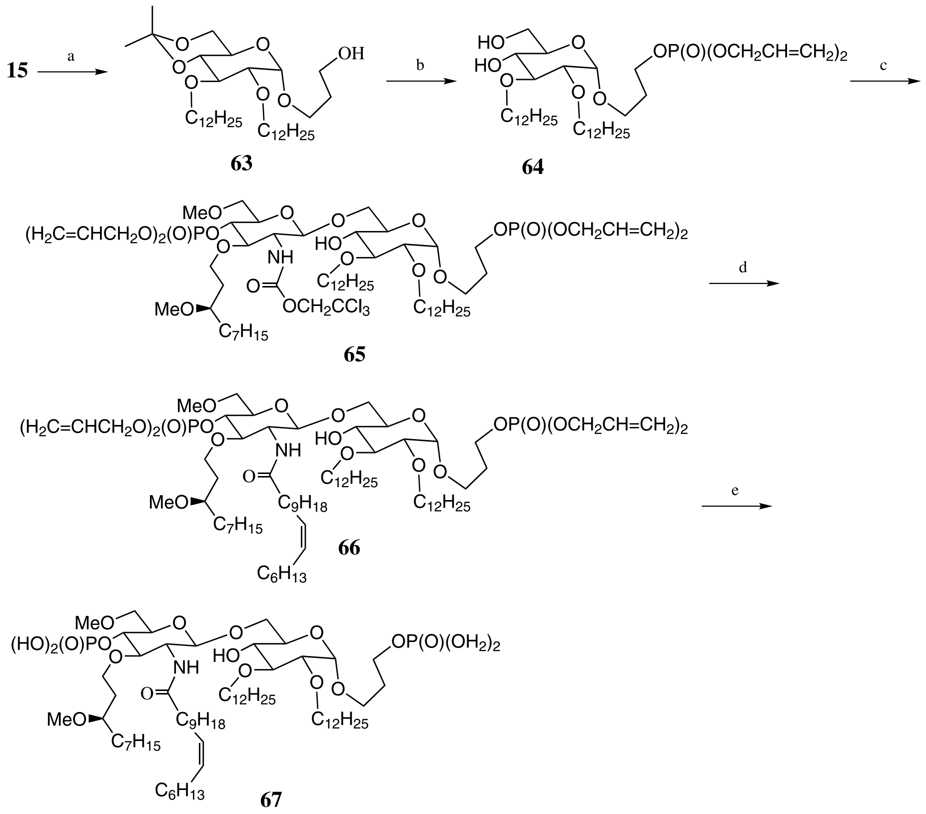
Fourthly, we synthesized compounds **67** having an α-3-(phosphonooxy)propyl group at the anomeric position, and **15**[6] was used as a starting material. Hydroboration of the allyl double bond of **15** with 9-borabicyclo[3.3.1]nonane (9-BBN), and subsequent oxidation of the borane compound with aq 30% H_2O_2 and aq 3 M NaOH gave alcohol **63**. After the alcohol of **63** was converted diallyl phosphate, the 4,6-*O*-isopropylidene group of **63** was deprotected with aq 80% acetic acid at 60 °C to yield diol **64**, which was coupled with imidate **5** at 0 °C using TMSOTf to afford β(1-6)-linked disaccharide **65** as mentioned for the formation of **49** from **48**. The 2,2,2-tri-chloroethoxycarbonyl group of **65** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoic acid using WSC·HCl as a dehydrating agent to afford amide **66**. The four allyl groups of **66** were deprotected with $Pd(PPh_3)_4$ and Et_3N–HCOOH in THF to yield **67** (Scheme 10).

Finally, we synthesized compound **72** having an α-3-(phosphono)propyl group at the anomeric position. The alcohol of **63** was brominated with CBr_4 and PPh_3 to give **68**. Treatment of **68** with triallylphosphite at 180 °C for 3 h, and then aq 80% AcOH at 60 °C for 1 h gave diol **69**, which was coupled with imidate **5** at 0 °C using TMSOTf to afford β(1-6)-linked disaccharide **70** as mentioned for the formation of **49** from **48**. The 2,2,2-trichloroethoxycarbonyl group of **70** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **71** as mentioned in the formation of **7** from **6**. The four allyl groups of **71** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **72** (Scheme 11).

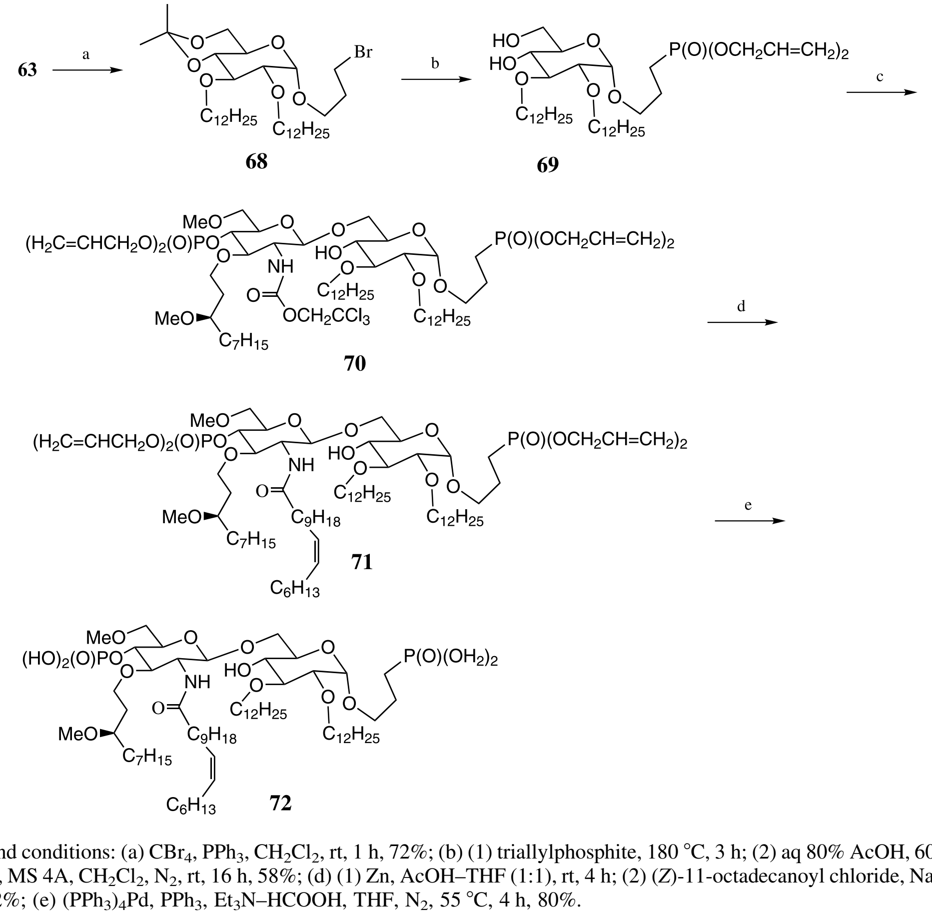
Thus, we could synthesize ten disaccharides (**8**, **14**, **21**, **31**, **40**, **51**, **57**, **62**, **67** and **72**).

2.2. Biological activity

The inhibitory activities on LPS-induced TNFα production in vitro (LPS-antagonistic activity) of ten synthetic compounds were investigated using human whole blood cells.[8] The IC_{50} values (nM) of these ten compounds, **8**, **14**, **21**, **31**, **40**, **51**, **57**, **62**, **67** and **72**, toward human whole blood cells were 11.2, 15.4, 2.7, 0.1, 0.4, 1.3, 3.2, 3.2, 1.4 and 14.4,



Scheme 10. Reagents and conditions: (a) 9-BBN, THF, rt, 18 h, then aq 3 M NaOH and aq 30% H_2O_2, rt, 3 h, 89%; (b) (1) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, THF, rt, 4 h, then aq 30% H_2O_2, THF, 0 °C, 1 h; (2) aq 80% AcOH, 60 °C, 1 h, 71%; (c) **5**, TMSOTf, MS 4A, CH_2Cl_2, under N_2, 0 °C, 1 h, 53%; (d) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoic acid, WSC.HCl, CH_2Cl_2, rt, 10 h, two steps 58%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 55 °C, 4 h, 84%.



Scheme 11. Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, rt, 1 h, 72%; (b) (1) triallylphosphite, 180 °C, 3 h; (2) aq 80% AcOH, 60 °C, 1 h, two steps 40%; (c) **5**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, N_2, rt, 16 h, 58%; (d) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecanoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 30 min, two steps 52%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 55 °C, 4 h, 80%.

respectively. The activities of the α-carboxymethyl compounds (**8** and **14**) in the anomeric position were relatively weak, and those of compounds **21**, **31**, **40**, **51** and **57** having an α-2-(phosphonooxy)ethyl group, **62** having an α-2-(phosphono)ethyl group and **67** having an α-3-(phosphonooxy)propyl group at the anomeric position were sufficiently strong. Above all, the activity ($IC_{50} = 0.1$ nM) of compounds **31** was strongest. The difference between the C6′ methoxyl group of **21** and the C6′ hydroxyl group of **31** largely affected the activity. Compounds **21** ($IC_{50} = 2.7$ nM) possessing an α-2-(phosphonooxy)ethyl group and **62** ($IC_{50} = 3.2$ nM) possessing an α-2-(phosphono)ethyl group at the anomeric position showed almost the same level of activities. On the contrary, the activity of 3-(phosphono)propyl **72** ($IC_{50} = 14.4$ nM) was much weaker than that of 3-(phosphonooxy)propyl **67** ($IC_{50} = 1.4$ nM). It is difficult to understand that the difference of one methylene length between compounds **62** and **72** or one oxygen existence between compounds **67** and **72** has such an influence on the LPS-antagonistic activity.

In addition, inhibitory activity (ID_{50}) on TNFα production toward galactosamine loaded C3H/HeN mice in vivo of compounds **21**, **31**, **57**, **62** and **67** was measured.[9] The values were 0.29, 0.50, 0.61, not dose-dependent and 0.33 mg/kg, respectively. These compounds were sufficiently strong except for anomeric 2-(phosphono)ethyl compound **62** toward C3H/HeN mice. However, judging from the result of C6′ methoxy compound **21** and C6′ hydroxy compound **31**, methoxy compound **21** was a little stronger than hydroxyl compound **31** toward C3H/HeN mice in spite of compound **31** having been much stronger activity than compound **21** toward human whole blood cells. The difference of one methylene length between compounds 2-(phosphonooxy)ethyl **21** and 3-(phosphonooxy)propyl **67** did not influence largely the inhibitory activities on LPS-induced TNFα production.

Usually, lipid A analogs having six fatty acids chains[10] show LPS-agonistic (endotoxic) activity toward both human and mouse macrophages, and lipid IVa (biosynthetic precur of lipid A)[11] having four fatty acid chains shows LPS-antagonistic activity toward human blood cells and adversely endotoxic activity toward mouse macrophages.[12] However, the synthetic compounds, this time, showed LPS-antagonistic activity toward both human whole blood cells and galactosamine loaded C3H/HeN mice. This tendency was the same as that for nontoxic natural RsDPLA[3] and synthetic E5564[5] containing a (*Z*)-double bond in one of the fatty acids.

3. Conclusion

Thus, we could synthesize ten E5564-related disaccharides containing a glucose instead of the glucosamine at the reducing end. As a result, it was proved that these novel synthetic compounds had almost the same or stronger activities towards both human blood cells and murine macrophages than against classic lipid A-type disaccharides having the glucosamine-glucosamine moieties.[13]

4. Experimental

4.1. General procedure

^1H NMR spectra were recorded with a JEOL-GSX 400 or a JNM-ECT 500 spectrometer using tetramethylsilane (TMS) as an internal standard. IR absorption spectra were measured with an IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-700 mass spectrometer. Separation of compounds by column chromatography was done with silica gel 60 (230–400 mesh ASTM) under a slightly elevated pressure (111–182 kPa) for easy elution. Commercially available anhydrous THF and dichloromethane were used for the reactions. DMF and pyridine were dried by storage over 4 Å molecular sieves.

4.1.1. Allyl 3-*O*-dodecyl-4,6-*O*-isopropylidene-2-*O*-tetradecyl-α-D-glucopyranoside (2). To a solution of allyl 3-*O*-docecyl-α-D-glucopyranoside **1** (2.84 g, 6.63 mmol) and tetradecyl methanesulfonate (2.33 g, 7.95 mmol) in DMF (20 ml) was added NaH (55% oil dispersion 347 mg, 7.95 mmol). After stirring for 16 h at room temperature, the reaction mixture was diluted with EtOAc, which was washed with ice water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **2** (3.10 g, 75%) as an oil. 400 MHz ^1H NMR ($CDCl_3$) 0.88 (6H, t, $J = 6.6$ Hz), 1.26 (40H, bs), 1.41 (3H, s), 1.48 (3H, s), 1.50–1.61 (4H, m), 3.30 (1H, m), 3.50–3.74 (8H, m), 3.84 (1H, m), 4.07 (1H, dd, $J = 6.6$, 13.2 Hz), 4.18 (1H, dd, $J = 5.1$, 13.2 Hz), 4.91 (1H, d, $J = 3.7$ Hz), 5.22 (1H, m), 5.32 (1H, m), 5.92 (1H, m)). FABMS (positive-ion) *m/z*, 625 $[M+H]^+$, 647 $[M+Na]^+$.

4.1.2. (Allyloxycarbonyl)methyl 3-*O*-dodecyl-4,6-*O*-isopropylidene-2-*O*-tetradecyl-α-D-glucopyranoside (3). To a solution of **2** (625 mg, 1.00 mmol) in $MeCN–CCl_4–H_2O$ (2:2:3, 35 ml) were added $RuO_2{\cdot}xH_2O$ (16 mg) and $NaIO_4$ (4.0 g). The mixture was stirred for 3 h at room temperature, and diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a carboxylic acid, which was dissolved in DMF (10 ml). Allyl bromide (1.2 ml) and Et_3N (0.8 ml) were added to this solution, and this mixture was stirred for 16 h at room temperature, and diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrate in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (9:1) gave **3** (490 mg, 72%) as an oil. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J = 6.6$ Hz), 1.25 (40H, bs), 1.40 (3H, s), 1.48 (3H, s), 1.48–1.65 (4H, m), 3.18 (1H, m), 3.33 (1H, m), 3.50–3.89 (8H, m), 4.18, 4.34 (2H, AB-q, $J = 16.2$ Hz), 4.62–4.67 (2H, m), 5.08 (1H, m), 5.22 (1H, m), 5.24–5.36 (2H, m), 5.91 (1H, m). FABMS (positive-ion) *m/z*, 683 $[M+H]^+$, 705 $[M+Na]^+$.

4.1.3. (Allyloxycarbonyl)methyl 3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (4). A solution of **3** (480 mg, 0.703 mmol) in $AcOH–H_2O$ (4:1, 5 ml) was stirred for 2 h at 65 °C, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3:2, then 2:3) gave triol **4** (319 mg, 71%) as a wax. IR ν_{max}(film) 3410, 2923, 2852, 1758, 1466 cm^{-1}. 400 MHz ^{1}H NMR (CDCl$_3$+D$_2$O) δ 0.88 (6H, t, *J*=6.6 Hz), 1.26 (40H, bs), 1.54–1.68 (4H, m), 3.19 (1H, m), 3.33 (1H, m), 3.47 (1H, m), 3.51–3.67 (2H, m), 3.72–3.88 (4H, m), 3.91–3.98 (2H, m), 4.21, 4.33 (2H, AB-q, *J*=16.8 Hz), 4.62–4.67 (2H, m), 5.12 (1H, m), 5.25–5.37 (2H, m), 5.91 (1H, m). FABMS (positive-ion) *m/z*, 665 [M+Na]$^+$. Anal. Calcd for $C_{37}H_{70}O_{8}$.: C, 69.12; H, 10.97. Found: C, 69.09; H, 10.81.

4.1.4. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (6). To a solution of imidate **5** (249.2 mg, 0.296 mmol) and **4** (190 mg, 0.296 mmol) in CH$_2$Cl$_2$ (7 ml) was added MS 4 Å (400 mg). After stirring for 30 min at room temperature, the mixture was cooled at -40 °C, and TMSOTf (10 mg, 0.045 mmol) was added to this mixture. The mixture was stirred for 2 h at -40 °C under nitrogen, and diluted with EtOAc, which was washed with satd NaHCO$_3$ and brine, dried over MgSO$_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1:1, then 1:2) gave **6** (169 mg, 43%) as a gum. IR ν_{max}(film) 3448, 3291, 3084, 2925, 2855, 1751, 1650 (w), 1546, 1465 cm^{-1}. 400 MHz ^{1}H NMR (CDCl$_3$) δ 0.89 (9H, t, *J*=6.6 Hz), 1.26 (50H, bs), 1.40–1.60 (8H, m), 3.25–3.91 (23H, m, containing 3H, s, at δ 3.28 ppm, and 3H, s, at δ 3.39 ppm), 4.05–4.19 (2H, m), 4.28–4.35 (3H, m), 4.55–4.81 (8H, m), 5.12 (1H, m), 5.09 (1H, d, *J*=3.7 Hz), 5.24–5.39 (6H, m), 5.90–5.99 (3H, m). FABMS (positive-ion) *m/z*, 1346 [M+Na]$^+$, 1344 [M+Na, ^{35}Cl]$^+$. HRFABMS, calcd for $C_{64}H_{115}NCl_3O_{18}PNa$: 1344.6818. Found: 1344.6826.

4.1.5. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (7). To a solution of **6** (154 mg, 0.116 mmol) in THF–AcOH (1:1, 6 ml) was added Zn powder (150 mg). The mixture was stirred vigorously with a magnetic stirrer at 25 °C for 4 h, and filtered. The filtrate was concentrated in vacuo below 30 °C, and diluted with EtOAc, which was washed with aq satd NaHCO$_3$ and brine, dried over MgSO$_4$ and filtered. The filtrate was concentrated in vacuo to give an amine, which was dissolved in THF (1.0 ml)–H$_2$O (0.3 ml) containing NaHCO$_3$ (30 mg, 0.357 mmol). To this solution was added a solution of (*Z*)-11-octadecenoyl chloride [obtained from (*Z*)-11-octadecenoic acid (40 mg, 0.142 mmol) by treatment of the excess oxalyl chloride in benzene at 25 °C for 2 h] in THF (0.5 ml) with vigorous stirring at 25 °C. After stirring for 0.5 h, the reaction mixture was diluted with EtOAc, which was washed with aq satd NaHCO$_3$ and brine, dried over MgSO$_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1:2) gave **7** (125 mg, 77%) as a gum. IR ν_{max}(KBr) 3306, 3082 (w), 2925, 2854, 1760, 1659, 1634, 1545 cm^{-1}. 400 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (12H, t, *J*=6.4 Hz), 1.25 (62H, bs), 1.35–1.80 (14H, m), 2.00–2.02 (6H, m), 2.10–2.25 (4H, m), 3.10–4.40 (29H, m, containing two 3H, s, at 3.28 and 3.38 ppm), 4.54–4.66 (6H, m), 5.09–5.39 (10H, m), 5.89–5.98 (3H, m), 5.96–6.06 (1H, m). FABMS (positive-ion) *m/z*, 1434 [M+Na]$^+$. HRFABMS, calcd for $C_{79}H_{146}NO_{17}PNa$: 1435.0226. Found: 1435.0234.

4.1.6. Carboxymethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (8). To a solution of **7** (104 mg, 0.074 mmol) in dry THF (6 ml) were added PPh$_3$ (10 mg, 0.034 mmol), Et$_3$N (37 mg, 0.365 mmol), HCOOH (34 mg, 0.728 mmol) and Pd(PPh$_3$)$_4$ (5 mg, 0.004 mmol) in this sequence. The solution was stirred for 4 h at 55 °C under nitrogen, and concentrated in vacuo to give a mixture, which was chromatographed on a DEAE-cellulose (Whatman ion-exchange cellulose, wet 3 g) column. The column was prepared by preliminary consecutive washing with 30 ml each of 0.5 M HCl, H$_2$O, 0.5 M NaOH, and H$_2$O, and 12 ml each of 1 M AcOH and H$_2$O, and 30 ml of 0.05 M AcONH$_4$, 30 ml each of CHCl$_3$–MeOH–H$_2$O (2:3:1) and finally CHCl$_3$–MeOH (2:1). The column was eluted with 3 ml each of CHCl$_3$–MeOH (2:1), then 0.05 M AcONH$_4$ in CHCl$_3$–MeOH–H$_2$O (2:3:1). The fractions containing **8** were collected. To this solution were added another volume of CHCl$_3$ and aq 0.15 M HCl to adjust the ratio of CHCl$_3$–H$_2$O–MeOH to 1:1:1, and the mixture was shaken well. The lower CHCl$_3$ layer was separated, and concentrated in vacuo to give **8** (83 mg, 95%) as a powder. [The fractions were analyzed by silica-gel TLC and a solvent system of CHCl$_3$–MeOH–AcOH–H$_2$O (8:4:1:1).] IR ν_{max}(KBr) 3500–3000 (br), 2924, 2853, 1735, 1655, 1630, 1549, 1466 cm^{-1}. 400 MHz ^{1}H NMR (CDCl$_3$+D$_2$O) δ 0.88 (12H, t, *J*=6.6 Hz), 1.26 (72H, bs), 1.40–1.80 (8H, m), 1.99–2.02 (4H, m), 2.20–2.40 (2H, m), 3.10–5.10 (29H, m, containing two 3H, s at δ 3.25 and 3.38 ppm), 5.31–5.38 (2H, m). FABMS (negative-ion) *m/z*, 1291 [M−H]$^-$. HRFABMS, calcd for $C_{70}H_{133}NO_{17}P$: 1290.9336. Found: 1290.9317. Anal. Calcd for $C_{70}H_{134}NO_{17}P$: C, 62.35; H, 10.09; N, 1.70; P, 2.08. Found: C, 62.78; H, 10.41; N, 1.10; P, 2.40.

