ELSEVIER

Tetrahedron

Tetrahedron Vol. 61, No. 11, 2005

Contents

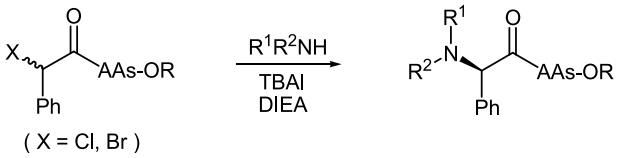
REPORT

On-microchip multiphase chemistry—a review of microreactor design principles and reagent contacting modes **pp 2733–2742**
George N. Doku, Willem Verboom, David N. Reinhoudt and Albert van den Berg*

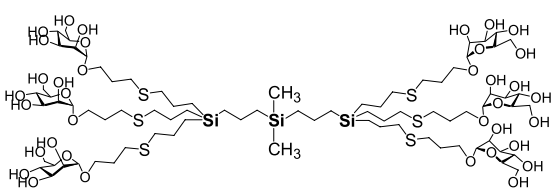
The design and operation of liquid–liquid and gas–liquid immiscible systems depend mainly on the method of dispersion/phase contacting, whilst gas–liquid–solid and gas–gas–solid systems depend not only on the phase contacting, but also on the solid integration principles employed. The microreactor design ideas employable, the methods of phase contacting and the consequent effects on the reaction yields are reviewed.

ARTICLES

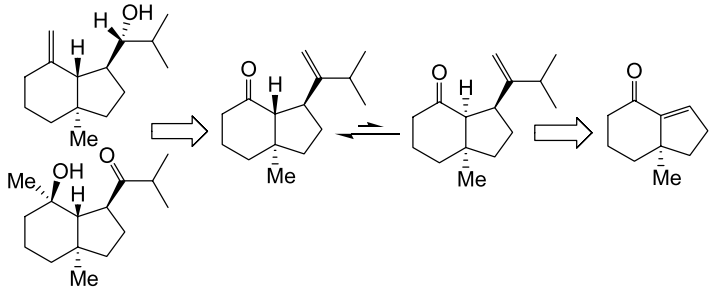
Stereoselective syntheses of tri- and tetrapeptide analogues by dynamic resolution of α-halo amides in nucleophilic substitution **pp 2743–2750**
Ji-yeon Chang, Eun-kyoung Shin, Hyun Jung Kim, Yangmee Kim and Yong Sun Park*



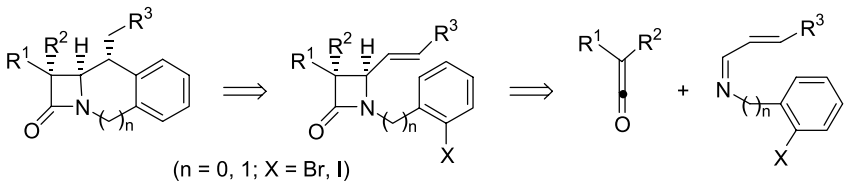
Synthesis of carbosilane dendrimers having peripheral mannose and mannobiose **pp 2751–2760**
Tomonori Mori,* Ken Hatano, Koji Matsuoka, Yasuaki Esumi, Eric J. Toone and Daiyo Terunuma*



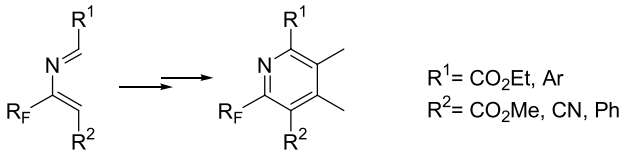
Total syntheses of the sesquiterpenoids (+)-*trans*-dracunculifoliol and (+)-4-hydroxyoppositan-7-one pp 2761–2766
Renata M. Oballa,* Rebekah Carson, Susan Lait, Jay A. Cadieux and Joël Robichaud