4.1.7. (Allyloxycarbonyl)methyl 2-*O*-[(*R*)-3-*tert*-butyldimethylsilyloxytetradecyl]-3-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (10). Compound **9** was treated as described for the formation of **3** from **2** to give **10** (72% yield) as an oil. IR ν_{max}(film) 2926, 2856, 1759, 1745 (shoulder) cm^{-1}. 400 MHz ^{1}H NMR (CDCl$_3$) δ 0.04 (3H, s), 0.05 (3H, s), 0.88 (9H, s, and 6H, t, *J*=6.6 Hz), 1.26 (38H, bs), 1.40 (3H, s), 1.48 (3H, s), 1.48–1.55 (2H, m), 1.74–1.79 (2H, m), 3.33 (1H, m), 3.50–3.86 (10H, m), 4.17, 4.30 (2H, AB-q, *J*=12.8 Hz), 4.64–4.65 (2H, m), 5.07 (1H, d, *J*=3.7 Hz), 5.24–5.36 (2H, m), 5.92 (1H, m). FABMS (positive-ion) *m/z*, 813 [M+H]$^+$, 835 [M+Na]$^+$. HRFABMS, calcd for $C_{46}H_{88}O_9SiNa$: 835.6095. Found: 835.6084.

4.1.8. (Allyloxycarbonyl)methyl 2-*O*-[(*R*)-3-hydroxytetradecyl]-3-*O*-dodecyl-α-D-glucopyranoside (11). Compound **10** was treated as described for the formation of **4** from **3** to give triol **11** (60%) as a solid, mp 55–56 °C (recrystallized from EtOAc/hexane=1/4). IR ν_{max}(KBr) 3426, 2919, 2850, 1753, 1468 cm^{-1}. 400 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (6H, t, *J*=6.6 Hz), 1.26 (36H, bs), 1.40–1.78

(8H, m), 2.10 (1H, bs, OH)), 2.62 (1H, bs, OH), 3.34 (1H, dd, $J=3.7$, 9.5 Hz), 3.49–4.00 (10H, m), 4.20, 4.34 (2H, AB-q, $J=16.8$ Hz), 4.62–4.66 (2H, m), 5.21 (1H, d, $J=2.9$ Hz), 5.25–5.36 (2H, m), 5.91 (1H, m). Anal. Calcd for $C_{37}H_{70}O_9$: C, 67.44; H, 10.71. Found: C, 67.41; H, 10.70.

4.1.9. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(R)-3-hydroxytetradecyl]-α-D-glucopyranoside (12). Compound **11** and imidate **5** were treated as described for the formation of **6** from **5** to give **12** (45%) as a gum, and imidate **5** (85 mg, 34%) and starting triol **11** (80 mg, 42%) were recovered. IR ν_{max}(film) 3457 (br), 3325 (br), 3085 (w), 2926, 2855, 1748, 1650 (w), 1545 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.6$ Hz), 1.26 (46H, bs), 1.36–1.80 (10H, m), 3.22–4.90 (37H, m, containing 3H, s, at δ 3.27 ppm, and 3H, s, at δ 3.39 ppm), 5.07–5.40 (7H, m), 5.87–5.99 (3H, m). FABMS (positive-ion) *m*/*z*, 1360 [M+Na, ^{35}Cl]$^+$. HRFABMS, calcd for $C_{64}H_{115}NCl_3O_{19}PNa$: 1360.6764. Found: 1360.6764.

4.1.10. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(Z)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(R)-3-hydroxytetradecyl]-α-D-glucopyranoside (13). Compound **12** was treated as described for the formation of **7** from **6** to give **13** (67%) as a gum. IR ν_{max}(film) 3500–3000 (br), 2926, 2855, 1752, 1652, 1549 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.26 (72H, bs), 1.37–1.85 (6H, m), 1.97–2.02 (4H, m), 2.12–2.24 (2H, m), 2.97–4.32 (30H, m, containing two 3H, s, at 3.27 and 3.38 ppm), 4.53–4.66 (6H, m), 5.02–5.40 (10H, m), 5.85–5.98 (3H, m), 6.09 (1H, d, $J=6.6$ Hz). FABMS (positive-ion) *m*/*z*, 1450 [M+Na]$^+$. HRFABMS, calcd for $C_{79}H_{146}NO_{18}PNa$: 1451.0174. Found: 1451.0171.

4.1.11. Carboxymethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(R)-3-hydroxytetradecyl]-α-D-glucopyranoside (14). Compound **13** (110 mg, 0.077 mmol) was treated as described for the formation of **8** from **7** to give **14** (55 mg, 55%) as a powder. IR ν_{max}(KBr) 3292 (br), 2925, 2854, 1737, 1654, 1631, 1552, 1466 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3+CD_3OD$, 5:1) δ 0.88 (12H, t, $J=6.6$ Hz), 1.27 (70H, bs), 1.40–1.80 (8H, m), 2.00–2.03 (4H, m), 2.15–2.23 (2H, m), 3.26–4.26 (28H, m, containing two 3H, s at δ 3.31 and 3.41 ppm), 4.65 (1H, d, $J=6.5$ Hz), 5.08 (1H, d, $J=2.9$ Hz), 5.35 (2H, m). FABMS (negative-ion) *m*/*z*, 1306 [M−H]$^-$, 1328 [M−2H+Na]$^-$. HRFABMS, calcd for $C_{70}H_{133}NO_{18}P$: 1306.9260. Found: 1306.9288. Anal. Calcd for $C_{70}H_{134}NO_{18}P$: C, 64.24; H, 10.32; N, 1.07; P, 2.37. Found: C, 64.46; H, 10.46; N, 1.27; P, 2.29.

4.1.12. 2-Hydroxyethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene α-D-glucopyranoside (16). To a solution of **15** (4.00 g, 6.70 mmol) in THF–H_2O (7:2, 66 ml) were added $NaIO_4$ (6.7 g) and a 2.5% solution of OsO_4 in *tert*-BuOH (1.4 ml). This mixture was stirred for 3 h at room temperature, and diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrate in vacuo to give an aldehyde (4 g), which was dissolved in EtOH (50 ml). To this solution was added $NaBH_4$ (270 mg), and the solution was stirred for 20 min, quenched with AcOH (200 mg), diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:1) gave alcohol **16** (3.00 g, 73%) as an oil. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.6$ Hz), 1.26 (36H, bs), 1.40 (3H, s), 1.41–1.43 (4H, m), 1.48 (3H, s), 2.82 (1H, bs, OH), 3.30 (1H, m), 3.51–3.85 (14H, m), 4.89 (1H, d, $J=3.7$ Hz). FABMS (positive-ion) *m*/*z*, 601 [M+H]$^+$, 623 [M+Na]$^+$.

4.1.13. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (17). To a solution of **16** (5.65 g, 9.2 mmol) in CH_2Cl_2 (70 ml) were added 1*H*-tetrazole (1.54 g, 22 mmol), diallyl diisopropylphosphoramidite (3.50 g, 1.128 mmol) and Na_2SO_4 (6 g). After stirring for 30 min at room temperature, to this reaction mixture were added THF (70 ml) and aq 30% H_2O_2 solution (2 ml). The mixture was stirred for 15 min at room temperature, and diluted with EtOAc, which was washed with aq 10% $Na_2S_2O_3$, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (2:1) gave alcohol **17** (6.60 g, 99%) as an oil. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, $J=6.6$ Hz), 1.21–1.38 (36H, m), 1.39 (3H, s), 1.47 (3H, s), 1.50–1.58 (4H, m), 3.25–3.3 (1H, m), 3.51–3.88 (11H, m) 4.23–4.27 (2H, m) 4.56–4.60 (4H, m), 4.89 (1H, d, $J=3.7$ Hz), 5.25–5.41 (4H, m), 5.91–6.00 (2H, m). FABMS (positive-ion) *m*/*z*, 761 [M+H]$^+$, 783 [M+Na]$^+$.

4.1.14. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (18). To a solution of **17** (6.60 g, 8.67 mmol) in MeOH (60 ml) was added *p*-TsOH·H_2O (412 mg, 2.17 mmol). After stirring for 2 h at room temperature, the reaction mixture was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc and then 10% MeOH in EtOAc gave **18** (6.01 g, 96%) as a wax. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.26 (36H,bs), 1.5–1.71 (4H,m), 3.27 (1H, dd, $J=3.7$, 8.8 Hz), 3.41–3.91 (11H, m), 4.24–4.28 (2H, m), 4.48–4.59 (4H, m), 4.95 (1H, d, $J=3.7$ Hz), 5.19–5.4 (4H,m), 5.9–6.00 (2H, m). FABMS (positive-ion) *m*/*z*, 721. [M+H]$^+$, 743 [M+Na]$^+$. HRFABMS, calcd for $C_{38}H_{74}O_{10}P$: 721.4941. Found: 721.4950.

4.1.15. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (19). To a solution of **5** (1.00 g, 1.18 mmol) and **18** (1.02 g, 1.49 mmol) in CH_2Cl_2 (30 ml) were added MS 4 Å (1.5 g), AgOTf (350 mg, 1.36 mmol) and TMSOTf (20 mg, 0.090 mmol). The mixture was stirred for 16 h at room temperature under nitrogen, and diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc–Hexane (2:1) gave **19** (1.01 g, 60%) as a gum. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.6$ Hz), 1.26 (48H, bs), 1.38–1.46 (2H, m), 1.53–1.58 (4H, m), 1.69–1.77 (2H, m), 2.60 (1H, bs, OH), 3.21–3.89 (23H, m, containing two 3H at δ 3.28 and 3.39 ppm), 4.09–4.32 (4H, m), 4.52–4.60 (8H, m), 4.95 (1H, d, $J=3.7$ Hz), 5.24–5.40 (8H, m), 5.89–5.99 (4H, m), 6.41 (1H, broad, NH). FABMS (positive-ion) *m/z*, 1422 $[M+Na, ^{35}Cl]^+$, 1424. HRFABMS, calcd for $C_{65}H_{118}Cl_3NO_{20}P_2Na$: 1422.6468. Found: 1422.6473.

4.1.16. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (20). Compound **19** was treated as described for the formation of **7** from **6** to give **20** (75%) as a gum. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.26 (66H, bs), 1.40–1.79 (10H, m), 1.99–2.04 (4H, m), 2.17–2.25 (2H, m), 3.01(1H, bs, OH), 3.17–3.82 (24H, m, containing two 3H, s, at 3.28 and 3.38 ppm), 3.97–4.31 (5H, m), 4.52–4.58 (8H, m), 4.88 (1H, d, $J=3.7$ Hz), 5.13 (1H, d, $J=8.1$ Hz), 5.23–5.38 (10H, m), 5.90–6.00 (4H, m), 6.62 (1H, d, $J=6.6$ Hz, NH). FABMS (positive-ion) *m/z*, 1512 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{149}NO_{19}P_2Na$: 1513.0097. Found: 1513.0121.

4.1.17. 2-(Phosphonooxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (21). Compound **20** was treated for 16 h as described for the formation of **8** from **7** to give **21** (45%) as a wax. IR ν_{max}(KBr) 3292 (br), 2954, 2853, 1630 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3+CD_3OD$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.26 (66H, bs), 1.40–1.79 (10H, m), 1.99–2.07 (4H, m), 2.17–2.25(2H, m), 3.20–3.90 (24H, m, containing two 3H, s at 3.30 and 3.40 ppm), 4.00–4.21 (5H, m), 4.65(1H, d, $J=8.1$ Hz), 4.88 (1H, d, $J=3.7$ Hz), 5.37–5.40 (2H, m). FABMS (negative-ion) *m/z*, 1328 $[M-H]^-$, 1350 $[M+Na\text{-}2H]^-$. Anal. Calcd for $C_{68}H_{133}NO_{19}P_{2,}$: C, 61.37; H, 10.07; N, 1.05; P, 4.66. Found: C, 60.89; H, 10.11; N, 1.13; P, 4.42.

4.1.18. Allyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-2-(trifluoroacetylamino)-β-D-glucopyranoside (23). Compound **22** and (*R*)-3-methoxydecyl *p*-toluenesulfonate were treated as described for the formation of **2** from **1** to give **23** (77%) as an amorphous solid. IR ν_{max}(KBr) 3304, 3114,2995, 2930,2877, 2858, 2825, 1705, 1674 cm^{-1} 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.8$ Hz), 1.27–1.47 (12H, m), 1.41 (3H, s), 1.50 (3H, s), 1.63–1.67 (2H, m), 3.25 (1H, m), 3.28 (3H, s), 3.32 (1H, td, $J=9.8$, 4.9 Hz), 3.43 (1H, m), 3.58–3.64 (2H, m), 3.77–3.87 (3H, m), 3.93 (1H, dd, $J=4.9$, 10.7 Hz), 4.06 (1H, dd, $J=5.9$, 12.7 Hz), 4.31 (1H, dd, $J=4.9$, 12.7 Hz), 4.88 (1H, d, $J=7.8$ Hz), 5.19–5.28 (2H, m), 5.83 (1H, m), 6.56 (1H, d, $J=7.8$ Hz, NH). FABMS (positive-ion) *m/z*, 548 $(M+Na)^+$, 526 $(M+H)^+$. HRFABMS, calcd for $C_{25}H_{42}F_3NO_7Na$: 548.2808. Found: 548.2815.

4.1.19. Allyl 2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-(trifluoroacetylamino)-β-D-glucopyranoside (24). Compound **23** was treated as described for the formation of **18** from **17** to give **24** (92%) as a powder. IR ν_{max}(KBr) 3548, 3375, 3265, 3116, 2927, 2874, 2858, 1702, 1672 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.6$ Hz), 1.27–1.77 (14H, m), 3.01 (2H, bs, OH), 3.29 (3H, s), 3.34–3.53 (3H, m), 3.59 (1H, t, $J=9.5$, 8.8 Hz), 3.67 (1H, m), 3.75–3.85 (3H, m), 3.92 (1H, dd, $J=3.7$, 11.7 Hz), 4.07 (1H, m), 4.31 (1H, m), 4.84 (1H, d, $J=8.8$ Hz), 5.19–5.29 (2H, m). FABMS (positive-ion) *m/z*, 508 $[M+Na]^+$, 486 $[M+H]^+$. HRFABMS, calcd for $C_{22}H_{38}F_3NO_7Na$: 508.2494. Found: 508.2503.

4.1.20. (*Z*)-1-Propenyl 2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (25). A solution of **24** (5.34 g, 11.0 mmol) in DMSO (30 ml) containing *tert*-BuOK (3.10 g, 27.6 mmol) was stirred for 85 °C for 2 h under nitrogen. To this solution was added H_2O (10 ml), and this solution was stirred at 85 °C for 6 h. After cooling, the aqueous solution was extracted with CH_2Cl_2 (three times), washed with H_2O and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an amine, which was dissolved in THF (40 ml), and aq satd $NaHCO_3$ (20 ml) was added. To this mixture was added 2,2,2-trichloroethyl chloroformate (2.58 g, 12.2 mmol), and this mixture was stirred at 0 °C for 30 min. After another amount of aq satd $NaHCO_3$ was added, the reaction mixture was extracted with CH_2Cl_2, which was washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (2:3) gave **25** (4.70 g, 76%) as a solid. IR ν_{max}(KBr) 3323, 3054, 2927, 2873, 2857, 1716, 1672, 1642 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, $J=6.8$ Hz), 1.28–1.62 (15H, m), 1.74–1.79 (2H, m), 2.36 (1H, s, OH), 3.30 (3H, s), 3.36–3.46 (3H, m), 3.58–3.71 (3H, m), 3.81 (1H, m), 3.90–3.94 (2H, m), 4.06 (1H, s, OH), 4.57 1H, m), 4.74 (2H, s), 4.86 (1H, d, $J=6.8$ Hz), 5.37 (1H, br, NH), 6.15 (1H, m). FABMS (positive-ion) *m/z*, 586 $[M+Na]^+$, 564 $[M+H]^+$. HRFABMS, calcd for $C_{23}H_{40}Cl_3NO_8Na$: 586.1726. Found: 586.1703.

4.1.21. (*Z*)-1-Propenyl 6-*O*-allyloxycarbonyl-2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (26). To a solution of **25** (4.60 g, 8.14 mmol) in CH_2Cl_2 (30 ml) were added pyridine (1.4 ml, 17.3 mmol) and allyl chloroformate (1.1 ml, 10.4 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$, H_2O and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave **26** (4.95 g, 94%) as a wax. IR ν_{max}(KBr) 3518, 3310, 3086, 3059, 2932, 2885, 2857, 1728, 1709, 1674, 1652 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, $J=6.8$ Hz), 1.25–1.62 (15H, m), 1.70–1.79 (2H, m), 3.30 (3H, s), 3.36–3.44 (2H, m), 3.50–3.71 (4H, m), 3.91–3.94 (2H, m, containing OH), 4.38 (1H, dd, $J=4.9$, 11.7 Hz), 4.50 (1H, dd, $J=2.0$, 11.7 Hz), 4.56 (1H, m), 4.63 (2H, d, $J=5.9$ Hz), 4.74 (2H, s), 4.85 (1H, m), 5.26–5.38 (3H, m, containing NH), 5.93 (1H, m), 6.16 (1H, dd, $J=2.0$, 5.9 Hz). FABMS (positive-ion) *m/z*, 670 $[M+Na]^+$, 648 $[M+H]^+$. HRFABMS, calcd for $C_{27}H_{44}Cl_3NO_{10}Na$: 670.1914. Found: 670.1959.

4.1.22. (Z)-1-Propenyl 6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (27). To a solution of **26** (4.80 g, 7.40 mmol) in THF (30 ml) were added 1*H*-tetrazole (830 mg, 11.8 mmol) and diallyl diisopropylphosphoramidite (2.50 g, 10.2 mmol) under nitrogen at room temperature. After stirring for 20 min, the mixture was cooled to 0 °C, and aq 30% H_2O_2 (10 ml) was added to this phosphite solution. The mixture was stirred for 30 min, and diluted with EtOAc, which was washed with aq satd $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave **27** (5.17 g, 86%) as a gum. IR ν_{max}($CHCl_3$) 3450, 3089, 2955, 2873, 2859, 2829, 1746, 1674, 1650 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.8$ Hz), 1.27–1.56 (15H, m), 1.68–1.81 (2H, m), 3.29 (3H, s), 3.31 (1H, m), 3.47 (1H, m), 3.72–3.82 (3H, m), 3.90 (1H, m), 4.32–4.38 (2H, m), 4.52–4.63 (8H, m), 4.73 (2H, s), 4.99 (1H, m), 5.19–5.38 (6H, m), 5.56 (1H, m, NH), 5.89–5.99 (3H, m), 6.13 (1H, m). FABMS (positive-ion) *m*/*z*, 830 $[M+Na]^+$, 808 $[M+H]^+$. HRFABMS, calcd for $C_{33}H_{53}Cl_3NO_{13}PNa$: 830.2215. Found: 830.2231.

4.1.23. 6-*O*-Allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (28). To a solution of **27** (4.80 g, 5.93 mmol) in THF (30 ml) were added I_2 (3.08 g, 12.1 mmol) and H_2O (6 ml). After stirring for 30 min at room temperature, the reaction mixture was diluted with EtOAc, washed with aq satd $Na_2S_2O_3$, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1) gave **28** (4.30 g, 94%) as a gum. IR ν_{max}($CHCl_3$) 3598, 3435, 3317, 3089, 2955, 2931, 2873, 2858, 1746, 1651 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.8$ Hz), 1.26–1.50 (12H, m), 1.67–1.78 (2H, m), 3.26 (3H, s), 3.32 (1H, m), 3.63–3.73 (2H, m), 3.86–3.94 (2H, m), 4.18 (1H, m), 4.30–4.38 (3H, m), 4.51–4.63 (7H, m), 4.67, 4.74 (2H, AB-q, $J=11.7$ Hz), 5.24–5.40 (7H, m), 5.81 (1H, d, $J=8.8$ Hz, NH), 5.89–5.98 (3H, m). FABMS (positive-ion) *m*/*z*, 790 $[M+Na]^+$, 768 $[M+H]^+$. HRFABMS, calcd for $C_{30}H_{50}Cl_3NO_{13}P$: 768.2085. Found: 768.2089.

4.1.24. 2-(Diallylphosphonooxy)ethyl 6-*O*-{6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosy}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (29). To a solution of **28** (164 mg, 0.213 mmol) in CH_2Cl_2 (5 ml) were added trichloroacetonitrile (0.15 ml, 1.4 mmol) and Cs_2CO_3 (15 mg). After stirring for 1 h at room temperature, this solution was diluted with CH_2Cl_2, and washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an imidate, which was dissolved in CH_2Cl_2 (5 ml). To this solution were added a solution of **18** (154 mg, 0.213 mol) and molecular sieves 4 Å (150 mg) under nitrogen. After stirring for 30 min at room temperature, to this mixture was added TMSOTf (10 mg) at 0 °C. After stirring for 16 h at rt, the reaction mixture was quenched with aq satd $NaHCO_3$, extracted with CH_2Cl_2, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:4) gave **29** (125 mg, 52%) as a gum. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.6$ Hz), 1.26–1.44 (39H, m), 1.53–1.60 (6H, m), 1.64–1.79 (3H, m), 2.50 (1H, bs), 3.23–3.36 (8H, m, containing 3H, s, at δ 3.28 ppm), 3.45–3.92 (15H, m), 4.10 (1H, br d, $J=8.1$ Hz), 4.19–4.36 (5H, m), 4.53–4.59 (10H, m), 4.63 (2H, d, $J=5.9$ Hz), 4.68 (1H, br.d, $J=11.7$ Hz), 4.78 (1H, d, $J=11.7$ Hz), 4.88–4.91 (2H, m), 5.23–5.40 (10H, m), 5.88–5.98 (5H, m), 6.54 (1H, m). FABMS (positive-ion) *m*/*z*, 1492 $[M+Na]^+$.