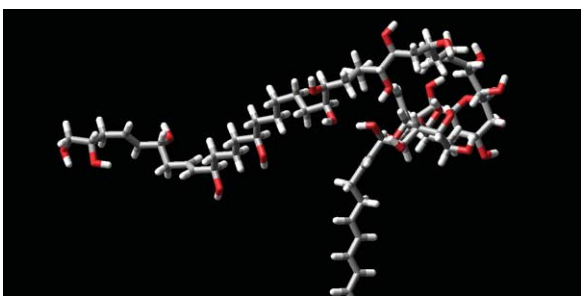
Free radical synthesis of benzofused tricyclic β-lactams by intramolecular cyclization of 2-azetidinone-tethered haloarenes pp 2767–2778
Benito Alcaide,* Pedro Almendros, Alberto Rodríguez-Vicente and M. Pilar Ruiz



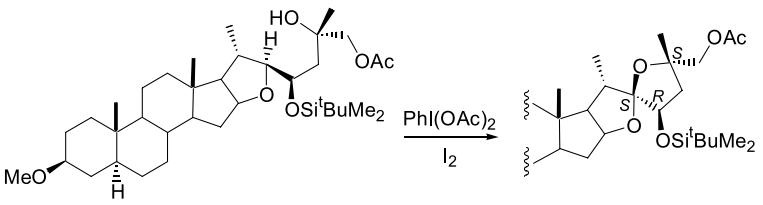
Aza-Wittig reaction of fluoroalkylated *N*-vinylic phosphazenes with carbonyl compounds. Usefulness of 2-azadienes for the preparation of fluoroalkyl pyridine derivatives pp 2779–2794
Francisco Palacios,* Concepción Alonso, Gloria Rubiales and Maite Villegas



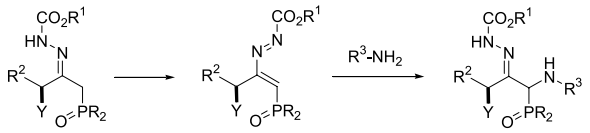
Hairpin conformation of amphidinols possibly accounting for potent membrane permeabilizing activities pp 2795–2802
Toshihiro Houdai, Shigeru Matsuoka, Nagy Morsy, Nobuaki Matsumori, Masayuki Satake and Michio Murata*



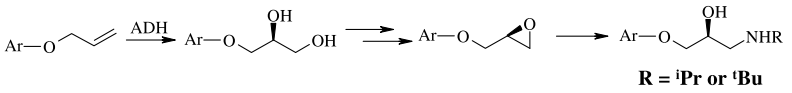
Hydrogen atom transfer methodology for the synthesis of C-22, C-23, and C-25 stereoisomers of cephalostatin north 1 side chain from spirostan sapogenins **pp 2803–2814**
Carmen Betancor, Raimundo Freire, Inés Pérez-Martín, Thierry Prangé and Ernesto Suárez*



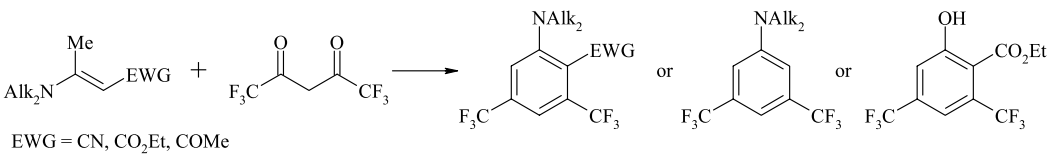
Synthesis of functionalized α-amino-phosphine oxides and -phosphonates by addition of amines and aminoesters to 4-phosphinyl- and 4-phosphonyl-1,2-diaza-1,3-butadienes **pp 2815–2830**
Francisco Palacios,* Domitila Aparicio, Yago López and Jesús M. de los Santos



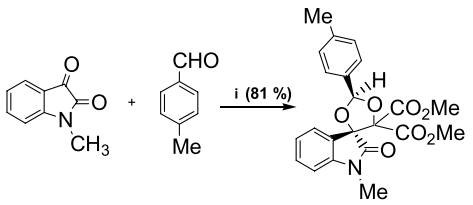
Asymmetric synthesis of aryloxypropanolamines via OsO_4-catalyzed asymmetric dihydroxylation **pp 2831–2838**
Iliyas A. Sayyed, Vinay V. Thakur, Milind D. Nikalje, Gajanan K. Dewkar, S. P. Kotkar and A. Sudalai*



Synthesis of functionalized *m*-bistrifluoromethylbenzenes via cyclocondensation of 1,1,1,5,5,5-hexafluoroacetylacetone with enamines **pp 2839–2847**
Dmitriy M. Volochnyuk, Alexander N. Kostyuk,* Dmitriy A. Sibgatulin and Alexander N. Chernega