4.1.25. 2-(Diallylphosphonooxy)ethyl 6-*O*-{6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (30). Compound **29** (125 mg, 0.085 mmol) was treated as described later in the formation of **50** from **49** to give **30** (62 mg, 47%) as an oil. IR ν_{max}(film) 3300, 3085, 2925, 2855, 1752, 1652 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.8$ Hz), 1.25–1.79 (77H, m), 1.99–2.01 (3H, m), 2.16–2.24 (2H, m), 2.87 (1H, d, $J=3.9$ Hz), 3.12–3.17 (1H, m), 3.24 (1H, dd, $J=9.8$, 2.9 Hz), 3.26–3.38 (5H, m, containing 3H, s, at δ 3.28 ppm), 3.45–3.55 (3H, m), 3.57–3.85 (8H, m), 4.02–4.06 (1H, m), 4.10 (1H, d, $J=10.7$ Hz), 4.16–4.33 (3H, m), 4.51–4.64 (12H, m), 4.88 (1H, d, $J=3.9$ Hz), 5.19 (1H, d, $J=7.8$ Hz), 5.23–5.40 (12H, m), 5.89–5.98 (5H, m), 6.74 (1H, d, $J=6.8$ Hz). FABMS (positive-ion) *m*/*z*, 1582 $[M+Na]^+$. HRFABMS, calcd for $C_{83}H_{151}O_{21}NP_2Na$: 1583.0161. Found: 1583.0179.

4.1.26. 2-(Phosphonooxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-[(*Z*)-11-(octadecenoyl)amino-4-*O*-phosphono]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (31). Compound **30** (136 mg, 0.087 mmol) was treated as described for the formation of **8** from **7** to give **31** (67 mg, 76%) as a powder. IR ν_{max}(KBr) 3285, 3064, 3005, 2955, 2923, 2853, 2327, 1716, 1657, 1632 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3+CD_3OD$) δ 0.90 (12H, t, $J=6.8$ Hz), 1.29–1.46 (70H, m), 1.56–1.74 (8H, m), 2.01–2.04 (4H, m), 2.24–2.28 (2H, m), 3.20 (1H, dd, $J=3.9$, 9.8 Hz), 3.30 (3H, s), 3.31–3.40 (2H, m), 3.45–3.49 (2H, m), 3.53–3.56 (1H, m), 3.62–3.88 (12H, m), 4.07–4.17 (4H, m), 4.49 (1H, d, $J=8.8$ Hz), 4.92 (1H, d, $J=3.9$ Hz), 5.33–5.35 (2H, m). ESIMS (negative-ion) *m*/*z*, 1314 $[M-H]^-$. HRESIMS, calcd for $C_{67}H_{130}NO_{19}P_2$: 1314.8708. Found: 1314.8694. Anal. Calcd for $C_{67}H_{131}NO_{19}P_2$: C, 61.12; H, 10.03; N, 1.06; P, 4.70. Found: C, 60.98; H, 10.01; N, 1.10; P, 4.62.

4.1.27. Allyl 2-*O*-acetyl-3-*O*-decyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (33). A solution of **32** (1.50 g, 4.244 mmol) in pyridine (5 ml) was allowed to stand for 5 h at room temperature, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3:1) gave **33** (1.50 g, 99%) as an oil. IR ν_{max}(film) 2995, 2926, 2857, 1748, 1647 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.9$ Hz), 1.26 (16H, bs), 1.41 (3H, s), 1.50 (3H, s), 2.11 (3H, s), 3.54 (1H, m), 3.60–3.77 (5H, m), 3.84 (1H, m), 3.98 (1H, dd, $J=6.6$, 13.2 Hz), 4.16 (1H, dd, $J=5.5$, 13.5 Hz), 4.77

(1H, dd, $J=4.5$, 9.5 Hz), 5.00 (1H, d, $J=3.7$ Hz), 5.20–5.32 (2H, m), 5.88 (1H, m). FABMS (positive-ion) *m/z*, 443 $[M+H]^+$, 465 $[M+Na]^+$. HRFABMS, calcd for $C_{24}H_{42}O_7Na$: 465.2828. Found: 465.2810.

4.1.28. 2-Hydroxyethyl 2-*O*-acetyl-3-*O*-decyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (34). To a solution of **33** (2.00 g, 4.519 mmol) in THF–H_2O (3:1, 40 ml) were added $NaIO_4$ (5.70 g, 26.65 mmol) and a 2.5% solution of OsO_4 in *tert*-BuOH (900 mg). This mixture was stirred for 2 h at room temperature, and diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an aldehyde, which was dissolved in EtOH (40 ml). To this solution was added $NaBH_4$ (200 mg, 5.286 mmol), and the solution was stirred for 10 min, quenched with AcOH (300 mg), diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave alcohol **34** (1.43 g, 71%) as an oil. IR ν_{max}(film) 3484, 2995, 2926, 2857, 1748 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.88 (3H, t, $J=6.8$ Hz), 1.26 (16H, bs), 1.42 (3H, s), 1.48–1.52 (5H, m, containing 3H, s, at δ 1.50 ppm), 2.11 (3H, s), 3.53–3.87 (11H, m), 4.80 (1H, dd, $J=3.9$, 9.8 Hz), 5.00 (1H, d, $J=3.9$ Hz). FABMS (positive-ion) *m/z*, 447 $[M+H]^+$, 469 $[M+Na]^+$. HRFABMS, calcd for $C_{23}H_{42}O_8Na$: 469.2777. Found: 469.2778.

4.1.29. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (35). A solution of **34** (430 mg, 0.972 mmol) in EtOH (99.5%, 15 ml) containing KOH (10 mg) was stirred for 5 h at room temperature, concentrated in vacuo and diluted with EtOAc, which was washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in CH_2Cl_2 (10 ml). To this solution Na_2SO_4 (300 mg), 1*H*-tetrazole (102 mg, 1.457 mmol) and diallyl diisopropylphosphoramidite (262 mg, 1.069 mmol) were added at room temperature. After stirring for 20 min, THF (10 ml) and aq 30% H_2O_2 (ca. 200 mg) were added. After stirring for 15 min at room temperature, the reaction mixture was diluted with EtOAc, washed with aq 10% $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc gave alcohol **35** (430 mg, 79%) as an oil. IR ν_{max}(film) 3409, 2994, 2926, 2857, 1744 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, $J=6.6$ Hz), 1.26 (14H, bs), 1.40 (3H, s), 1.49 (3H, s), 1.55–1.59 (2H, m), 3.09 (1H, d, $J=8.1$ Hz, OH), 3.44–3.94 (10H, m), 4.23–4.28 (2H, m) 4.55–4.60 (4H, m), 4.88 (1H, d, $J=3.7$ Hz), 5.25–5.30 (2H, m), 5.36–5.43 (2H, m), 5.91–6.02 (2H, m).

4.1.30. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-4,6-*O*-isopropylidene-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (36). To a solution of **35** (440 mg, 0.779 mmol) in CH_2Cl_2 (30 ml) were added 3-oxotetradecanoic acid (208 mg, 0.858 mmol) and WSC·HCl (179 mg, 0.935 mmol). The mixture was stirred for 40 min, and concentrated in vacuo to give a residue, which was diluted with EtOAc. The solution was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (1:1) gave **36** (464 mg, 75%) as a gum. IR ν_{max}(film) 2926, 2856, 1750, 1719, 1650 (w), 1627 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, $J=6.6$ Hz), 1.26 (30H, bs), 1.39 (3H, s), 1.40–1.63 (7H, m, containing 3H, s, at δ 1.49 ppm), 2.53 (2H, t, $J=7.3$ Hz), 3.40–3.54 (3H, m), 3.61–3.75 (6H, m), 3.82–3.88 (2H, m), 4.19–4.23 (2H, m) 4.56–4.59 (4H, m), 4.77 (1H, dd, $J=3.7$, 9.5 Hz), 5.03 (1H, d, $J=4.4$ Hz), 5.26–5.41 (4H, m), 5.93–6.00 (2H, m). FABMS (positive-ion) *m/z*, 789 $[M+H]^+$, 811 $[M+Na]^+$. HRFABMS, calcd for $C_{41}H_{73}O_{12}PNa$: 811.4737. Found: 811.4724.

4.1.31. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (37). A solution of **36** (460 mg, 0.583 mmol) in aq 80% AcOH (46 ml) was heated at 85 °C for 1.5 h, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with EtOAc–MeOH (19:1) gave **37** (374 mg, 86%) as a gum. IR ν_{max}(film) 3407 (br), 2925, 2855, 1747, 1718, 1650 (w), 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, $J=6.6$ Hz), 1.26 (30H, bs), 1.53–1.60 (4H, m), 2.51–2.55 (3H, m, containing OH), 2.66 (1H, d, $J=2.9$ Hz, OH), 3.46–3.90 (11H, m, containing 2H, s at δ 3.50 ppm), 4.20–4.25 (2H, m) 4.55–4.59 (4H, m), 4.72 (1H, dd, $J=3.7$, 9.5 Hz), 5.08 (1H, d, $J=3.7$ Hz), 5.26–5.41 (4H, m), 5.91–5.99 (2H, m). FABMS (positive-ion) *m/z*, 749 $[M+H]^+$, 771 $[M+Na]^+$. HRFABMS, calcd for $C_{38}H_{69}O_{12}PNa$: 771.4425. Found: 771.4418.

4.1.32. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (38). Compound **37** (374 mg, 0.499 mmol) was treated as described for the formation of **19** from **5** and **18** to give **38** (486 mg, 68%) as a gum after silica gel column chromathography with EtOAc. IR ν_{max}(film) 3292 (br), 3085 (w), 2927, 2856, 1748, 1721, 1650 (w), 1545, 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.6$ Hz), 1.26 (42H, bs), 1.40–1.80 (8H, m), 2.52 (1H, t, $J=7.7$ Hz), 2.80 (1H, bs, OH), 3.24–3.84 (24H, m, containing two 3H at δ 3.29 and 3.39 ppm), 4.09–4.32 (4H, m), 4.53–4.59 (8H, m), 4.69–4.71 (3H, m), 4.87 (1H, m), 5.03 (1H, d, $J=3.7$ Hz), 5.24–5.41 (8H, m), 5.90–5.99 (4H, m), 6.36 (1H, bs, NH). FABMS (positive-ion) *m/z*, 1428 $[M+H]^+$, 1450 $[M+Na, {}^{35}Cl]^+$. HRFABMS, calcd for $C_{65}H_{114}Cl_3NO_{22}P_2Na$: 1450.6298. Found: 1450.6301.

4.1.33. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (39). Compound **38** was treated as described for the formation of **7** from **6** to give **39** (81%) as a gum. IR ν_{max}(film) 3302, 3086 (w), 2926, 2855, 1746, 1719, 1654, 1553, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.26 (60H, bs), 1.40–1.80 (10H, m), 1.99–2.02 (4H, m), 2.17–2.27 (2H, m), 2.52 (2H, m), 3.20–3.91 (26H, m, containing two 3H, s, at 3.29 and 3.38 ppm), 4.07–4.27 (6H, m), 4.54–4.57 (8H, m), 4.70 (1H, dd, $J=3.7$, 9.5 Hz), 5.02 (1H, d, $J=3.7$ Hz), 5.19 (1H, d, $J=8.1$ Hz), 5.23–5.40 (10H, m), 5.88–5.99 (4H, m), 6.51 (1H, d, $J=6.6$ Hz, NH). FABMS (positive-ion) *m/z*, 1518 $[M+H]^+$, 1540 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{145}NO_{21}P_2Na$: 1540.9682. Found: 1540.9653.

4.1.34. 2-(Phosphonooxy)ethyl 3-*O*-decyl-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (40). Compound **39** (200 mg, 0.132 mmol) was treated for 16 h at 55 °C as described for the formation of **8** from **7** to give **40** (150 mg, 84%) as a wax. IR ν_{max}(KBr) 3286 (br), 2825, 2854, 1742, 1716, 1629, 1552, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$–CD_3OD, 5:1) δ 0.88 (12H, t, $J=6.6$ Hz), 1.26 (60H, bs), 1.40–1.63 (8H, m), 1.72–1.77 (2H, m), 1.99–2.04 (4H, m), 2.18–2.22 (2H, m), 2.53–2.57 (2H, m), 3.30–3.83 (24H, m, containing two 3H, s at 3.30 and 3.41 ppm), 4.01–4.14 (4H, m), 4.65–4.72 (2H, m), 4.98 (1H, d, $J=3.7$ Hz), 5.30–5.36 (2H, m). FABMS (negative-ion) *m/z*, 1356 $[M-H]^-$. Anal. Calcd for $C_{68}H_{129}NO_{21}P_2H_2O$: C, 59.33; H, 9.59; N, 1.02; P, 4.50. Found: C, 59.24; H, 9.66; N, 1.20; P, 4.32.

4.1.35. Allyl 4,6-*O*-benzylidene-3-*O*-dodecyl-α-D-glucopyranoside (42). To a solution of **41** (2.85 g, 7.33 mmol) in DMF (30 ml) were added benzaldehyde dimethyl acetal (3.3 ml) and *p*-toluenesulfonic acid monohydrate (140 mg). The mixture was stirred for 16 h at room temperature, diluted with ether, washed with aq. sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (5:1) gave **42** (3.05 g, 87%) as a solid. IR ν_{max}(KBr) 3441, 1371, 1072 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $J=6.8$ Hz), 1.23–1.33 (18H, m), 1.57–1.61 (2H, m), 2.29 (1H, d, $J=6.8$ Hz, OH), 3.52–3.56 (2H, m), 3.66–3.75 (3H, m), 3.84–3.90 (2H, m), 4.07 (1H, dd, $J=6.8$, 12.7 Hz), 4.24 (1H, dd, $J=4.9$, 12.7 Hz), 4.27 (1H, dd, $J=4.9$, 9.8 Hz), 4.96 (1H, d, $J=2.9$ Hz), 5.24 (1H, d, $J=10.7$ Hz), 5.34 (1H, dd, $J=2.0$, 15.6 Hz), 5.55 (1H, s), 5.94 (1H, m), 7.33–7.39 (3H, m), 7.47–7.49 (2H, m). FABMS (positive-ion): *m/z* 477 $(M+H)^+$, 499 $(M+Na)^+$. HRFABMS, calcd for $C_{28}H_{44}O_6Na$: 499.3036. Found: 499.3040. Anal. Calcd for $C_{28}H_{44}O_6$: C, 70.56; H, 9.30. Found: C, 70.21; H, 9.00.

4.1.36. Allyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-(4-methoxybenzyloxy)tetradecyl]-α-D-glucopyranoside (43). To a solution of **42** (3.54 g, 7.43 mmol) in DMF (100 ml) was gradually added NaH (60% oil dispersion, 1.62 g, 40.5 mmol) at 0 °C with stirring. After 15 min at 0 °C, (*R*)-3-(4-methoxybenzyloxy)tetradecyl methanesulfonate (4.02 g, 9.38 mmol) was added to the mixture. After stirring for 6 h at room temperature, the mixture was quenched with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (4:1) gave **43** (4.01 g, 67%) as a white solid. IR ν_{max}(KBr) 3067, 3038, 2920, 2851, 1615 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.21–1.59 (40H, m), 1.76–1.87 (2H, m), 3.34 (1H, dd, $J=3.9$, 8.8 Hz), 3.51 (1H, dd, $J=8.8$, 9.8 Hz), 3.56 (1H, m), 3.68–3.82 (9H, m, containing 3H, s, at 3.80 ppm), 3.86 (1H, dt, $J=4.9$, 9.8 Hz), 4.07 (1H, dd, $J=6.8$, 12.7 Hz), 4.20 (1H, dd, $J=5.9$, 12.7 Hz), 4.26 (1H, dd, $J=4.9$, 9.8 Hz), 4.43, 4.47 (2H, ABq, $J=11.7$ Hz), 4.96 (1H, d, $J=3.9$ Hz), 5.21–5.35 (2H, m), 5.55 (1H, s), 5.92 (1H, m), 6.87 (2H, d, $J=7.8$ Hz), 7.25–7.27 (2H, m), 7.33–7.38 (3H, m), 7.59–7.50 (2H, m). FABMS (positive-ion): *m/z* 831 $(M+Na)^+$. HRFABMS, calcd for $C_{50}H_{80}O_8Na$: 831.5755. Found: 831.5731.

4.1.37. 2-Hydroxyethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-(4- methoxybenzyloxy)tetradecyl]-α-D-glucopyranoside (44). To a solution of **43** (1.63 g, 2.01 mmol) in acetone (16 ml) and H_2O (4 ml) were added $NaIO_4$ (1.28 g, 5.98 mmol) and OsO_4 (2.5 wt% solution in *t*-BuOH, 1.3 ml, 0.104 mmol). After stirring for 4 h at room temperature, the reaction mixture was quenched with aq satd $Na_2S_2O_3$, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in EtOH (15 ml). To this solution was added $NaBH_4$ (102 mg, 2.71 mmol) at 0 °C. After stirring for 1 h at room temperature, the mixture was concentrated in vacuo, diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave **44** (1.05 g, 64%) as a gum. IR ν_{max}($CHCl_3$) 3516, 2928, 2856 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.22–1.62 (40H, m), 1.77–1.83 (2H, m), 2.82 (1H, dd, $J=5.9$, 6.8 Hz), 3.35 (1H, d, $J=3.9$, 9.8 Hz), 3.50 (1H, dd, $J=8.8$, 9.8 Hz), 3.53 (1H, m), 3.63 (1H, m), 3.68–3.91 (13H, m, containing 3H, s, at 3.80 ppm), 4.27 (1H, dd, $J=4.9$, 9.8 Hz), 4.42, 4.47 (2H, ABq, $J=11.7$ Hz), 4.95 (1H, d, $J=3.9$ Hz), 5.54 (1H, s), 6.87 (2H, d, $J=7.8$ Hz), 7.25–7.27 (2H, m), 7.33–7.38 (3H, m), 7.48–7.50 (2H, m). FABMS (positive-ion): *m/z* 835 $(M+Na)^+$.

4.1.38. 2-(Diallylphosphonoxy)ethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-(4-methoxybenzyloxy)tetradecyl]-α-D-glucopyranoside (45). To a solution of **44** (930 mg, 1.14 mmol) and 1*H*-tetrazole (157 mg, 2.25 mmol) in THF (10 ml) was added diallyl diisopropylphosphoramidite (379 mg, 1.54 mmol). After stirring for 4 h at room temperature, aq 30% H_2O_2 (1 ml) was added to the reaction mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq satd $Na_2S_2O_3$, extracted with EtOAc, washed with water, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1) gave **45** (1.07 g, 96%) as a gum. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.21–1.59 (40H, m), 1.73–1.85 (2H, m), 3.33 (1H, dd, $J=3.9$, 8.8 Hz), 3.50 (1H, t, $J=9.8$ Hz), 3.55 (1H, m), 3.66–3.91 (12H, m, containing 3H, s, at 3.80 ppm), 4.22–4.29 (3H, m), 4.42, 4.47 (2H, AB-q, $J=11.7$ Hz), 4.54–4.57 (4H, m), 4.94 (1H, d, $J=3.9$ Hz), 5.21–5.39 (4H, m), 5.54 (1H, s), 5.87–5.98 (2H, m), 6.86–6.91 (2H, m), 7.25–7.27 (2H, m), 7.32–7.38 (3H, m), 7.47–7.49 (2H, m). FABMS (positive-ion): *m/z* 995 $(M+Na)^+$, 973 $(M+H)^+$.

4.1.39. 2-(Diallylphosphonoxy)ethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (46). To a solution of **45** (1.04 g, 1.07 mmol) in CH_2Cl_2 (10 ml) and H_2O (1 ml) was added DDQ (291 mg, 1.28 mmol). After stirring for 1.5 h at room temperature, the solution was diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane-EtOAc (3:7) gave **46** (821 mg, 90%) as a gum. IR $\nu_{max}(CHCl_3)$ 3502, 2927, 2855, 1732 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, *J* = 6.8 Hz), 1.23–1.59 (40H, m), 1.65–1.75 (2H, m), 3.19 (1H, d, *J* = 2.9 Hz, OH), 3.37 (1H, dd, *J* = 3.9, 9.8 Hz), 3.52 (1H, t, *J* = 9.8 Hz), 3.66–3.92 (10H, m), 4.23–4.29 (3H, m), 4.55–4.59 (4H, m), 5.01 (1H, d, *J* = 3.9 Hz), 5.22–5.24 (2H, m), 5.33–5.37 (2H, m), 5.53 (1H, s), 5.89–5.97 (2H, m), 7.33–7.38 (3H, m), 7.46–7.48 (2H, m). FABMS (positive-ion): *m/z* 875 $(M+Na)^+$, 853 $(M+H)^+$. HRFABMS, calcd for $C_{47}H_{81}O_{11}PNa$: 875.5413. Found: 875.5426.

4.1.40. 2-(Diallylphosphonoxy)ethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (47). To a solution of **46** (810 mg, 0.950 mmol) in CH_2Cl_2 (10 ml) was added PCC (413 mg, 1.92 mmol). After stirring for 5 h at room temperature, the solution was filtered through Celite. The filtrate was concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (3:7) gave **47** (753 mg, 93%) as a gum. IR $\nu_{max}(CHCl_3)$ 2927, 2855, 1713 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, *J* = 6.8 Hz), 1.23–1.30 (34H, m), 1.52–1.56 (4H, m), 2.43 (2H, dd, *J* = 6.8, 7.8 Hz), 2.64, 2.74 (2H, ABqt, *J* = 16.6, 6.8 Hz), 3.38 (1H, dd, *J* = 3.9, 9.8 Hz), 3.49 (1H, dd, *J* = 8.8, 9.8 Hz), 3.63–3.71 (3H, m), 3.75–3.78 (2H, m), 3.83–3.92 (4H, m), 4.22–4.28 (3H, m), 4.55–4.57 (4H, m), 4.95 (1H, d, *J* = 3.9 Hz), 5.22–5.24 (2H, m), 5.33–5.37 (2H, m), 5.52 (1H, s), 5.89–5.97 (2H, m), 7.34–7.37 (3H, m), 7.46–7.48 (2H, m). FABMS (positive-ion): *m/z* 873 $(M+Na)^+$, 851 $(M+H)^+$. HRFABMS, calcd for $C_{47}H_{79}O_{11}PNa$: 873.5257. Found: 873.5265.

4.1.41. 2-(Diallylphosphonoxy)ethyl 3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (48). Compound **47** was treated as described for the formation of **4** from **3** to give diol **48** (92%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3599, 3409, 2927, 2872, 2855, 1713 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, *J* = 6.8 Hz), 1.26–1.31 (34H, m), 1.51–1.56 (4H, m), 1.72 (1H, bs, OH), 2.43 (2H, t, *J* = 6.8, 7.8 Hz), 2.62 (1H, bs, OH), 2.64, 2.71 (2H, ABqt, *J* = 16.6, 6.8 Hz), 3.30 (1H, dd, *J* = 2.9, 9.8 Hz), 3.44 (1H, t, *J* = 8.8 Hz), 3.50 (1H, t, *J* = 8.8 Hz), 3.58 (1H, m), 3.72–3.91 (8H, m), 4.20–4.30 (2H, m), 4.55–4.59 (4H, m), 4.97 (1H, d, *J* = 2.9 Hz), 5.25–5.40 (4H, m), 5.91–5.99 (2H, m). FABMS (positive-ion): *m/z* 785$(M+Na)^+$. HRFABMS, calcd for $C_{40}H_{75}O_{11}PNa$: 785.4943. Found: 785.4951.

4.1.42. 2-(Diallylphosphonoxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (49). A solution of imidate **5** (844 mg, 1.00 mmol), diol **48** (626 mg, 0.821 mmol) and molecular sieves 4 Å (590 mg) in CH_2Cl_2 (10 ml) was stirred at room temperature. After stirring for 1 h, cat. TMSOTf (6 μl, 0.033 mmol) was added to the mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq satd $NaHCO_3$, diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:3) gave **49** (627 mg, 53%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3588, 3450, 3272, 3088, 2928, 2873, 2856, 1732, 1651 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, *J* = 6.8 Hz), 1.25–1.78 (52H, m), 2.42 (2H, dd, *J* = 6.8, 7.8 Hz), 2.60–2.73 (3H, m, containing 1H, OH), 3.26–3.36 (8H, m, containing 3H, s, at 3.28 ppm), 3.39 (3H, s), 3.44 (1H, t, *J* = 8.8 Hz), 3.54 (1H, m), 3.60–3.64 (2H, m), 3.68–3.89 (11H, m), 4.10 (1H, d, *J* = 11.7 Hz), 4.20 (1H, m), 4.25–4.31 (2H, m), 4.53–4.59 (8H, m), 4.70, 4.77 (2H, AB-q, *J* = 12.8 Hz), 4.85 (1H, d, *J* = 7.8 Hz), 4.92 (1H, d, *J* = 2.9 Hz), 5.24–5.39 (8H, m), 5.90–5.99 (4H, m), 6.45 (1H, bs, NH). FABMS (positive-ion): *m/z* 1464 $(M+Na)^+$. HRFABMS, calcd for $C_{67}H_{120}Cl_3NO_{21}P_2Na$: 1464.6798. Found: 1464.6812.

4.1.43. 2-(Diallylphosphonoxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (50). To a solution of **49** (432 mg, 0.300 mmol) in THF (9 ml) and acetic acid (1 ml) was added zinc dust (776 mg, 11.872 mmol). After vigorously stirring for 4 h at room temperature, the solution was filtered to remove the Zn powder and concentrated in vacuo to give a crude product. The product was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude product, which was dissolved in CH_2Cl_2 (10 ml). (*Z*)-11-Octadecenoic acid (198 mg, 0.697 mmol) and WSC·HCl (167 mg, 0.873 mmol) were added to this solution. After stirring for 10 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:9) gave **50** (256 mg, 56%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3605, 3453, 3316, 2928, 2856, 1712, 1662 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, *J* = 6.8 Hz), 1.18–1.80 (74H, m), 1.99–2.01 (4H, m), 2.14–2.26 (2H, m), 2.41 (2H, dd, *J* = 6.8, 7.8 Hz), 2.62, 2.70 (2H, AB-q, t, *J* = 16.6, 6.8 Hz), 3.07 (1H, d, *J* = 2.9 Hz, OH), 3.20 (1H, m), 3.25–3.32 (5H, m, containing 3H, s, at 3.28 ppm), 3.35–3.40 (4H, m, containing 3H, s, at 3.38 ppm), 3.43 (1H, dd, *J* = 8.8, 9.8 Hz), 3.56–3.88 (13H, m), 3.96 (1H, dd, *J* = 8.8, 9.8 Hz), 4.09 (1H, d, *J* = 9.8 Hz), 4.17 (1H, m), 4.22–4.29 (2H, m), 4.53–4.58 (8H, m), 4.90 (1H, d, *J* = 2.9 Hz), 5.15 (1H, d, *J* = 7.8 Hz), 5.23–5.40 (10H, m). FABMS (positive-ion): *m/z* 1554 $(M+Na)^+$. HRFABMS, calcd for $C_{82}H_{151}NO_{20}P_2Na$: 1555.0201. Found: 1555.0200.

4.1.44. 2-(Phosphonoxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (51). Compound **50** (204 mg, 0.133 mmol) was treated as described for the formation of **8** from **7** to give **51** (164 mg, 90%) as a white powder. IR $\nu_{max}(KBr)$ 3287, 3070, 2954, 2925, 2854, 2324, 1714, 1629 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.90 (12H, t, *J* = 6.8 Hz), 1.30–1.67 (72H, m), 1.72–1.76 (2H, m), 1.99–2.04 (4H, m), 2.20–2.30 (2H, m), 2.50 (2H, t, *J* = 7.3 Hz), 2.67–2.69 (2H, m), 3.23 (1H, dd, *J* = 3.9, 9.8 Hz), 3.29–3.31 (4H, m, containing 3H, s, at 3.29 ppm), 3.35 (1H, t, *J* = 8.8 Hz), 3.39 (3H, s), 3.42

(1H, dd, $J=8.8$, 9.8 Hz), 3.52–3.77 (11H, m), 3.79–3.91 (5H, m), 4.04–4.15 (4H, m), 4.48 (1H, d, $J=8.8$ Hz), 4.93 (1H, d, $J=3.9$ Hz), 5.31–5.37 (2H, m). ESIMS (negative-ion): *m/z* 1370 (M$-$H)$^-$. HRESIMS, calcd for $C_{70}H_{134}NO_{20}P_2$: 1370.8966. Found: 1370.8944.

4.1.45. 2-Hydroxyethyl 2-*O*-[(*R*)-3-(*tert*-butyldimethylsilyloxy)tetradecyl]-3-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (52). To a solution of **9** (1.45 g, 1.925 mmol) in THF (35 ml) and H_2O (10 ml) were added $NaIO_4$ (3.00 g, 14.03 mmol) and OsO_4 (2.5 wt% solution in *t*-BuOH, 1.3 ml, 1.04 mmol). After stirring for 2 h at room temperature, the reaction mixture was diluted with ether, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in EtOH (40 ml). To this solution was added $NaBH_4$ (150 mg, 3.965 mmol) at 0 °C. After stirring for 20 min at 0 °C, diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (4:1) gave **52** (598 mg, 41%) as a gum. IR ν_{max}(film) 3468 (br), 2926, 2856, 1465, 1380, 1370 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.88 (9H, s), 0.89 (6H, t, $J=6.3$ Hz), 1.26 (36H, bs), 1.40 (3H, s), 1.41–1.43 (2H, m), 1.48 (3H, s), 1.52–1.56 (2H, m), 1.71–1.75 (2H, m), 2.82 (1H, br, OH), 3.30 (1H, m), 3.51–3.85 (14H, m), 4.89 (1H, d, $J=3.7$ Hz). FABMS (positive-ion): *m/z* 759 (M$+$H)$^+$, 781 (M$+$Na)$^+$. HRFABMS, calcd for $C_{43}H_{86}O_8SiNa$: 781.5990. Found: 781.5977.

4.1.46. 2-(Diallylphosphonooxy)ethyl 2-*O*-[(*R*)-3-(*tert*-butyldimethylsilyloxy)tetradecyl]-3-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (53). Compound **52** (428 mg, 0.654 mmol) was treated as described for the formation of **17** from **16** to give **53** (504 mg, 97%) as a gum. IR ν_{max}(film) 2927, 2856, 1464 cm^{-1}.400 MHz ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.88 (15H, bs), 1.26 (36H, bs), 1.39 (3H, s), 1.41–1.43 (2H, m), 1.48 (3H, s), 1.51–1.55 (2H, m), 1.64–1.76 (2H, m), 3.28 (1H, m), 3.41–3.89 (12H, m), 4.22–4.27 (2H, m), 4.56–4.59 (4H, m), 4.88 (1H, d, $J=3.7$ Hz), 5.21–5.41 (4H, m), 5.91–6.01 (2H, m). FABMS (positive-ion): *m/z* 919 (M$+$H)$^+$, 941 (M$+$Na)$^+$. HRFABMS, calcd for $C_{49}H_{96}O_{11}PSi$: 919.6459. Found: 919.6484.

4.1.47. 2–(Diallylphosphonooxy)ethyl 3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (54). Compound **53** (489 mg, 0.532 mmol) was treated as described for the formation of **18** from **17** to give triol **54** (340 mg, 84%) as a gum. IR ν_{max}(film) 3388 (br), 2918, 2850, 1467 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.26 (36H, bs), 1.41–1.73 (6H, m), 2.56 (1H, m, OH), 2.68 (1H, d, $J=2.9$ Hz, OH), 3.12 (1H, bs, OH), 3.29 (1H, m), 3.44–3.92 (12H, m), 4.22–4.28 (2H, m), 4.55–4.59 (4H, m), 5.00 (1H, d, $J=3.7$ Hz), 5.25–5.28 (2H, m), 5.35–5.41 (2H, m), 5.90–6.00 (2H, m). FABMS (positive-ion): *m/z* 765 (M$+$H)$^+$, 787 (M$+$Na)$^+$. HRFABMS, calcd for $C_{40}H_{77}O_{11}PNa$: 787.5101. Found: 787.5118.

4.1.48. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (55). Compound **54** was treated as described for the formation of **19** from imidate **5** and **18** to give compound **55** as a gum in 67% yield. IR ν_{max}(film) 3500–3250, 2926, 2856, 1746, 1547, 1464 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.6$ Hz), 1.26 (51H, bs), 1.38–1.77 (8H, m), 2.72 (1H, bs, OH), 3.13 (1H, bs, OH), 3.21–3.90 (24H, m, containing two 3H, s, at δ 3.28 and 3.39 ppm), 4.07–4.30 (4H, m), 4.53–4.60 (8H, m), 4.65–4.90 (3H, m), 4.95 (1H, d, $J=3.7$ Hz), 5.24–5.40 (8H, m), 5.89–5.99 (4H, m), 6.40 (1H, bs, NH). FABMS (positive-ion): *m/z* 1466 (M$+$Na)$^+$, 1468. HRFABMS, calcd for $C_{67}H_{122}Cl_3NO_{21}P_2Na$: 1466.6984. Found: 1466.6962.

4.1.49. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (56). Compound **55** was treated as described for the formation of **7** from **6** to give **56** (82%) as a gum. IR ν_{max}(film) 3398 (br), 2925, 2855, 1640, 1555, 1465 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.26 (68H, bs), 1.40–1.79 (10H, m), 1.99–2.04 (4H, m), 2.17–2.21 (2H, m), 3.18 (1H, bs, OH), 3.20 (1H, m), 3.27–3.95 (26H, m, containing two 3H, s, at δ 3.28 and 3.38 ppm), 4.07–4.27 (4H, m), 4.55–4.65 (8H, m), 4.94 (1H, d, $J=3.7$ Hz), 5.13 (1H, d, $J=8.1$ Hz), 5.25–5.39 (10H, m), 5.90–5.99 (4H, m), 6.58 (1H, d, $J=7.3$ Hz, NH). FABMS (positive-ion): *m/z* 1556 (M$+$Na)$^+$. HRFABMS, calcd for $C_{82}H_{153}NO_{20}P_2Na$: 1557.0359. Found: 1557.0341.

4.1.50. 2-(Phosphonoxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (57). Compound **56** was treated as described for the formation of **21** from **20** to give **57** (67%) as a powder. IR ν_{max}(KBr) 3289 (br), 2924, 2853, 1629, 1554 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.90 (12H, t, $J=6.6$ Hz), 1.26 (68H, bs), 1.40–1.77 (10H, m), 1.98–2.04 (4H, m), 2.17–2.22 (2H, m), 3.23–3.85 (26H, m, containing two 3H, s, at δ 3.30 and 3.40 ppm), 4.00–4.18 (4H, m), 4.69 (1H, d, $J=8.1$ Hz), 4.94 (1H, d, $J=3.7$ Hz), 5.33–5.38 (2H, m). FABMS (negative-ion): *m/z* 1372 (M$-$H)$^-$. HRFABMS, calcd for $C_{70}H_{136}NO_{20}P_2$: 1372.9131. Found: 1372.9121. Anal. Calcd for $C_{70}H_{137}NO_{20}P_2$: C, 60.36; H, 10.06; N, 1.01; P, 4.45. Found: C, 60.11; H, 10.15; N, 1.28; P, 4.34.

4.1.51. 2-Bromoethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (58). To a solution of **16** (1.10 g, 1.83 mmol) in CH_2Cl_2 (10 mL) was added CBr_4 (731 mg, 2.20 mmol) and PPh_3 (671 mg, 2.56 mmol). After stirring for 2 h at room temperature, the solution was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **58** (1.20 g, 99%) as a gum. IR ν_{max}($CHCl_3$) 2927, 2855 cm^{-1}. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.25–1.60 (46H, m, containing two 3H, s, δ 1.41 and 1.48 ppm), 3.30 (1H, dd, $J=2.9$, 8.8 Hz), 3.50–3.57 (4H, m), 3.59–3.75 (6H, m), 3.83–3.96 (3H, m), 4.91 (1H, d, $J=3.9$ Hz). FABMS (positive-ion) *m/z* 685 (M$+$Na)$^+$. HRFABMS, calcd for $C_{35}H_{67}BrO_6Na$: 685.4002. Found: 685.4056.

4.1.52. 2-(Diallylphosphono)ethyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (59). A mixture of **58** (1.20 g, 1.81 mmol) and triallylphosphite (5 mL) was stirred at 180 °C for 3 h. The mixture was cooled to room temperature, and chromatographed on a silica gel short column. Elution with hexane-EtOAc (2:3) gave a compound, which was dissolved in aq 80% AcOH (15 mL) and stirred at 60 °C for 1 h. The solution was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was concentrated in vacuo and chromatographed on a silica gel column. Elution with $CHCl_3$–EtOH (20:1) gave **59** (760 mg, 60%) as a white powder. IR $\nu_{max}(CHCl_3)$ 3598, 3420, 3089, 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.26–1.61 (40 H, m), 2.28(1H, bs, OH), 2.58 (1H, bs, OH), 3.27 (1H, dd, $J=2.9$, 9.8 Hz), 3.44–3.53 (3 H, m), 3.57–3.62 (2H, m), 3.70–3.80 (3H, m), 3.84–3.92 (2H, m), 3.98 (1H, m), 4.51–4.60 (4H, m), 4.90 (1H, d, $J=2.9$ Hz), 5.24–5.38 (4H, m), 5.90–5.98 (2H, m). FABMS (positive-ion) *m/z* 727 $(M+Na)^+$; HRFABMS, calcd for $C_{38}H_{73}O_9PNa$: 727.4889. Found: 727.4892.

4.1.53. 2-(Diallylphosphono)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (60). To a solution of imidate **5** (338 mg, 0.401 mmol) and diol **59** (189 mg, 0.268 mmol) were dissolved in CH_2Cl_2 (5 mL). To this solution was added AgOTf (155 mg, 0.604 mmol) at room temperature. After stirring for 1 h at room temperature, the mixture was quenched with aq. sat. $NaHCO_3$, diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:9) gave **60** (273 mg, 74%) as an amorphous compound. IR $\nu_{max}(CHCl_3)$ 3449, 3255, 3088, 2928, 2873, 2856, 1734 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.8$ Hz), 1.26–1.80 (54H, m), 2.24–2.32 (2H, m), 2.55 (1H, s, OH), 3.21–3.36 (7H, m, containing 3H, s, δ 3.29), 3.39 (3H, s), 3.42–3.66 (7H, m), 3.71–3.88 (6H, m), 3.92–3.99 (2H, m), 4.16 (1H, d, $J=10.7$ Hz), 4.30 (1H, q, $J=8.8$, 9.8 Hz), 4.53–4.59 (8H, m), 4.70, 4.78 (2H, AB-q, $J=11.7$ Hz), 4.87 (1H, d, $J=2.9$ Hz), 4.93 (1H, d, $J=7.8$ Hz), 5.23–5.39 (8H, m), 5.88–5.98 (4H, m), 6.75 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion) *m/z* 1406 $(M+Na)^+$. HRFABMS, calcd for $C_{65}H_{118}Cl_3NO_{19}P_2Na$: 1406.6732. Found: 1406.6720.

4.1.54. 2-(Diallylphosphono)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(Z)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (61). To a solution of **60** (220 mg, 0.159 mmol) in THF (4 mL) and acetic acid (0.5 mL) was added zinc dust (210 mg). After vigorously stirring for 3 h at room temperature, the solution was filtered to remove the Zn dust, and concentrated in vacuo to give a crude product. The product was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude product, which was dissolved in CH_2Cl_2 (5 mL), and (*Z*)-11-octadecenoic acid (68 mg, 0.239 mmol) and WSC·HCl (55 mg, 0.287 mmol) were added to this solution. After stirring for 20 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc gave **61** (122 mg, 52%) as an amorphous compound. IR $\nu_{max}(CHCl_3)$ 3004, 2928, 2856, 1662 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.8$ Hz), 1.25–1.80 (76H, m), 1.99–2.01 (4H, m), 2.16–2.33 (4H, m), 2.85 (1H, d, $J=3.9$ Hz, OH), 3.07 (1H, m), 3.23–3,25 (2H,m), 3.29 (3H, s), 3.36 (1H, m), 3.38 (3H, s), 3.44 (1H, t, $J=8.8$ Hz), 3.48–3.84 (12H, m), 3.91 (1H, m), 4.09 (1H, dd, $J=9.8$, 8.8 Hz), 4.17 (1H, d, $J=10.7$ Hz), 4.25 (1H, dd, $J=9.8$,8.8 Hz), 4.50–4.58 (8H, m), 4.85 (1H, d, $J=3.9$ Hz), 5.19 (1H, d, $J=7.8$ Hz), 5.23–5.38 (10H, m), 5.89–5.98 (4H,m), 6.91 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion) *m/z* 1496 $(M+Na)^+$; HRFABMS, calcd for $C_{80}H_{149}NO_{18}P_2Na$: 1497.0139. Found: 1497.0123.

4.1.55. 2-(Phosphono)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (62). Compound **61** (110 mg, 0.075 mmol) was treated as described in the formation of **8** from **7** to give **62** (80 mg, 82%) as a white powder. IR ν_{max}(KBr) 3284, 3218, 3178, 3116, 2955, 2924, 2853, 1655, 1630 cm^{-1}. 500 MHz ^1H NMR ($CD_3OD:CDCl_3=5:1$) δ 0.90 (12H, t, $J=6.8$ Hz), 1.28–1.67 (74H, m), 1.73–1.77 (2H, m), 2.01–2.03 (4H, m), 2.16 (2H, dt, $J=18.6$, 7.8 Hz), 2.25 (2H, t, $J=6.8$ Hz), 3.22 (1H, dd, $J=2.9$, 9.8 Hz), 3.31 (3H, s), 3.34 (1H, m), 3.41 (3H, s), 3.46 (1H, dd, $J=9.8$, 8.8 Hz), 3.54–3.85 (15H, m), 3.96 (1H, m), 4.07–4.12 (2H, m), 4.54 (1H, d, $J=8.8$ Hz). 4.87 (1H, d, $J=3.9$ Hz), 5.31–5.37 (2H, m).

4.1.56. 3-Hydroxypropyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (63). To a solution of **15** (3.30 g, 5.53 mmol) in THF (25 ml) was added 9-BBN (0.5 M solution in THF, 28 ml, 14.0 mmol). After stirring for 18 h at room temperature, aq 3 M NaOH (50 ml) and aq 30% H_2O_2 (18 ml) were added to the reaction mixture at 0 °C. After stirring for 3 h at room temperature, the mixture was extracted with EtOAc, washed with satd NH_4Cl and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane–EtOAc (7:3) gave **63** (3.03 g, 89%) as a gum. IR $\nu_{max}(CHCl_3)$ 3506, 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.25–1.94 (48H, m, containing two 3H, s, at 1.40 and 1.48 ppm), 2.88 (1H, dd, $J=4.9$, 6.8 Hz, OH), 3.28 (1H, dd, $J=3.9$, 7.8 Hz), 3.49–3.85 (12H, m), 3.96 (1H, m), 4.87 (1H, d, $J=3.9$ Hz). FABMS (positive-ion): *m/z* 637 $(M+Na)^+$, 615 $(M+H)^+$. HRFABMS, calcd for $C_{36}H_{70}O_7Na$: 637.5011. Found: 637.5034.

4.1.57. 3-(Diallylphosphonooxy)propyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (64). To a solution of **63** (981 mg, 1.59 mmol) and 1*H*-tetrazole (168 mg, 2.40 mmol) in THF (8 ml) was added diallyl diisopropylphosphoramidite (512 mg, 2.09 mmol). After stirring for 4 h at room temperature, aq 30% H_2O_2 (1 ml) was added to the reaction mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq 10% $Na_2S_2O_3$, diluted with EtOAc, washed with water, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a residue, which was dissolved in aq 80% AcOH (10 ml) and stirred at 60 °C for 1 h. The solution was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane–EtOAc (1:9) gave **64** (828 mg, 71%) as a white powder. IR ν_{max}(CHCl$_3$) 3600, 3393, 2927, 2855 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.25–1.33 (36H, m), 1.53–1.59 (4H, m), 1.71 (1H, bs, OH), 1.97–2.09 (3H, m, containing OH), 3.26 (1H, dd, $J=3.9$, 9.8 Hz), 3.43 (1H, t, $J=8.8$ Hz), 3.51–3.75 (7H, m), 3.83–3.91 (3H, m), 4.13 (1H, m), 4.27 (1H, m), 4.53–4.57 (4H, m), 4.89 (1H, d, $J=3.9$ Hz), 5.25–5.39 (4H, m), 5.90–5.98 (2H, m). FABMS (positive-ion): m/z 757 $(M+Na)^+$, 735 $(M+H)^+$. HRFABMS, calcd for $C_{39}H_{76}O_{10}P$: 735.5175. Found: 735.5180.