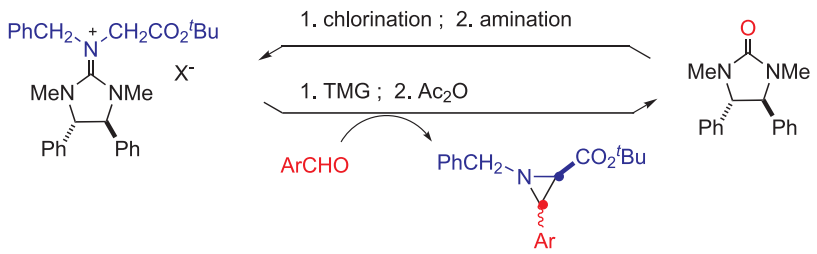


A stereoselective synthesis of spiro-dioxolanes via the multicomponent reaction of dicarbomethoxycarbene, aldehydes and 1,2- or 1,4-diones **pp 2849–2856**
Vijay Nair,* Sindhu Mathai, Smitha C. Mathew and Nigam P. Rath

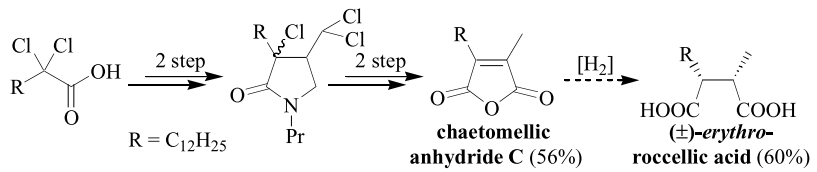


i. $N_2(CO_2Me)_2$, $Rh_2(OAc)_4$, dry benzene, argon, 16 h

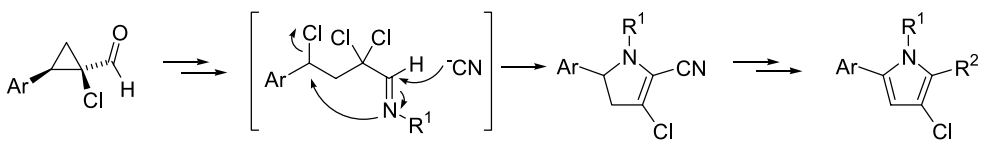
Mechanistic approaches to asymmetric synthesis of aziridines from guanidinium ylides and aryl aldehydes **pp 2857–2869**
Toyokazu Haga and Tsutomu Ishikawa*



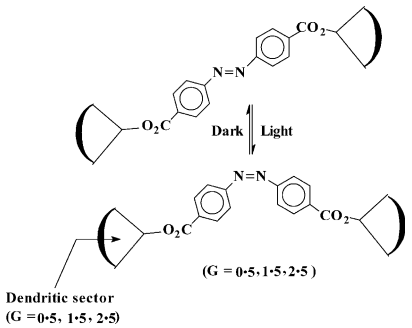
A new route to (±)-*erythro*-roccellic acid and chaetomellic anhydride C through functional rearrangement, promoted by *n*-propylamine or CH_3ONa/CH_3OH, of *N*-propyl-3-chloro-4-dichloromethyl-3-dodecylpyrrolidin-2-one **pp 2871–2877**
Laurent De Buyck, Chiara Danieli, Franco Ghelfi,* Ugo M. Pagnoni, Andrew F. Parsons, Mariella Pattarozzi and Fabrizio Roncaglia



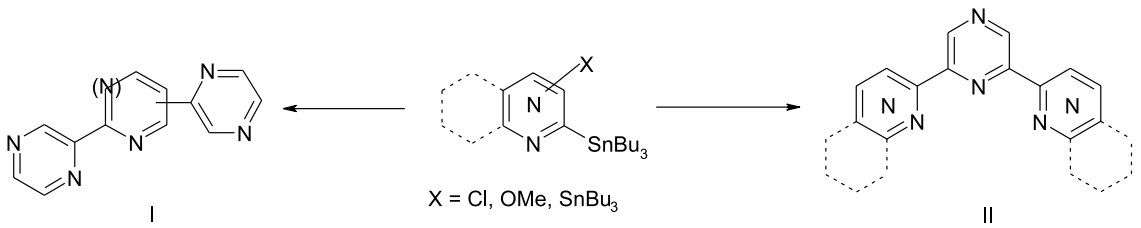
Synthesis of 2-aryl-4-chloropyrroles via ring expansion of 2-aryl-1-chlorocyclopropanecarbaldehydes **pp 2879–2887**
Guido Verniest, Sven Claessens, Filip Bombeke, Tinneke Van Thienen and Norbert De Kimpe*