4.1.58. 3-(Diallylphosphonoxy)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (65). Compound **64** was treated as described for the formation of **49** from **48** to give **65** (53%) as an amorphous solid. IR ν_{max} (CHCl$_3$) 3450, 3088, 2928, 2873, 2856, 1737 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (9H, t, $J=6.8$ Hz), 1.25–1.79 (54H, m), 1.95–2.06 (2H, m), 2.78 (1H, d, $J=2.9$ Hz, OH), 3.24 (1H, dd, $J=3.9$, 9.8 Hz), 3.28 (3H, s), 3.30 (1H, m), 3.39 (3H, s), 3.41–3.86 (17H, m), 4.08 (1H, d, $J=8.8$ Hz), 4.12–4.24 (2H, m), 4.29 (1H, m), 4.52–4.60 (8H, m), 4.72, 4.76 (2H, AB-q, $J=11.7$ Hz), 4.84 (1H, d, $J=3.9$ Hz), 4.87 (1H, d, $J=7.8$ Hz), 5.24–5.39 (8H, m), 5.90–5.98 (4H, m), 6.03 (1H, bs, NH). FABMS (positive-ion): m/z 1436 $(M+Na)^+$. HRFABMS, calcd for $C_{66}H_{120}Cl_3NO_{20}P_2Na$: 1436.6844. Found: 1436.6847.

4.1.59. 3-(Diallylphosphonoxy)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (66). Compound **65** was treated as described for the formation of **50** from **49** to give **66** as an amorphous solid. IR ν_{max}(CHCl$_3$) 3454, 3319, 3089, 2928, 2856, 1665 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (12H, t, $J=6.8$ Hz), 1.25–1.80 (76H, m), 1.95–2.05 (6H, m), 2.14–2.27 (2H, m), 3.17 (1H, d, $J=3.9$ Hz, OH), 3.21–3.24 (2H, m), 3.28 (3H, s), 3.30 (1H, m), 3.38 (3H, s), 3.41–3.82 (15H, m), 3.94 (1H, t, $J=9.8$, 8.8 Hz), 4.07 (1H, d, $J=8.8$ Hz), 4.11–4.20 (2H, m), 4.25 (1H, q, $J=8.8$, 9.8 Hz), 4.52–4.58 (8H, m), 4.84 (1H, d, $J=3.9$ Hz), 5.16 (1H, d, $J=7.8$ Hz), 5.23–5.39 (10H, m), 5.90–5.98 (4H, m), 6.36 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion): m/z 1526 $(M+Na)^+$. HRFABMS, calcd for $C_{81}H_{151}NO_{19}P_2Na$: 1527.0261. Found: 1527.0277.

4.1.60. 3-(Phosphonoxy)propyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(Z)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (67). Compound **66** was treated as described for the formation of **8** from **7** to give **67** (84%) as a white powder. IR ν_{max}(KBr) 3285, 3228, 3069, 3004, 2955, 2924, 2853, 2318, 1656, 1630 cm^{-1}. 500 MHz ^{1}H NMR (CD$_3$OD+CDCl$_3$) δ 0.89 (12H, t, $J=6.8$ Hz), 1.30–1.67 (74H, m), 1.72–1.75 (2H, m), 1.93–1.99 (2H, m), 2.01–2.04 (4H, m), 2.20–2.30 (2H, m), 3.17 (1H, dd, $J=3.9$, 9.8 Hz), 3.29 (3H, s), 3.33 (1H, m), 3.39 (3H, s), 3.44 (1H, dd, $J=9.8$, 8.8 Hz), 3.48–3.67 (10H, m), 3.73–3.85 (6H, m), 4.04–4.12 (4H, m), 4.48 (1H, d, $J=8.8$ Hz), 4.85 (1H, d, $J=2.9$ Hz), 5.31–5.38 (2H, m). ESIMS (negative-ion): m/z 1342 $(M-H)^-$. HRESIMS (negative-ion): calcd for $C_{69}H_{134}NO_{19}P_2$: 1342.9031. Found: 1342.9047.

4.1.61. 3-Bromopropyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidine-α-D-glucopyranoside (68). Compound **63** was treated as described for the formation of **58** from **16** to give **68** (72%) as a gum. IR ν_{max}(CHCl$_3$) 2927, 2855 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.21–1.32 (36H, m), 1.41 (3H, s), 1.48 (3H, s), 1.50–1.60 (4H, m), 2.13–2.19 (2H, m), 3.29 (1H, m), 3.49–3.75 (11H, m), 3.81–3.87 (2H, m), 4.86 (1H, d, $J=4.4$ Hz). FABMS (positive-ion): m/z 699 $(M+Na)^+$. HRFABMS, calcd for $C_{36}H_{69}BrO_6Na$: 699.4173. Found: 699.4180.

4.1.62. 3-(Diallylphosphono)propyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (69). Compound **68** was treated as described for the formation of **59** from **58** to give **69** (40%) as a white powder. IR ν_{max}(CHCl$_3$) 3600, 2927, 2856 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.26–1.33 (36H, m), 1.53–1.60 (4H, m), 1.83–2.00 (4H, m), 2.64–2.75 (2H, bs, 2OH), 3.26 (1H, dd, $J=3.9$, 9.8 Hz), 3.44 (1H, dd, $J=8.8$, 9.8 Hz), 3.48–3.68 (6H, m), 3.72–3.80 (2H, m), 3.84–3.93 (2H, m), 4.49–4.58 (4H, m), 4.90 (1H, d, $J=3.9$ Hz), 5.23–5.38 (4H, m), 5.90–5.98 (2H, m). FABMS (positive-ion): m/z 741 $(M+Na)^+$, 719 $(M+H)^+$. HRFABMS, calcd for $C_{39}H_{76}O_9P$: 719.5222. Found: 719.5242.

4.1.63. 3-(Diallylphosphono)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (70). Compound **69** (470 mg, 0.654 mmol) was treated as described in the formation of **19** from **18** to give **70** (533 mg, 58%) as an amorphous solid. IR ν_{max}(CHCl$_3$) 3588, 3450, 3253, 3088, 2928, 2873, 2856, 1733 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (9H, t, $J=6,8$ Hz), 1.26–1.79 (54H, m), 1.83–2.03 (4H, m), 2.64 (1H, s, OH), 3.22–3.27 (3H, m, containing 1H, dd, $J=3.9$, 9.8 Hz, at 3.23 ppm), 3.28 (3H, s), 3.36 (1H, m), 3.39 (3H, s), 3.44–3.88 (15H, m), 4.11 (1H, d, $J=9.8$ Hz), 4.29 (1H, m), 4.48–4.59 (8H, m), 4.73, 4.75 (2H, ABq, $J=12.7$ Hz), 4.84 (1H, d, $J=2.9$ Hz), 4.90 (1H, d, $J=7.8$ Hz), 5.24–5.39 (8H, m), 5.90–5.98 (4H, m), 6.36 (1H, d, $J=5.9$ Hz). FABMS (positive-ion): m/z 1420 $(M+Na)^+$. HRFABMS, calcd for $C_{66}H_{120}NO_{19}Cl_3P_2Na$: 1420.6896. Found: 1420.6904.

4.1.64. 3-(Diallylphosphono)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (71). Compound **70** (480 mg, 0.343 mmol) was treated as described in the formation of **7** from **6** to give **71** (266 mg, 52%) as an amorphous solid. IR ν_{max}(CHCl$_3$) 3454, 3300, 3088, 2928, 2856, 1662 cm^{-1}. 500 MHz ^{1}H NMR (CDCl$_3$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.20-1.96 (80H, m), 1.99-2.03 (4H, m), 2.14–2.26 (2H, m), 3.08 (1H, d, $J=3.9$ Hz, OH), 3.18 (1H, m), 3.22 (1H, dd, $J=3.9$, 9.8 Hz), 3.26–3.32 (4H, m, containing

3H, s, at 3.28 ppm), 3.35–3.42 (4H, m, containing 3H, s, at 3.38 ppm), 3.46 (1H, dd, $J=8.8$, 9.8 Hz), 3.48–3.83 (13H, m), 3.98 (1H, dd, $J=7.8$, 8.8 Hz), 4.09 (1H, d, $J=9.8$ Hz), 4.25 (1H, t, $J=8.8$ Hz), 4.48–4.58 (8H, m), 4.83 (1H, d, $J=3.9$ Hz), 5.17 (1H, d, $J=7.8$ Hz), 5.23–5.38 (10H, m), 5.90–5.98 (4H, m), 6.52 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion): *m/z* 1510 $(M+Na)^+$. HRFABMS, calcd for $C_{81}H_{151}NO_{18}P_2Na$: 1511.0304. Found: 1511.0305.

4.1.65. 3-(Phosphono)propyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside. (72). Compound **71** (251 mg, 0.168 mmol) was treated as described in the formation of **8** from **7** to give **72** (177 mg, 80%) as a white powder. IR ν_{max}(KBr) 3286, 3069, 3004, 2954, 2925, 2854, 1630 cm^{-1}. 500 MHz ^1H NMR (CD_3OD) δ 0.89 (12H, t, $J=6.8$ Hz), 1.30–1.92 (80H, m), 2.01–2.05 (4H, m), 2.20–2.29 (2H, m), 3.18 (1H, dd, $J=3.9$, 9.8 Hz), 3.29 (3H, s), 3.33 (1H, m), 3.39 (3H, s), 3.43 (1H, dd, $J=3.3$, 9.8 Hz), 3.46 (1H, m), 3.50–3.67 (9H, m), 3.71–3.85 (6H, m), 4.04–4.12 (2H, m), 4.48 (1H, d, $J=7.8$ Hz), 4.85 (1H, d, $J=3.9$ Hz), 5.31–5.38 (2H, m). ESIMS (negative-ion): *m/z* 1326 $(M-H)^-$. HRESIMS: calcd for $C_{69}H_{134}NO_{18}P_2$: 1326.9069. Found: 1326.9047.

4.2. Methods for measurement of biological activity

The sources of the materials used in the study are as follows: lipopolysaccharide (LPS) from *E. coli* serotype 026:B6 and 12-*O*-tetradecanoylphorbol acetate (TPA) were from Sigma, St. Louis, MO; RPMI-1640 medium, fetal bovine serum (FBS) and newborn calf serum (NBCS) were from Gibco, Grand Island, NY; and human TNFα ELISA kit and mouse TNFα ELISA kit were from Genzyme-Techne, Minneapolis, MN.

4.2.1. Production of TNFα by human whole blood.[8] *Materials*: Lipopolysaccharide (LPS, lot 50K4117, *E. coli* 026:B6), human tumor necrosis factor alpha (TNFα) immunoassay kit and 96-well assay plates were purchased from Sigma, BioSource International, Inc. and Corning Inc. (Cat. No. 3956), respectively.

Whole blood TNFα production: Fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. The subjects did not have any apparent inflammatory conditions and had taken no drugs for at least 7 days prior to blood collection. Written informed consent was obtained from all volunteers before the experiment. In each well of the plates, 360 μL aliquots of blood were mixed with 20 μL of LPS solution (200 ng/mL, final concentration: 10 ng/mL) dissolved in PBS in the presence (for test sample) or absence (for positive control sample) of the test compounds solution (dissolved in DMSO/PBS solution). For the negative control samples, the same amount of blood was cultured without either LPS or a test compound solution. After 6 h of incubation at 37 °C, the plates were centrifuged at $490\times g$ for 15 min, and the plasma was collected and stored at −20 °C. The concentrations of TNFα in the plasma were measured with commercially available immunoassay kits.

Statistical analysis: The percentage of inhibition of TNFα production was calculated by the following formula: 1 − (concentration of TNFα in the test sample − concentration of TNFα in the negative control sample)/(concentration of TNFα in the positive control sample − concentration of TNFα in the negative control sample) × 100. The suppressive activities of test compounds are expressed as the fifty percent inhibitory concentration (IC_{50}) of the test compound, the concentration at which the test compound suppresses TNFα production by 50%. The IC_{50} was calculated from the percentage of inhibition using the SAS System for Windows. The results are expressed as the mean IC_{50} of triplicate experiments.

4.2.2. Production of TNFα by galactosamine loaded C3H/HeN mice in vivo.[9] *Materials: Animals*: Male C3H/HeN mice were purchased from Charles River Japan (Tokyo, Japan). All mice were used at the age of 7 weeks, and housed at Sankyo Laboratories (Tokyo, Japan) with free access to standard rodent chow diet.

Reagents: Lipopolysaccharide (LPS, from *Escherichia coli* O26:B6) and D-Galactosamine (GalN) were purchased from Sigma (St Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits of murine TNFα were from R&D Systems (Minneapolis, MN).

TNFα production: Naïve C3H/HeN mice (five per group) were intravenously injected with the test compound solution (10 ml/kg; dissolved in 0.1% triethylamine /saline solution), and immediately after, mice were intravenously injected with a mixture of LPS (0.05 mg/10 ml saline/kg) and GalN (1 g/10 ml saline/kg). Mice were injected with vehicle (0.1% triethylamine/saline solution) and saline for negative control samples, and with vehicle and LPS/GalN for positive control samples. One hour after injection, venous blood was collected under ether anesthesia with heparinized syringes fitted with 23-gauge needles from the abdominal vena, and was centrifuged at 4 °C for 3 min at $13{,}230\times g$ to obtain the plasma. Plasma was stored at −30 °C before measuring TNFα levels by ELISA. The concentrations of TNFα of mouse plasma were measured using ELISA analysis according to the manufacturer's instructions.

Statistical analysis: The percentage of inhibition of TNFα production was calculated by the following formula: [1 − (concentration of TNFα in the test sample − concentration of TNFα in the negative control sample)/(concentration of TNFα in the positive control sample − concentration of TNFα in the negative control sample)] × 100. The suppressive activities of test compounds are expressed as the mean of fifty percent inhibitory dose (ID_{50}) of the test compound, at which the test compound suppresses TNFα production by 50%. The ID_{50} was calculated from the percentage of inhibition using the SAS System for Windows (version 5).

References and notes

1. Brade, H.; Opal, S. M.; Vogel, S. N.; Morrison, D. C.

Endotoxin in Health and Diseases; Marcel Dekker Inc.: New York, Basel, 1999; pp 915–926.

2. (a) Imoto, M.; Yoshimura, H.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Tetrahedron Lett.* **1985**, *26*, 1545–1548. (b) Imoto, M.; Yoshimura, H.; Shimamoto, T.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2205–2214. (c) Cf. Takahashi, T.; Nakamoto, S.; Ikeda, K.; Achiwa, K. *Tetrahedron Lett.* **1986**, *27*, 1819–1822.
3. (a) Qureshi, N.; Honovich, J. P.; Hara, H.; Cotter, R. J.; Takayama, K. *J. Biol. Chem.* **1988**, *263*, 5502–5504. (b) Qureshi, N.; Takayama, K.; Kurtz, R. *Infect. Immun.* **1991**, *59*, 441–444. (c) Qureshi, N.; Takayama, K.; Meyer, K. C.; Kirkland, T. N.; Bush, C. A.; Chen, L.; Wang, R.; Cotter, R. J. *J. Biol. Chem.* **1991**, *266*, 6532–6538. (d) Christ, W. J.; McGuinness, P. D.; Asano, O.; Wang, Y.; Mullarkey, M. A.; Perez, M.; Hawkins, L. D.; Blythe, T. A.; Dubuc, G. R.; Robidoux, A. L. *J. Am. Chem. Soc.* **1994**, *116*, 3637–3638. (e) Kaltashov, I. A.; Doroshenko, V.; Cotter, R. J.; Takayama, K.; Qureshi, N. *Anal. Chem.* **1997**, *69*, 2317–2322.
4. Rossignol, D. P.; Hawkins, L. D.; Christ, W. J.; Kobayashi, K.; Kawata, T.; Lynn, M.; Yamatsu, I.; Kishi, Y. *Endotoxin in Health and Diseases*; Marcel Dekker Inc.: New York, Basel, 1999; Chapter 47.
5. Christ, W. J.; Rossignol, D. P.; Kobayashi, S.; Kawata, T. U.S. Patent 5,935,938, August 10, 1999.
6. Shiozaki, M.; Doi, H.; Tanaka, D.; Shimozato, T.; Kurakata, S. *Bull. Chem. Soc. Jpn.,* accepted for publication.
7. Shiozaki, M.; Kobayashi, Y.; Ishida, N.; Arai, M.; Hiraoka, T.; Nishijima, M.; Kuge, S.; Otsuka, T.; Akamatsu, Y. *Carbohydr. Res.* **1991**, *222*, 57–68.
8. Jagger, M. P.; Huo, Z.; Riches, P. G. *Clin. Exp. Immunol.* **2002**, *130*, 467–474.
9. Endo, Y.; Shibasaki, M.; Yamaguchi, K.; Kai, K.; Sugawara, S.; Takada, H.; Kikuchi, H.; Kumagai, K.; Br *J. Pharmacol.* **1999**, *128*, 5–12.
10. (a) Liu, W.-C.; Oikawa, M.; Fukase, K.; Suda, Y.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1377–1385. (b) Fukase, K.; Ueno, A.; Fukase, Y.; Oikawa, M.; Suda, Y.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 485–500. (c) Fukase, K.; Kirikae, T.; Kirikae, F.; Liu, W.-C.; Oikawa, M.; Suda, Y.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 2189–2197.
11. (a) Golenbock, D. T.; Hampton, R. Y.; Qureshi, N.; Takayama, K.; Raetz, C. R. *J. Biol. Chem.* **1991**, *266*, 19490–19498 cf. (b) Oikawa, M.; Shintaku, T.; Yoshizaki, H.; Fukase, K.; Adachi, S.; Lee, K.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 1455–1461.
12. Saitoh, S.; Akashi, S.; Yamada, T.; Tanimura, N.; Kobayashi, M.; Konno, K.; Matsumoto, F.; Fukase, K.; Kusumoto, S.; Nagai, Y.; Kusumoto, Y.; Kosugi, A.; Miyake, K. *Int. Immunol.* **2004**, *16*, 961–969.
13. (a) Watanabe, Y.; Shiozaki, M.; Tanaka, D.; Shimozato, T.; Kurakata, S. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 2341–2352. (b) Watanabe, Y.; Miura, K.; Shiozaki, M.; Kanai, S.; Kurakata, S.; Nishijima, M. *Carbohydr. Res.* **2003**, *338*, 47–54.

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A ^{1}H NMR study on the complexation of tetraalkylammonium cations, mono and diprotonated amines, and amino acids with a derivatized cyclotetrachromotropylene in an aqueous solution

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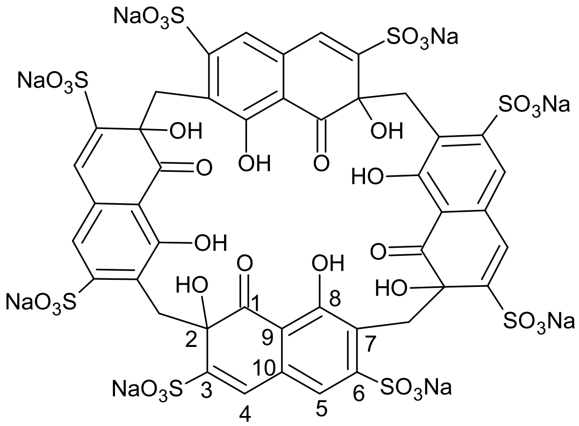
Abstract—The derivatized cyclotetrachromotropylene host forms complexes of 1:1 host to guest stoichiometry with tetraalkylammonium cations and amino acids whereas complexes of 1:2 host to guest stoichiometry are formed with mono and diprotonated amines in an aqueous solution. Both electrostatic and hydrophobic interactions are involved in the complexation.

1. Introduction

The study of host–guest complexation in an aqueous medium is of great importance because it provides an understanding of the chemistry in the biological system. Organic cations as guests have received extensive attention[1–13] in the last decade, especially after the report that acetylcholine can be bound to acetylcholine esterase through interaction with aromatic residues present in the enzyme.[14] Several reviews on the complexation of organic cations have also been published.[15–19] Depending on the kind of host used, the non-covalent forces involved in the binding of the organic cations could be hydrogen bonding, π–π, CH–π, cation–π or cation–anion interactions. Until now only a few host molecules capable of binding organic cations in an aqueous medium have been reported and they are mainly from the sulfonatocalixarenes.

The water-soluble derivative of cyclotetrachromotropylene (**1**) that we have synthesized recently, appeared to be a good host for organic cations. It has a rigid shallow bowl structure with exposed π surfaces for hydrophobic interaction and eight adjacent sulfonic anions on the upper rim for electrostatic interaction (CPK molecular models shown in **1a–1c**). We were interested to gain an understanding of the interactions involved in the complexation of organic cations with **1**. This paper reports our study on the complexation of four tetraalkylammonium cations, four monoprotonated amines, three diprotonated amines and four amino acids with **1** in an aqueous medium at 25 °C using ^{1}H NMR spectroscopy.



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Keywords: Complexation; Stability constant; Tetraalkylammonium cations; Protonated amines; Amino acids; Derivatized cyclotetrachromotropylene.

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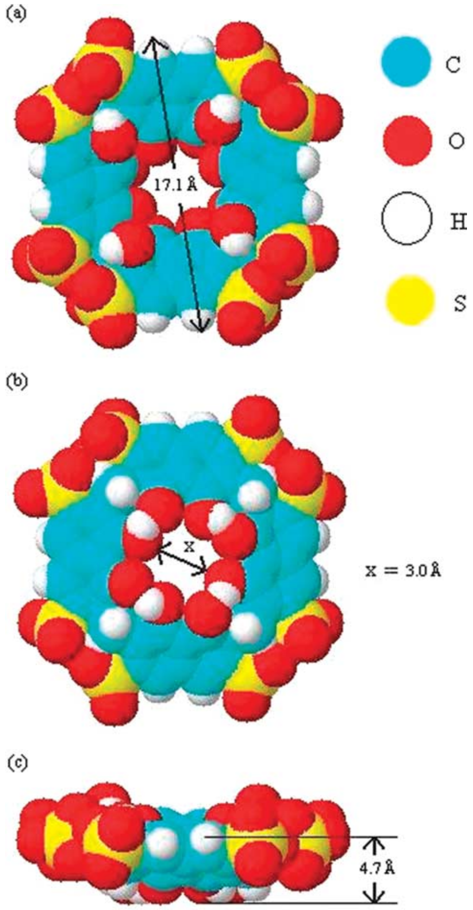
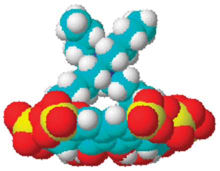


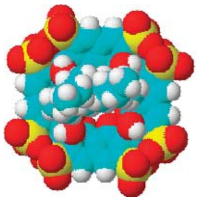
2. Results and discussion

2.1. Complexation of tetraalkylammonium cations

Four quaternary ammonium cations (tetramethylammonium, tetraethylammonium, tetrapropylammonium and tetrabutylammonium) were studied. Their proton chemical shifts in D_2O are shifted upfield and the resonance peaks broadened in the presence of **1** as shown in Figure 1, indicating that they are included in the cavity of **1**. All the proton chemical shift titration curves show two tangents meeting at a point where the molar ratio of host to guest is unity (Fig. 2), indicating that the complexes are of 1:1 host to guest stoichiometry. CPK molecular models of the complexes indicate that of the four alkyl chains of the spherical guest cations, at any given time two are pointing inside the host cavity and the other two pointing away from the cavity (see **2** for an illustration of the 1:1 complex of tetrabutylammonium cation and **1**). The average position of each guest proton determines the magnitude of the induced chemical shift change. For a short alkyl chain like ethyl, the induced chemical shift of H_1 is larger than that of H_2 (the subscript in H indicates the carbon bonded to the proton, the carbon atom attached to N is numbered 1). As the alkyl chain becomes longer, the differences in the induced chemical shifts of the various alkyl protons diminish (Table 1).



2 (Side view)



2 (Top view)

The stability constant *K* of each 1:1 host to guest complex was obtained by a non-linear regression fitting procedure.[20] A representative calculated titration curve together with the experimental chemical shifts is shown in Figure 3 for the tetramethylammonium cation. The *K* values obtained from different protons of the same tetraalkylammonium cation are in good agreement with one another (Table 1). The trend

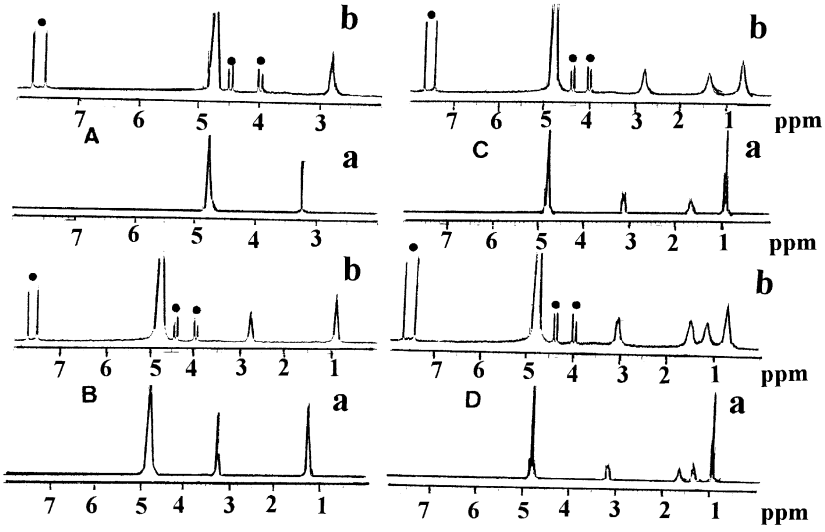


Figure 1. 400 MHz ^1H NMR spectra in D_2O at 25 °C of (A) 1.16×10^{-3} M of tetramethylammonium chloride: (a) no host; (b) in the presence of 1.23×10^{-3} M of **1**; (B) 1.17×10^{-3} M of tetraethylammonium chloride: (a) no host; (b) in the presence of 1.29×10^{-3} M of **1**; (C) 1.15×10^{-3} M of tetrapropylammonium bromide: (a) no host; (b) in the presence of 1.14×10^{-3} M of **1**; (D) 1.14×10^{-3} M of tetrabutylammonium iodide: (a) no host; (b) in the presence of 1.11×10^{-3} M of **1**. Solvent peak at 4.70 ppm used as internal reference. Peaks marked with ● are host peaks.

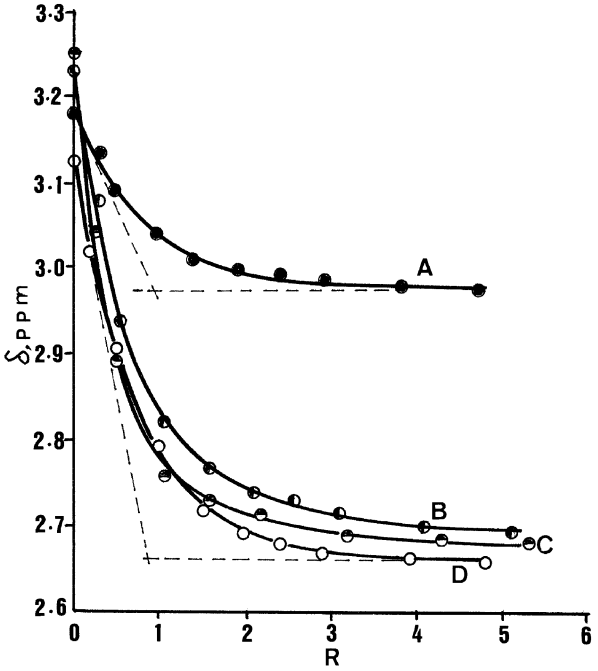


Figure 2. Variation of H_1 chemical shift of tetraalkyl ammonium cations with the molar ratio **R** of host to guest in D_2O at 25 °C: A, tetrabutylammonium (1.14×10^{-3} M); B, tetramethylammonium (1.16×10^{-3} M); C, tetraethylammonium (1.17×10^{-3} M) and D, tetrapropylammonium (1.15×10^{-3} M).

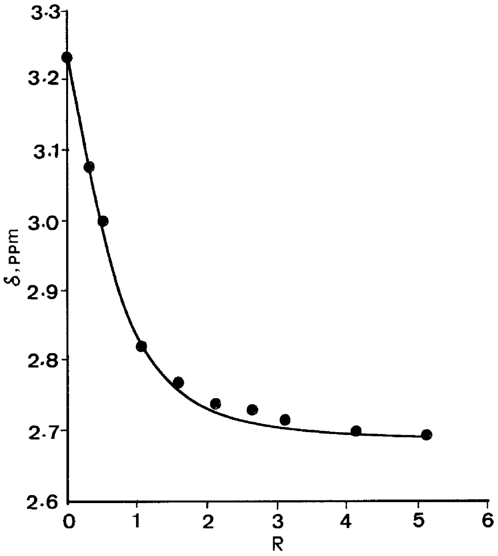


Figure 3. Calculated proton chemical shift titration curve of tetramethylammonium chloride (1.16×10^{-3} M) in D_2O at 25 °C. **R** is the molar ratio of host to guest and the points are experimental values. The complex is of 1:1 host to guest stoichiometry. The chemical shifts of the free and complexed guest and the *K* value used to produce the curve are given in Table 1.

in the *K* values, tetraethyl > tetrapropyl > tetramethyl > tetrabutyl, indicates that electrostatic (between the cation and the sulfonic anions) and CH–π interactions (between the alkyl chains of the guest and the aromatic walls of the host) are involved in the complexation. If only the electrostatic interaction were present, the expected trend in the *K* values (based on the distance of the guest cation from the sulfonic anions of the host) is tetramethyl > tetraethyl > tetrapropyl > tetrabutyl. If only CH–π interactions were present, the expected trend in the *K* values is tetrabutyl > tetrapropyl > tetraethyl > tetramethyl.[21] The same stability trend has been reported for the 1:1 complexes of the same guests with *p*-sulfonatocalix[4]arene as host and the *K* values are also of the same magnitude.[9]

2.2. Complexation of mono and diprotonated amines

The proton chemical shifts of the four monoprotonated amines (*t*-butylamine, *n*-propylamine, di-*n*-butylamine and tri-*n*-butylamine) and three diprotonated amines (1,2-diaminoethane, 1,3-diaminopropane and 1,5-diaminopentane) in D_2O are shifted upfield and the resonance peaks broadened in the presence of **1**, indicating that they are included in the cavity of **1**. Some representative spectra are shown in Figure 4. In contrast to the tetralkylammonium cations, all the proton chemical shift titration curves show two tangents meeting at a point where the molar ratio of host to guest is 0.5 (Fig. 5), indicating that the complexes are of 1:2 host to guest stoichiometry. The same stoichiometry was also indicated in Job's plots[22] (Fig. 6 shows the Job's plot for protonated *n*-propylamine as guest). CPK molecular models indicate that the large cavity size of the host is able to accommodate two guests. This is the first time that mono and diprotonated amines have been observed to form complexes of 1:2 host to guest stoichiometry. Quaternary ammonium cations have been reported to form complexes of 1:2 host to guest stoichiometry with *p*-sulfonatocalix[8]-arene.[15] In all the complexes, the NH_3^+ group is outside the aromatic wall of the host to be close to the SO_3^- group for

Table 1. ^{1}H NMR Chemical shifts of tetraalkylammonium salts and stability constants *K* of their 1:1 complexes with host **1** in D_2O at 25 °C

Guest	Proton	δ_u[a], ppm	$\Delta\delta$[b], ppm	K[c], M^{-1}	sd[d], ppm
$(Me)_4NCl$	H_1	3.233	0.557	7500	0.018
$(Et)_4NCl$	H_1	3.250	0.574	27,000	0.031
	H_2	1.258	0.425	27,000	0.025
$(n\text{-Pr})_4NBr$	H_1	3.125	0.475	9000	0.012
	H_2	1.664	0.394	9000	0.009
	H_3	0.908	0.295	15,000	0.011
$(n\text{-Bu})_4NI$	H_1	3.181	0.215	5000	0.007
	H_2	1.633	0.209	5000	0.007
	H_3	1.341	0.278	6100	0.007
	H_4	0.928	0.274	6700	0.007

[a] Chemical shift of free guest.

[b] Difference between the chemical shifts of free and complexed guest (upfield shift).

[c] Calculated by the non-linear regression fitting method; error about 15%.

[d] Standard deviation between experimental and calculated chemical shifts.

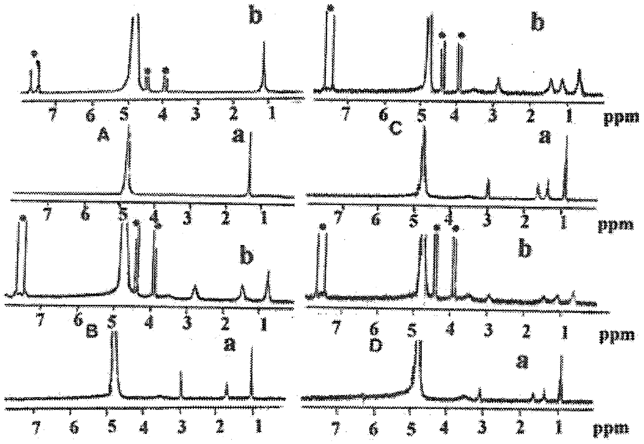


Figure 4. 400 MHz ^{1}H NMR spectra in D_2O in the presence of 1.70×10^{-3} M of DCl at 25 °C of (A) 1.67×10^{-3} M of *t*-butylamine: (a) no host; (b) in the presence of 1.62×10^{-3} M of **1**; (B) 1.61×10^{-3} M of *n*-propylamine: (a) no host; (b) in the presence of 1.67×10^{-3} M of **1**; (C) 1.47×10^{-3} M of di-*n*-butylamine: (a) no host; (b) in the presence of 6.56×10^{-4} M of **1**, (D) 1.52×10^{-3} M of tri-*n*-butylamine: (a) no host; (b) in the presence of 6.56×10^{-4} M of **1**. Solvent peak at 4.70 ppm used as internal reference. Peaks marked with ● are host peaks.

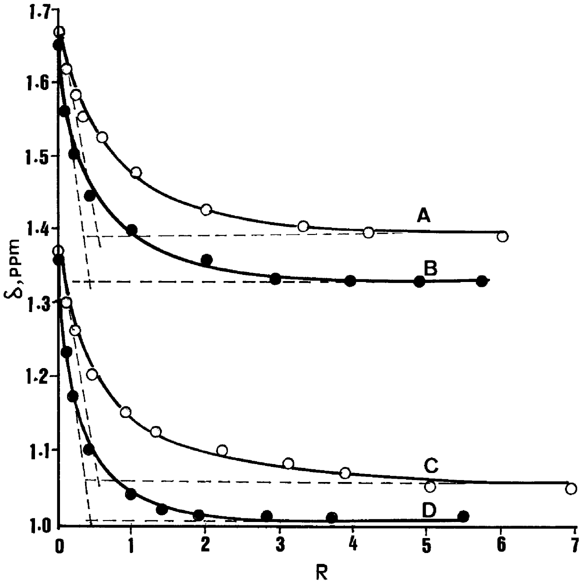


Figure 5. Variation of the proton chemical shift of A (*n*-propylammonium, H_2, 1.61×10^{-3} M); B (di-*n*-butylammonium, H_2, 1.47×10^{-3} M); C (*t*-butylammonium, 1.67×10^{-3} M) and **D** (tri-*n*-butylammonium, H_3, 1.52×10^{-3} M) with the molar ratio **R** of host to guest in D_2O at 25 °C.

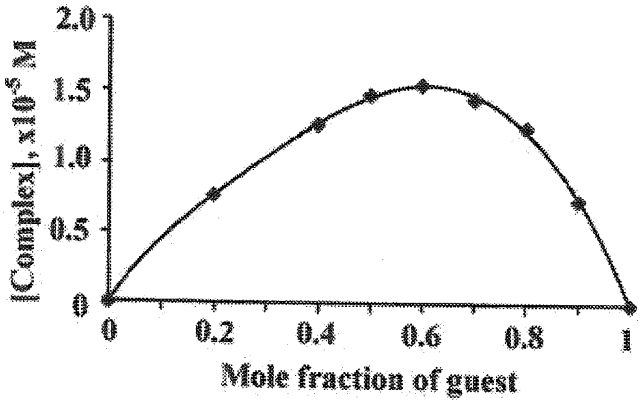


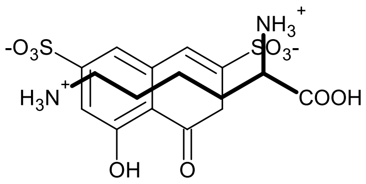
Figure 6. A Job's plot for protonated *n*-propylamine as guest.

electrostatic interaction and the alkyl chain in the cavity for CH–π interaction as indicated by the least induced chemical shift for H_1. For example, the induced chemical shifts for $n\text{-PrNH}_3^+$ are $H_1 < H_2 < H_3$. A similar observation has been reported for the complexation of protonated amines by *p*-sulfonatocalix[4]arene as host.[8,12] Similarly, the induced chemical shifts in the diprotonated 1,5-diaminopentane indicate that the two NH_3^+ groups are outside the aromatic wall of the host to be close to two SO_3^- groups for electrostatic interaction and the methylene moieties in the cavity for CH–π interaction. For example, when the ratio of host to guest used is 0.24 the induced chemical shifts were 0.295, 0.451 and 0.509 ppm for H_1, H_2 and H_3 respectively.

The stability constant *K* of each 1:2 host to guest complex was obtained by a non-linear regression fitting procedure (Table 2). A representative calculated titration curve together with the experimental chemical shifts is shown in Figure 7 for protonated *t*-butylamine. The calculated titration curves for 1:2 host to guest stoichiometry fit the experimental chemical shifts well in all the protonated amines. However, no calculated titration curves for 1:1 host to guest stoichiometry could fit the experimental chemical shifts well, thereby further supporting the conclusion that the complexes are of 1:2 host to guest stoichiometry. The *K* values obtained from different protons of the same protonated amine are in good agreement with one another (Table 2). The increase in the *K* values with increase in the number of CH bonds indicates the importance of CH–π interactions in the complexation. For example, the *K* value of protonated di-*n*-butylamine (7×10^6 M^{-2}) is larger than that of *n*-propylamine (8×10^5 M^{-2}) and diprotonated 1,3-diaminopropane (1×10^6 M^{-2}) larger than that of 1,2-diaminoethane (2×10^5 M^{-2}). The *K* value of diprotonated 1,3-diaminopropane (1×10^6 M^{-2}) is not much larger than that of protonated *n*-propylamine (8×10^5 M^{-2}) probably because the second NH_3^+ group in the former cannot come close to a second SO_3^- group of the host for a strong electrostatic interaction.

2.3. Complexation of amino acids

The complexation of four amino acids (D,L-alanine, D,L-aspartic acid, D,L-asparagine and L-lysine) at pD 1.0 in D_2O was studied. Their proton chemical shifts except aspartic acid, were shifted upfield in the presence of **1**, indicating that they are included in the cavity of **1**. A representative spectrum is shown in Figure 8 for lysine as guest. Job's plots indicate that the complexes are of 1:1 host to guest stoichiometry. A representative plot is shown in Figure 9 for asparagine.



3

For lysine which has two NH_3^+ groups, the trend in the

Table 2. ^{1}H NMR Chemical shifts of monoprotonated and diprotonated amines and stability constants K of their 1:2 host to guest complexes with host **1** in D_2O at 25 °C

Guest	Proton	δ_u[a], ppm	$\Delta\delta$[b], ppm	K[c], M^{-2}	sd[d], ppm
t-BuNH$_3^+$	H_2	1.368	0.333	1.0×10^6	0.006
n-PrNH$_3^+$	H_1	2.961	0.293	8.0×10^5	0.006
	H_2	1.679	0.366	8.0×10^5	0.008
	H_3	0.973	0.391	7.5×10^5	0.007
(n-Bu)$_2$ NH$_2^+$	H_1	3.024	0.276	6.0×10^6	0.010
	H_2	1.645	0.341	6.0×10^6	0.010
	H_3	1.380	0.393	8.0×10^6	0.014
	H_4	0.921	0.389	8.0×10^6	0.014
(n-Bu)$_3$NH$^+$	H_1	3.094	0.254	1.0×10^7	0.011
	H_2	1.633	0.270	1.5×10^7	0.012
	H_3	1.335	0.338	3.0×10^7	0.014
	H_4	0.896	0.324	3.0×10^7	0.013
$^+NH_3(CH_2)_2NH_3^+$	H_1	3.304	0.431	2.2×10^5	0.010
$^+NH_3(CH_2)_3NH_3^+$	H_1[e]	3.075	0.431	1.2×10^6	0.010
$^+NH_3(CH_2)_5NH_3^+$	H_1[e]	2.990	0.431	8.0×10^6	0.009

[a] Chemical shift of free guest.

[b] Difference between the chemical shifts of free and complexed guest (upfield shift).

[c] Calculated by the non-linear regression fitting method; error about 15%.

[d] Standard deviation between experimental and calculated chemical shifts.

[e] Other proton peaks too broad to discern.

induced chemical shifts of $H_5>H_1$ (the subscript in H indicates the carbon bonded to the proton, the carbon atom attached to COOH is numbered 1) indicates that the α-NH$_3^+$ group is located outside the host cavity having electrostatic interaction with the SO_3^- group and the alkyl chain inside the cavity (depicted in **3**, for clarity only one wall of the host cavity shown). For example, for a 1:1 molar ratio of host to guest, the induced chemical shifts are 0.095, 0.190, 0.229, 0.284 and 0.216 ppm for H_1, H_2, H_3, H_4 and H_5 respectively. Since, the α-NH$_3^+$ group is close to the SO_3^- group outside the host cavity, the CH moieties in the short chain amino acids such as alanine and asparagine cannot penetrate deep inside the host cavity, as reflected in their relatively smaller induced chemical shifts compared with those of lysine. The proton chemical shifts of aspartic acid are not affected by the presence of **1**, indicating the absence of any complexation. A possible explanation is that the COOH group in the CH_2COOH chain favours the aqueous environment rather than the hydrophobic cavity of **1**.

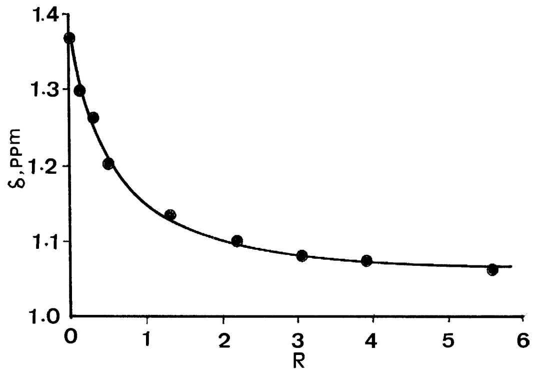


Figure 7. Calculated proton chemical shift titration curve of t-butylamine (1.67×10^{-3} M) in D_2O in the presence of 1.70×10^{-3} M of DCl at 25 °C. **R** is the molar ratio of host to guest and the points are experimental values. The complex is of 1:2 host to guest stoichiometry. The chemical shifts of the free and complexed guest and the K value used to produce the curve are given in Table 2.

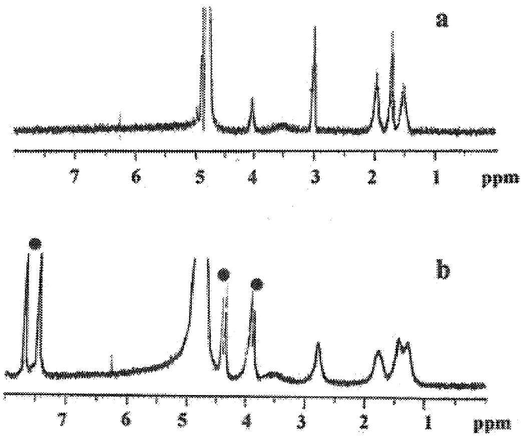


Figure 8. 400 MHz ^{1}H NMR spectra in D_2O at 25 °C of 1.61×10^{-3} M of lysine: (a) no host; (b) in the presence of 1.57×10^{-3} M of **1**. Peaks marked with ● are host peaks.

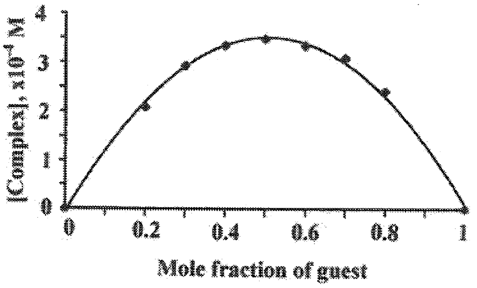


Figure 9. A Job's plot for asparagine as guest.

The stability constant K of each 1:1 host to guest complex was obtained by a non-linear regression fitting procedure (Table 3). A representative calculated titration curve together with the experimental chemical shifts is shown in Figure 10 for lysine. It is interesting to note that the K value for lysine is of the same magnitude as that reported for the 1:1 complex of lysine with p-sulfonatocalix[4]arene as host,[8] just like in the case of tetraalkylammonium cations discussed above.

Table 3. ^1H NMR Chemical shifts of amino acids $RCH(NH_3^+)COOH$ and stability constants K of their 1:1 complexes with host **1** in D_2O at pD 1.0 and 25 °C

R	Proton	δ_u[a], ppm	$\Delta\delta$[b], ppm	K[c], M^{-1}	sd[d], ppm
CH_3	H_1	4.101	0.182	70	0.002
Alanine	H_2	1.523	0.161	70	0.002
CH_2CONH_2	H_1	4.326	0.086	250	0.003
Asparagine	H_2	3.004	0.094	250	0.002
$(CH_2)_4NH_3^+$	H_2[e]	1.980	0.339	2000	0.005
Lysine	H_5	3.014	0.376	2100	0.007

[a] Chemical shift of free guest.

[b] Difference between the chemical shifts of free and complexed guest (upfield shift).

[c] Calculated by the non-linear regression fitting method; error about 15%.

[d] Standard deviation between experimental and calculated chemical shifts.

[e] Other proton peaks (H_1, H_3 and H_4) could not be discerned at higher host to guest ratio used because of broadening and overlap.

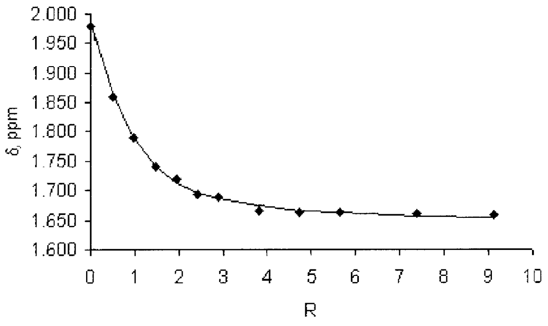


Figure 10. Calculated H_2 chemical shift titration curve of lysine (1.61×10^{-3} M) in D_2O at pD 1.0 and 25 °C **R** is the molar ratio of host to guest and the points are experimental values. The complex is of 1:1 host to guest stoichiometry. The chemical shifts of the free and complexed guest and the K value used to produce the curve are given in Table 3.

3. Experimental

3.1. General

Materials. Chromotropic acid, disodium salt, formaldehyde, tetraalkylammonium salts, amines, diaminoalkane dihydrochlorides and amino acids were commercial samples.

Instruments: Bruker–Avance 300 and Bruker–Avance 400 NMR spectrometers.

3.1.1. Synthesis of compound 1. A mixture of cyclotetrachromotropylene (0.25 g, 0.624 mmol, synthesized as described earlier[20]) and dichromate (0.08 g) in distilled water (2.5 mL) was heated at 78 °C for 5 h. The resulting solution was filtered to remove any presence of fine solid. The filtrate was concentrated to about 1 mL on a hot plate, cooled and then added to ethanol (15 mL). The precipitated crude product was dissolved in distilled water and purified by column chromatography with Sephadex LH20 using water as eluting solvent, yielding 20% of **1** as a brown solid. Anal. Found: C 28.80; H 3.08; Na 10.1; Calcd for $C_{44}H_{24}Na_8O_{36}S_8\cdot15H_2O$: C 28.73; H 2.94; Na 10.0. ^1H NMR (in D_2O at 25 °C, solvent peak at 4.70 ppm as internal reference) δ 3.85, 4.36 (8H, 2d, J 13.8 Hz, CH_2), 7.44 (4H, s, H_5), 7.65 (4H, s, H_4). ^{13}C NMR (in D_2O at 25 °C, TMS as external reference) δ 34.6 (methylene C), 86.9 (C_2), 111.9 (C_9), 121.8 (C_5), 133.2 (C_3), 134.4 (C_4), 134.6 (C_{10}), 140.0 (C_7), 144.5 (C_6), 159.9 (C_8) and 190.5 (C_1). MALDI mass spectrum, m/z 1604 ($M\cdot2H_2O$).

For ^1H chemical shifts, the assignment of δ 7.44 H_5 and 7.65 to H_4 is based on the presence of a cross peak at 7.65/86.9 in the HMBC spectrum. For ^{13}C, the characteristic chemical shifts of C_1 (190.5), C_2 (86.9), C_8 (59.9) and methylene C (34.6) are easily identified. DEPT 135 identifies the two C–H carbons, C_4 and C_5 (121.8 and 134.4) which are then assigned as C_5 (121.8) and C_4 (134.4) from the HMQC spectrum. Analysis of the cross peaks arising from H and CH_2 interactions enables us to separate C_3, C_6 and C_7 (33.2, 140.0, 144.5) as one group and C_9 and C_{10} (111.9, 134.6) as another. Analysis of the cross peaks arising from H_5 interactions separates C_3 (133.2) from C_6 and C_7 (140.0, 144.5). Since, C_6 is bonded to an electron withdrawing SO_3^- group whereas C_7 to a CH_2 group, the former is assigned a lower field (144.5) and the latter a higher field (140.0). Based on resonance structures of **1**, we assign C_9 (111.9) and C_{10} (134.6).

^1H NMR spectra in D_2O at 25 °C were recorded with a Bruker–Avance 400 MHz spectrometer. The solvent peak (unaffected by the concentration variation of the host and guests) at 4.70 ppm was used as the internal reference. 75 MHz ^{13}C NMR spectra in D_2O were recorded with a Bruker Avance 300 NMR spectrometer.

^1H NMR chemical shift titrations in D_2O were carried out by varying the concentration of the host **1** for a fixed concentration of the guest (about 1×10^{-3} M). An appropriate amount of DCl was added to monoprotonate the amines. Diaminoalkane dihydrochlorides provided the diprotonated amines. In the case of amino acids as guests, the complexation was studied at pD 1.0.

Stability constant K for 1:1 and 1:2 host to guest complexes were determined using the non-linear regression fitting method.[20]

Job's method of continuous variations was carried out according to reported procedure.[22]

Acknowledgements

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References and notes

1. Lehn, J.-M.; Meric, R.; Vigneron, J.-P.; Cesario, M.; Guilhem, J.; Pascard, C.; Asfari, Z.; Vicens *J. Supramol. Chem.* **1997**, *8*, 309.
2. Meric, R.; Vigneron, J.-P.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 129.
3. Kearny, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907.
4. Poh, B.-L.; Lim, C. S. *Tetrahedron* **1990**, *46*, 3651.
5. Garel, L.; Lozach, B.; Dutasta, J.-P.; Collet, A. J. *J. Am. Chem. Soc.* **1993**, *115*, 11652.
6. Kobayashi, K.; Asakawa, Y.; Aoyama, Y. *Supramol. Chem.* **1993**, *2*, 133.
7. Arena, G.; Casnati, A.; Contino, A.; Gulina, F. G.; Sciotto, D.; Ungaro, R. J. *J. Chem. Soc., Perkin Trans. 2* **2000**, 419.
8. Douteau-Guevel, N.; Coleman, A. W.; Morel, J.-P.; Morel-Desrosiers, N. J. *J. Chem. Soc., Perkin Trans. 2* **1999**, 629.
9. Bonal, C.; Israeli, Y.; Morel, J.-P.; Morel-Desrosiers, N. J. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1075.
10. Leverd, P. C.; Berthault, P.; Lance, M.; Nierlich, M. *Eur. J. Org. Chem.* **2000**, 133.
11. Stodeman, M.; Dhar, N. *Therm. Acta* **1998**, *320*, 33.
12. Stodeman, M.; Dhar, N. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 899.
13. Poh, B.-L.; Tan, C. M. *Tetrahedron* **1994**, *50*, 3453.
14. Sussman, J-L. ; Harel, M.; Frolow, F.; Gefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
15. Shinkai, S. *Tetrahedron* **1993**, *49*, 8933.
16. Abraham, W. *J. Incl. Phenom.* **2002**, *43*, 159.
17. Ikeda, A.; Shinkai, S. *Chem. Rev.* **1997**, *97*, 1713.
18. Gokel, G. W.; Abel, E.; Comprehensive Supramolecular Chemistry; Pergamon: Exeter, UK, 1996; Vol. 1, p 511.
19. Lhotak, P.; Shinkai, S. *J. Phys. Org. Chem.* **1997**, *10*, 273.
20. Poh, B.-L.; Tan, C. M.; Loh, C. L. *Tetrahedron* **1993**, *49*, 3849.
21. Poh, B.-L.; Tan, C. M. *Tetrahedron* **1993**, *49*, 9581.
22. Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626.

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2-Ethoxy-3-pyridylboronic acid: a versatile reagent for the synthesis of highly-functionalised 3-aryl/heteroaryl-pyridines via Suzuki cross-coupling reactions

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Abstract—This paper describes the commercially-viable synthesis and isolation of 2-ethoxy-3-pyridylboronic acid on a ca. 70 g scale via a directed *ortho*-metalation reaction on readily-available 2-ethoxypyridine. A range of efficient cross-coupling reactions of 2-ethoxy-3-pyridylboronic acid with selected aryl/heteroaryl halides under palladium-catalysed Suzuki–Miyaura conditions yield novel 2-ethoxy-3-aryl/heteroaryl-pyridines in high yield (heteroaryl = pyridyl, pyrimidyl, pyrazyl). The X-ray crystal structure of 2-ethoxy-3-pyridylboronic acid reveals that the boronic acid group takes part in an intramolecular O–H···O bond with the adjacent ethoxy substituent, and an intermolecular O–H···N bond.

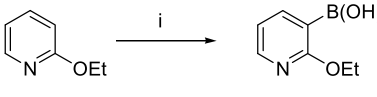
1. Introduction

Highly-functionalised pyridines, including aryl- and heteroaryl-substituted derivatives, are widespread in the pharmaceutical and agrochemical sectors.[1] They also have important applications as ligands for metal-coordination and supramolecular assemblies[2] and as fluorophores.[3] Efficient methodology for the synthesis of new derivatives, with broad functional group compatibility, is, therefore, of considerable current interest.[4] The use of catalytic cross-coupling protocols for preparing aryl-functionalised heterocycles is very topical,[5] and in this context heterocyclic boronic acids are emerging as important reagents for Suzuki-Miyaura reactions.[5c]

The syntheses of the parent (unsubstituted) pyridylboronic acids require precisely-controlled conditions and their purification is difficult (especially 2-pyridylboronic acid which readily deboronates).[6] This has restricted their use as routine reagents.[7] However, it has recently been shown that many halo- and dihalo-pyridylboronic acids (or their corresponding pinacol esters) are more stable,[8] although isolation is sometimes difficult due to partial solubility in water, and for some derivatives precise control of pH during workup is needed to avoid deboronation.[9] Cross-coupling reactions of bromopyridylboronic acids can suffer from competing self-coupling reactions[8a] or debromination.[8f]

2. Results and discussion

We now report that 2-ethoxy-3-pyridylboronic acid **2** is a versatile new reagent. The synthesis of **2** was readily achieved by a directed ortho-metalation reaction[10] of 2-ethoxypyridine **1** (LDA in THF at −50 °C) followed by addition of triisopropylborate and aqueous workup (Scheme 1) (70% yield on a 5 g scale). This procedure has been scaled-up to give ca. 70 g batches of analytically-pure **2** in 48% yield (see Section 4). In our hands, comparable scale-up reactions for many other pyridylboronic acid derivatives have proved to be practically far more difficult.



1 **2**

Scheme 1. Reagents and conditions: i LDA, THF, − 50 °C, triisopropylborate, H_2O.

Keywords: Boronic acid; Cross-coupling; Directed *ortho*-metalation; Pyridine.

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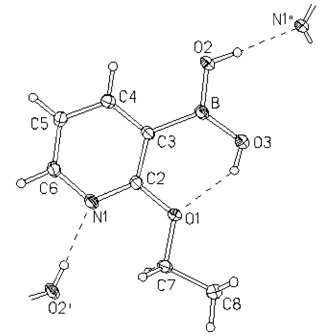
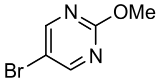
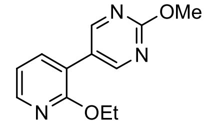
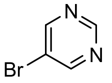
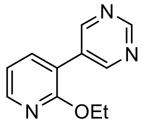
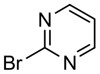
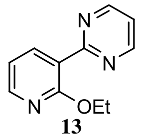
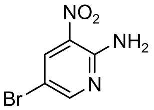
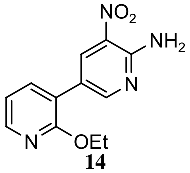
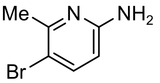
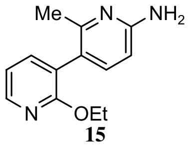
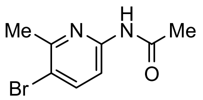
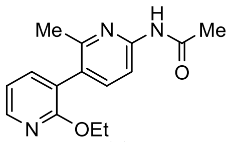
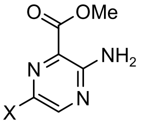
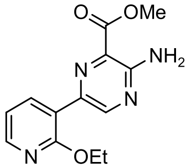
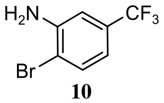
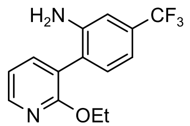


Figure 1. X-ray structure of **2**, showing thermal ellipsoids (50% probability) and hydrogen bonds. Symmetry transformations: (i) x+½, ½–y, z–½; (ii) x–½, ½–y, z+½.

Compound **2** has the following attractive features: (i) it is shelf-stable at room temperature (no observable decomposition after 9 months' storage); (ii) 2-ethoxypyridine is cheap and readily-available from commercial suppliers; (iii) the hydrophobic ethoxy substituent ensures good solubility of **2** in organic solvents which makes extraction from the aqueous phase easier than for most other pyridylboronic acid derivatives we have studied; (iv) in the cross-coupled products the 2-ethoxy group leads to sterically-induced twisting of the biaryl system (*peri*-interactions) and hence increased solubility, which assists chromatographic separation from any minor byproducts.

The X-ray crystal structure[11] of **2** shows a nearly planar conformation of the molecule, stabilised by an

Table 1. $\mathbf{1}+\mathrm{R{-}X}\xrightarrow{i}\mathbf{11{-}18}$

Entry	Boronic acid	R–X	Product	Isolated yield (%)
1	**2**	 **3**	 **11**	82
2	**2**	 **4**	 **12**	90
3	**2**	 **5**	 **13**	89
4	**2**	 **6**	 **14**	74
5	**2**	 **7**	 **15**	75
6	**2**	 **8**	 **16**	78
7	**2**	 **9** **a** X=Br, **b** X=Cl	 **17**	90 (from **9a**) 62 (from **9b**)
8	**2**	 **10**	 **18**	71

Reagents and conditions: $Pd(PPh_3)_2Cl_2$, 1,4-dioxane, Na_2CO_3 (1 M), reflux, 24 h; for **9a**, 20 °C, 16 h; for **9b**, 60 °C, 15 min.

intramolecular O–H···O hydrogen bond (Fig. 1), while the other hydroxyl group forms an intermolecular O–H···N bond linking the molecules into an infinite chain. This structure is unusual as arylboronic acids (like carboxylic acids) typically form H-bonded dimers in crystals;[8a,12] this does not occur with compound **2**.

Suzuki cross-coupling reactions of **2** were carried out with a range of aryl/heteroaryl halides **3–10** under standard conditions [Pd(PPh$_3$)$_2$Cl$_2$, dioxane, reflux] to yield products **11–18**, respectively. The results are collated in Table 1. There are some important features of these results. (i) The reactions are high-yielding with a variety of halogenated coupling partners (viz. pyridyl, pyrimidyl, pyrazyl and phenyl derivatives). (ii) The reaction is versatile with respect to functional group tolerance (viz. nitro, amine, amide, ester and trifluoromethyl) thereby allowing access to highly-functionalised 2-ethoxy-3-aryl/heteroaryl-pyridine derivatives, which are attractive drug and agrochemical candidates. (iii) The high-yielding reactions in the presence of a primary amine substituent (entries 4, 5 and 7) are notable:[8a,13] protection of the amino group is sometimes necessary for Suzuki reactions.[14]

Substrates **3–8** and **10** gave the optimised yield of products after 24 h at reflux, as judged by tlc and ^{1}H NMR monitoring of the reaction mixture. Reagent **9a** is a notable exception. A high yield of product **17** (78%) was obtained after only 15 min reflux; the yield decreased after 2 h and 8 h to 40 and 12%, respectively; 24 h reflux gave an intractable green product. Indeed, the reaction of **2** with **9a** at 20 °C for 16 h gave **17** in 90% yield. The chloro analogue **9b** was also very reactive: 62% yield of **17** was obtained after only 15 min reaction at 60 °C. Refluxing analytically-pure compound **17** in dioxane in the presence of Pd(PPh$_3$)$_2$Cl$_2$ led to the decomposition of **17** to yield an insoluble green solid which accounts for the reduced isolated yield of **17** after longer reaction times. The reasons for the increased reactivity of **9a** and **9b**, compared to **3–8** and **10** are not clear at present.

3. Conclusions

We have described an efficient synthesis of 2-ethoxy-3-pyridylboronic acid **2** which serves as a versatile compound for the preparation of functionalised aryl/heteroaryl-pyridine libraries. Reagent **2** offers significant practical advantages over other pyridylboronic acid derivatives we have studied in this context: notably, ease of synthesis and purification, efficient scale-up to 70 g batches, shelf-stability at 20 °C, and high-yielding cross-coupling reactions. Compound **2** is, therefore, highly recommended as a new reagent in pyridine chemistry.

4. Experimental

4.1. General

General details of equipment and techniques used are the same as those we have reported previously.[8a] All synthetic reagents were used as supplied. Solvents were dried and distilled using standard procedures.

4.1.1. 2-Ethoxy-3-pyridylboronic acid (2). Procedure A. To a solution of diisopropylamine (5.9 mL, 41.69 mmol) in anhydrous THF (50 mL) at −10 °C, n-BuLi (2.5 M in hexane, 17.4 mL, 43.59 mmol) was added dropwise. The reaction was stirred for 0.5 h at −10 °C then cooled to −50 °C. 2-Ethoxypyridine **1** (4.5 mL, 37.9 mmol) in anhydrous THF (10 mL) was added dropwise. The reaction was stirred for 1 h at −50 °C then triisopropylborate (10.5 mL, 45.5 mmol) was added slowly. The reaction mixture was stirred at −50 °C for another 1 h, then allowed to warm to −10 °C and quenched with water (50 mL). The reaction was left at room temperature with stirring overnight. The organic solvent was evaporated in vacuo and the remaining aqueous layer, pH 10, was filtered. The filtrate was washed with diethyl ether (2×50 mL). The aqueous layer was then acidified to pH 4 (with 48% HBr) to give **2** as a white solid (4.4 g, 70%), mp 103.0–103.8 °C; υ_{max}(KBr) 3200, 2992, 2912, 1585, 1439 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d$_6$) δ 8.16 (1H, dd, J=2.0, 4.8 Hz), 7.87 (1H, dd, J=2.0, 6.8 Hz), 7.83 (2H, s), 6.94 (1H, dd, J=4.8, 7.2 Hz), 4.33 (2H, q, J=6.8 Hz), 1.31 (3H, t, J=6.8 Hz); ^{13}C NMR (100 MHz, acetone-d$_6$) δ 166.0, 148.4, 144.8, 116.9, 115.7 (br C-B), 61.2, 14.5; MS (EI) *m/z* 166.9 (M$^+$, 100%). Anal. Calcd. for $C_7H_{10}BNO_3$: C, 50.35; H, 6.04; N, 8.39. Found: C, 50.32; H, 5.92; N, 8.34%.

Procedure B. Following procedure A, diisopropylamine (96.7 g, 0.96 mol) in anhydrous THF (500 mL), n-BuLi (2.5 M in hexane, 400 mL, 1.00 mol), 2-ethoxypyridine **1** (107 g, 0.87 mol) and triisopropylborate (196 g, 1.04 mol) were used. After quenching with water the reaction was left at room temperature with stirring overnight. The organic layer was discarded and the aqueous layer, pH 10, was then filtered. The filtrate was washed with diethyl ether (200 mL) until no starting material was detected in the ether washings by tlc. The aqueous layer was then acidified to pH 7 (with 48% HBr) to give **2** as an analytically-pure off-white solid (69.7 g, 48%) spectroscopically identical with the sample from Procedure A.

4.2. General procedure for the cross-coupling reactions

The boronic acid **2** (1.0 equiv) the arylhalide (0.9 equiv) and Pd(PPh$_3$)$_2$Cl$_2$ (ca. 5 mol%) were sequentially added to degassed 1,4-dioxane (10 mL) and the mixture was stirred at 20 °C for 30 min. Degassed aqueous Na_2CO_3 solution (1 M, 3.0 equiv.) was added and the reaction mixture was heated under argon at reflux (typically for 24 h; entry **7** is the only exception). The solvent was removed in vacuo then ethyl acetate was added and the organic layer was washed with brine, separated, and dried over $MgSO_4$. The mixture was purified by chromatography on a silica gel column. On some occasions an additional recrystallisation was necessary. Other products isolated were small amounts of the 'self-coupled' boronic acid, i.e. 2,2′-diethoxy-[3,3′]bipyridine, which was usually the first material to elute, and unreacted arylhalide.

4.2.1. 5-(2-Ethoxy-3-pyridyl)-2-methoxypyrimidine (11). Boronic acid **2** (284 mg, 1.7 mmol), 5-bromo-2-methoxypyrimidine **3** (260 mg, 1.4 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na_2CO_3 (4 mL); reaction time 24 h; eluent EtOAc:hexane (1:2 v/v), gave **11**

as a white solid (260 mg, 82%) mp 70.9–71.6 °C; υ_{max}(KBr) 3054, 2988, 2952, 1568, 1546, 1450 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d$_6$) δ 8.79 (2H, s), 8.17 (1H, dd, J = 1.6, 4.8 Hz), 7.82 (1H, dd, J = 1.6, 7.2 Hz), 7.06 (1H, dd, J = 4.8, 7.2 Hz), 4.42 (2H, q, J = 7.2 Hz), 3.98 (3H, s), 1.34 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 164.70, 160.42, 158.80, 146.80, 137.38, 124.10, 117.69, 116.91, 96.11, 61.99, 54.82, 14.57; MS (EI) *m/z* 231.1 (M$^+$, 100%). Anal. Calcd. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.42; H, 5.74; N, 17.93%.

4.2.2. 5-(2-Ethoxy-3-pyridyl)pyrimidine (12). Boronic acid **2** (284 mg, 1.7 mmol), 5-bromopyrimidine **4** (239 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 24 h; eluent EtOAc:hexane (1:1 v/v), gave **12** as a white solid (271 mg, 90%) mp 119.0–119.8 °C; υ_{max}(KBr) 2992, 2966, 2908, 1579, 1555, 1439 cm^{-1};^{1}H NMR (400 MHz, acetone-d$_6$) δ 9.10 (1H, s), 9.01 (2H, s), 8.23 (1H, dd, J = 1.6, 4.8 Hz), 7.89 (1H, dd, J = 1.6, 8.0 Hz), 7.11 (1H, dd, J = 5.2, 7.6 Hz), 4.43 (2H, q, J = 7.2 Hz), 1.34 (3H, t, J = 6.8 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 160.5, 157.3, 156.4, 147.7, 138.0, 130.6, 117.5, 117.0, 96.2, 62.2, 14.6; MS (EI) *m/z* 201.1 (M$^+$, 100%). Anal. Calcd. for $C_{11}H_{11}N_3O$: C, 65.66; H, 5.51; N, 20.88. Found: C, 65.46; H, 5.51; N, 20.63%.

4.2.3. 2-(2-Ethoxy-3-pyridyl)pyrimidine (13). Boronic acid **2** (284 mg, 1.7 mmol), 2-bromopyrimidine **5** (239 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 24 h; eluent EtOAc:hexane (1:2 v/v), gave **13** as a colourless viscous oil (268 mg, 89%); υ_{max}(KBr) 2977, 1571, 1445, 1415 cm^{-1}; ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.68 (2H, d, J = 4.8 Hz), 8.11 (1H, d, J = 4.8 Hz), 7.94 (1H, d, J = 7.3 Hz), 7.03 (1H, t, J = 4.8 Hz), 6.83 (1H, dd, J = 5.1, 7.2 Hz), 4.37 (2H, q, J = 7.0 Hz), 1.23 (3H, t, J = 7.0 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 164.5, 161.1, 156.8 (2C), 148.0, 140.1, 122.3, 118.7, 116.3, 62.0, 14.2; HRMS (EI) calcd for $C_{11}H_{11}N_3O$ (M$^+$) 201.09121, found 201.09133.

4.2.4. 2′-Ethoxy-5-nitro-[3,3′]bipyridinyl-6-ylamine (14). Boronic acid **2** (284 mg, 1.7 mmol), 5-bromo-2-amino-3-nitropyridine **6** (327 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 24 h; eluent EtOAc, gave **14** as a yellow solid (289 mg, 74%) mp 193.0–193.9 °C; υ_{max}(KBr) 3470, 3275, 3121, 2984, 1654, 1561, 1351 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.67 (1H, d, J = 2.0 Hz), 8.63 (1H, d, J = 2.0 Hz), 8.15 (1H, dd, J = 2.0 Hz, J = 4.8 Hz), 8.03 (2H, br s, NH$_2$), 7.90 (1H, dd, J = 2.0, 4.8 Hz), 7.07 (1H, dd, J = 4.8, 7.6 Hz), 4.37 (2H, q, J = 7.2 Hz), 1.31 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 159.7, 156.0, 152.7, 146.0, 137.9, 134.2, 126.2, 120.5, 118.7, 117.4, 61.5, 14.3; MS (EI) *m/z* 260.0 (M$^+$, 100%). Anal. Calcd. for $C_{12}H_{12}N_4O_3$: C, 55.38; H, 4.65; N, 21.53. Found: C, 55.12; H, 4.58; N, 21.36%.

4.2.5. 2′-Ethoxy-5-methyl-[3,3′]bipyridinyl-6-ylamine (15). Boronic acid **2** (284 mg, 1.7 mmol), 6-amino-3-bromo-2-methylpyridine **7** (281 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 24 h; eluent EtOAc, gave **15** as a crystalline solid (258 mg, 75%) mp 138.6–139.0 °C; υ_{max}(KBr) 3466, 3299, 3151, 2975, 1639 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.10 (1H, dd, J = 2.0, 4.8 Hz), 7.47 (1H, dd, J = 2.0, 7.2 Hz), 7.12 (1H, d, J = 8.4 Hz), 6.99 (1H, dd, J = 4.8, 7.2 Hz), 6.30 (1H, d, J = 8.4 Hz), 5.88 (2H, br s, NH$_2$), 4.29 (2H, q, J = 7.2 Hz), 2.05 (3H, s), 1.22 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 160.2, 158.5, 153.6, 145.3, 139.6, 139.0, 123.3, 119.0, 116.8, 104.9, 60.9, 22.3, 14.5; MS (EI) *m/z* 229.1 (M$^+$, 100%). Anal. Calcd. for $C_{13}H_{15}N_3O$: C, 68.10; H, 6.59; N, 18.23. Found: C, 67.85; H, 6.54; N, 17.96%.

4.2.6. N-(2′-Ethoxy-2-methyl-[3,3′]bipyridinyl-6-yl)-acetamide (16). Boronic acid **2** (284 mg, 1.7 mmol), N-(5-bromo-6-methyl-pyridin-2-yl)-acetamide **8**[15] (334 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 24 h; eluent EtOAc, gave **16** as a crystalline solid (317 mg, 78%) mp 147.7–148.6 °C; υ_{max}(KBr) 3241, 3042, 2981, 1669, 1577, 1533 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.50 (1H, br s, NH), 8.17 (1H, dd, J = 2.0, 4.8 Hz), 7.94 (1H, d, J = 8.4 Hz), 7.57 (1H, dd, J = 2.0, 7.2 Hz), 7.51 (1H, d, J = 8.4 Hz), 7.04 (1H, dd, J = 4.8, 7.2 Hz), 4.31 (2H, q, J = 7.2 Hz), 2.20 (3H, s), 2.08 (3H, s), 1.20 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 169.2, 160.0, 154.3, 150.7, 146.3, 139.7, 139.6, 126.7, 122.0, 116.9, 110.4, 61.1, 23.3, 22.1, 14.4; MS (EI) *m/z* 271.0 (M$^+$, 100%). Anal. Calcd. for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.18; H, 6.25; N, 15.31%.

4.2.7. Methyl 2-amino-5-(2-ethoxy-3-pyridyl)-3-pyrazinoate (17). Boronic acid **2** (284 mg, 1.7 mmol), 3-amino-6-bromopyrazine-2-carboxylic acid methyl ester **9a**[16] (345 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 16 h at 20 °C; eluent EtOAc, gave **17** as a yellow solid (440 mg, 90%) mp 156.6–157.0 °C; υ_{max}(KBr) 3447, 3264, 3146, 2979, 1693, 1618 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.89 (1H, s), 8.17 (1H, dd, J = 2.0, 4.8 Hz), 8.12 (1H, dd, J = 2.0, 7.6 Hz), 7.46 (2H, br s, NH$_2$), 7.11 (1H, dd, J = 4.8, 7.6 Hz), 4.41 (2H, q, J = 7.2 Hz), 3.87 (3H, s), 1.35 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 166.3, 159.8, 154.3, 148.0, 146.5, 138.0, 136.7, 122.4, 119.3, 117.4, 61.6, 52.2, 14.4; MS (EI) *m/z* 274.0 (M$^+$, 100%). Anal. Calcd. for $C_{13}H_{14}N_4O_3$: C, 56.93; H, 5.14; N, 20.43. Found: C, 56.63; H, 4.97; N, 20.33%.

When the reaction was carried out at reflux for 15 min, product **17** (321 mg, 78% yield) was obtained.

A comparable reaction with 3-amino-6-chloropyrazine-2-carboxylic acid methyl ester **9b**,[16] 15 min at 60 °C, gave **17** in 62% yield.

4.2.8. 3-(2-Amino-4-trifluoromethylbenzene)-2-ethoxypyridine (18). Boronic acid **2** (284 mg, 1.7 mmol), 1-amino-2-bromo-5-(trifluoromethyl)benzene **10** (360 mg, 1.5 mmol), Pd(PPh$_3$)$_2$Cl$_2$ (59.6 mg, 0.085 mmol), 1,4-dioxane (10 mL) and Na$_2$CO$_3$ (4 mL); reaction time 24 h; eluent DCM, gave **18** as a clear brown oil (300 mg, 71%); υ_{max}(KBr) 3464, 3378, 3054, 2982, 1633, 1579 cm^{-1}; ^{1}H NMR (400 MHz, acetone-d$_6$) δ 8.27 (1H, dd, J = 2.0, 3.2 Hz), 7.70 (1H, dd, J = 2.0, 5.2 Hz), 7.26 (1H, d, J =

7.8 Hz), 7.20 (1H, s), 7.12 (1H, dd, J = 2.0, 4.8 Hz), 7.04 (1H, d, J = 7.8 Hz), 4.90 (2H, t, s), 4.48 (2H, q, J = 7.2 Hz), 1.36 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, acetone-d$_6$) δ 116.3, 147.5, 140.8, 132.4, 130.9 (q, J = 32 Hz), 125.5 (q, J = 270 Hz), 122.2, 117.8, 113.5, 112.2, 62.2, 14.8; HRMS (EI) calcd for $C_{14}H_{13}F_3N_2O$ (M^+) 282.09800, found 282.09820.

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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.2005.02.070

References and notes

1. (a) Bashford, K. E.; Burton, M. B.; Cameron, S.; Cooper, A. L.; Hogg, R. D.; Kane, P. D.; MacManus, D. A.; Matrunola, C. A.; Moody, C. J.; Robertson, A. A. B.; Warne, M. R. *Tetrahedron Lett.* **2003**, *44*, 1627–1629. (b) Raw, S. A.; Taylor, R. J. K. *Chem. Commun.* **2004**, 508–509.
2. (a) Yoshizawa, M.; Nagao, M.; Umemoto, K.; Biradha, K.; Fujita, M.; Sakamoto, S.; Yamaguchi, K. *Chem. Commun.* **2003**, 1808–1809. (b) Constable, E. C.; Housecroft, C. E.; Neuburger, M.; Reymann, S.; Schaffner, S. *Chem. Commun.* **2004**, 1056–1057.
3. Review: Mitsche, U.; Bauerle, P. *J. Mater. Chem.* **2000**, *10*, 1471–1507.
4. For a review of pyridine ring synthesis see: Henry, G. D. *Tetrahedron* **2004**, *60*, 6043–6061.
5. Reviews of cross-coupling methodology: (a) Stanforth, S. P. *Tetrahedron* **1998**, *54*, 263–303. (b) Hassan, J.; Sévignon, M.; Gozzi, C.; Schultz, E.; Lemaire, M. *Chem. Rev.* **2002**, *102*, 1359–1470. (c) For a review of heterocyclic boronic acids see: Tyrrell, E.; Brookes, P. *Synthesis* **2003**, 469–483. (d) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483.
6. Fischer, F. C.; Havinger, E. *Recl. Trav. Chim. Pays-Bas* **1965**, *84*, 439–440.
7. (a) Cai, D.; Larsen, R. D.; Reider, P. J. *Tetrahedron Lett.* **2002**, *43*, 4285–4287. (b) Li, W.; Nelson, D. P.; Jensen, M. S.; Hoerrner, R. S.; Cai, D.; Larsen, R. D.; Reider, P. J. *J. Org. Chem.* **2002**, *67*, 5394–5397. (c) Chan, D. M. T.; Monaco, K. L.; Li, R.; Bonne, D.; Clark, C. G.; Lam, P. Y. S. *Tetrahedron Lett.* **2003**, *44*, 3863–3865. (d) Cioffi, C. L.; Spencer, W. T.; Richards, J.J; Herr, R. J. *J. Org. Chem.* **2004**, *69*, 2210–2212.
8. (a) Parry, P.; Wang, C.; Batsanov, A. S.; Bryce, M. R.; Tarbit, B. *J. Org. Chem.* **2002**, *67*, 7541–7543. (b) Bouillon, A.; Lancelot, J.-C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 2885–2890. (c) Bouillon, A.; Lancelot, J.-C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 3323–3328. (d) Bouillon, A.; Lancelot, J.-C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 4369–4373. (e) Parry, P. R.; Bryce, M. R.; Tarbit, B. *Synthesis* **2003**, 1035–1038. (f) Sutherland, A.; Gallagher, T. *J. Org. Chem.* **2003**, *68*, 3352–3355. (g) Bouillon, A.; Lancelot, J.-C.; de Oliveira Santos, J. S.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2003**, *59*, 10043–10049. (h) Leflemme, N.; Dallemagne, P.; Rault, S. *Tetrahedron* **2004**, *60*, 4861–4865.
9. For example, 2,3-dichloro-4-pyridylboronic acid is particularly susceptible to deboronation during workup. Thompson, A. E.; Bryce, M. R.; Batsanov, A. B.; Parry, P. R.; Tarbit, B. Manuscript in preparation.
10. Reviews: (a) Mongin, M.; Quéguiner, G. *Tetrahedron* **2001**, *57*, 4059–4090. (b) Anctil, E. J.-G.; Snieckus, V. *J. Organomet. Chem.* **2002**, *653*, 150–160. (c) Whisler, M. C.; MacNeil, S.; Snieckus, V.; Beak, P. *Angew. Chem., Int. Ed.* **2004**, *43*, 2206–2225. (d) Chinchilla, R.; Nájera, C.; Yus, M. *Chem. Rev.* **2004**, *104*, 2667–2722.
11. Crystal data: $C_7H_{10}BNO_3$ **2**, $M = 166.97$, monoclinic, space group $P2_1/n$ (No. 14), $T = 120$ K, $a = 3.940(1)$, $b = 14.335(2)$, $c = 14.226(2)$ Å, $\beta = 94.60(1)°$, $V = 800.9(3)$ Å^3, $Z = 4$, $R = 0.040$ on 2028 unique data with $I > 2\sigma(I)$. CCDC-256029. See supplementary information in CIF format.
12. Pedireddi, V. R.; SeethaLekshmi, N. *Tetrahedron Lett.* **2004**, *45*, 1903–1906.
13. (a) Li, J. J.; Yue, W. S. *Tetrahedron Lett.* **1999**, *40*, 4507–4510. (b) Maes, B. V. W.; Lemiere, G. L. F.; Dommisse, R.; Augustyns, K.; Haemers, A. *Tetrahedron* **2000**, *56*, 1777–1781. (c) Cooke, G.; de Cremiers, H. A.; Rotello, V. M.; Tarbit, B.; Vanderstraeten, P. E. *Tetrahedron* **2001**, *57*, 2787–2789.
14. (a) Caron, S.; Massett, S. S.; Bogle, D. E.; Castaldi, M. J.; Braish, T. F. *Org. Proc. Res. Dev.* **2001**, *5*, 254–256. For efficient reactions in the presence of a free amine group see: (b) Thompson, A. E.; Hughes, G.; Batsanov, A. S.; Bryce, M. R.; Parry, P. R.; Tarbit, B. *J. Org. Chem.* **2005**, *70*, 388–390. (c) Bracher, F.; Daab, J. *Eur. J. Org. Chem.* **2002**, 2288–2291.
15. For the synthesis of **8** we used a modification of the procedure reported in Wright, S. W.; Hageman, D. L.; McClure, L. D. *J. Heterocycl. Chem.* **1998**, *35*, 719. 2-Amino-5-bromo-6-methylpyridine was dissolved in pyridine at 0 °C. Acetyl chloride (5 equiv) was added and the mixture was stirred at 20 °C for 30 min. Addition of water precipitated analytically-pure compound **8** in 93% yield.
16. Ellingson, R. C.; Henry, R. L. *J. Am. Chem. Soc.* **1949**, *71*, 2798–2800.

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Corrigendum

Corrigendum to "Aspartame analogues containing 1-amino-2-phenylcyclohexanecarboxylic acids (c_6Phe). Part 2"

[Tetrahedron 61 (2005) 2913]

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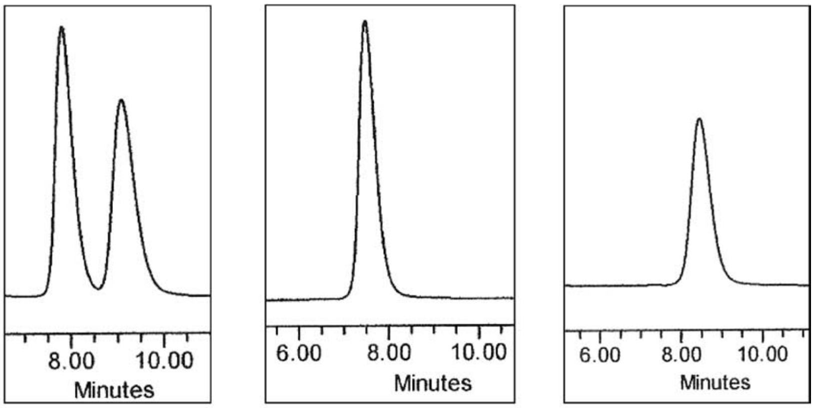
On page 2915, of the above paper, Figure 1 should be replaced by the following one:



(*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6**   (*S*,1*R*,2*S*)-**6**   (*S*,1*S*,2*R*)-**6**

The authors apologise for the errors.

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Corrigendum

Corrigendum to “Enantioselective syntheses of two 5, 9*E* diastereomers of 223V, an alkaloid from the poison frog *Dendrobates pumilio*”

[Tetrahedron 61 (2005) 1187]

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The authors regret that 20 of the 22 references[1–22] in the References and notes for the above paper were numbered incorrectly. The correct References and notes are as follows:

References and notes

1. Daly, J. W.; Garraffo, H. M.; Spande, T. F. In *Alkaloids from Amphibian Skin*; Pelletier, S. W., Ed.; Alkaloids: Chemical and Biological Perspectives; Pergamon: New York, 1999; Vol. 13, pp 1–161.
2. Garraffo, H. M.; Spande, T. F.; Jones, T. H.; Daly, J. W. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1553–1563.
3. Toyooka, N.; Tanaka, K.; Momose, T.; Daly, J. W.; Garraffo, H. M. *Tetrahedron* **1997**, *53*, 9553.
4. Toyooka, N.; Okumura, M.; Nemoto, H. *J. Org. Chem.* **2002**, *67*, 6078.
5. Toyooka, N.; Nemoto, H. *Studies Nat. Product. Chem.* **2003**, *29*, 419.
6. Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: New York, 1983.
7. Cieplak, A. S. *J. Am. Chem. Soc.* **1981**, *103*, 4540.
8. Ferraboschi, P.; Casati, S.; Grandi, S.; Grisenti, P.; Santaniello, E. *Biocatalysis* **1994**, *10*, 279.
9. Kolb, H. C.; Van Nieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
10. Comins, D. L.; Dehghani, A. *Tetrahedron Lett.* **1992**, *33*, 6299.
11. Okita, T.; Isobe, M. *Synlett* **1994**, 589. Okita, T.; Isobe, M. *Tetrahedreon* **1995**, *51*, 3737. Foti, C. J.; Comins, D. L. *J. Org. Chem.* **1995**, *60*, 2656. Nicolaou, K. C.; Shi, G.-Q.; Namoto, K.; Bernal, F. *Chem. Commun.* **1998**, 1757.
12. Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595.
13. Tokuyama, T.; Tsujita, T.; Shimada, A.; Garraffo, H. M.; Spande, T. E.; Daly, J. W. *Tetrahedron* **1991**, *47*, 5401.
14. Tokuyama, T.; Nishimori, N.; Shimada, A.; Edwards, M. W.; Daly, J. W. *Tetrahedron* **1987**, *43*, 643–652.
15. Edwards, M. W.; Daly, J. W.; Myers, C. W. *J. Nat. Prod.* **1988**, *51*, 1188.
16. Momose, T.; Toyooka, N. *J. Org. Chem.* **1994**, *59*, 943.
17. Comins, D. L.; LaMunyon, D. H.; Chen, X. *J. Org. Chem.* **1997**, *62*, 8182.
18. Smith, A. L.; Williams, S. F.; Holmes, A. B.; Hughes, L. R.; Lidert, Z.; Swithenbank, C. *J. Am. Chem. Soc.* **1998**, *110*, 8696. Holmes, A. B.; Smith, A. L.; Williams, S. F.; Hughes, L. R.; Lidert, Z.; Swithenbank, C. *J. Org. Chem.* **1991**, *56*, 1393.
19. Michel, P.; Rassatt, A.; Daly, J. W.; Spande, T. F. *J. Org. Chem.* **2000**, *65*, 8908.
20. Enders, D.; Theibes, C. *Synlett* **2000**, 1745.
21. Åhman, J.; Somfai, P. *Tetrahedron* **1995**, *35*, 9747.
22. Song, Y.; Okamoto, S.; Sato, F. *Tetrahedron Lett.* **2002**, *43*, 8635.

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