Synthesis and photoresponsive study of azobenzene centered polyamidoamine dendrimers **pp 2889–2896**
Samaresh Ghosh, Ajit K. Banthia* and Zhu Chen

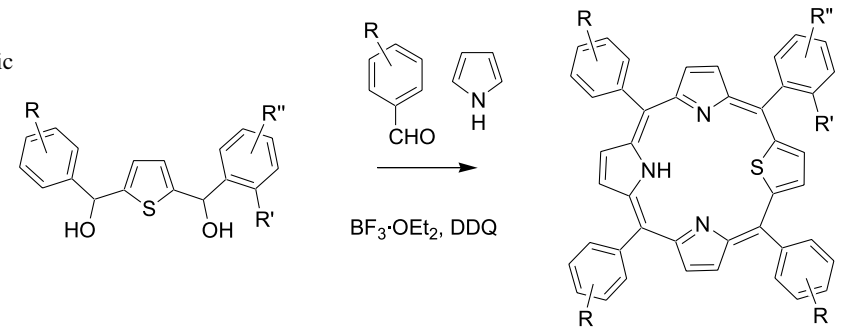


Synthesis of new polyaza heterocycles. Part 42: Diazines **pp 2897–2905**
Mircea Darabantu, Ludovic Boully, Alain Turck and Nelly Plé*

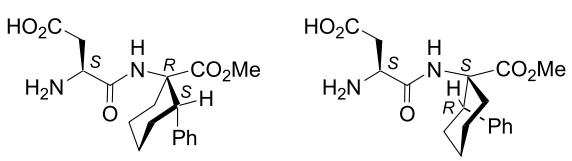


Convenient synthetic route of versatile 21-monothiatetraphenylporphyrins of the A_4 and AB_3 type **pp 2907–2912**
Silvio Stute,* Kerstin Gloe and Karsten Gloe

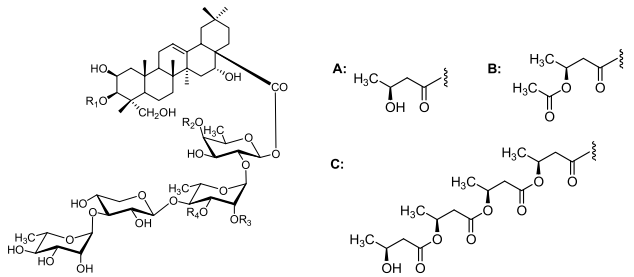
Modified synthetic method to novel symmetric and asymmetric 21-thiaporphyrins.



Aspartame analogues containing 1-amino-2-phenylcyclohexanecarboxylic acids (c_6Phe). Part 2 **pp 2913–2919**
Miriam Alías, Marta Lasa, Pilar López, José I. García and Carlos Cativiela*



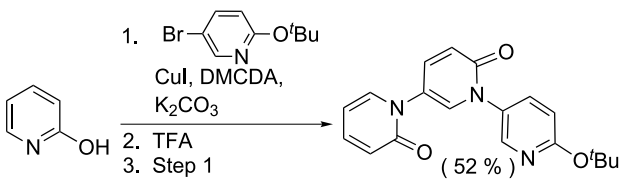
Bellisosides A–F, six novel acylated triterpenoid saponins from *Bellis perennis* (compositae) **pp 2921–2929**
Wei Li, Yoshihisa Asada,* Kazuo Koike, Tamotsu Nikaido, Tsutomu Furuya and Takafumi Yoshikawa



	1	2	3	4	5	6	7
R_1	Glc	Glc	Rha	Rha	Rha	Rha	Glc
R_2	Ac	Ac	A	B	C	C	H
R_3	Ac	H	H	H	H	Ac	H
R_4	H	Ac	H	H	H	H	H

Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl; Ac, acetyl.

An improved Ullmann–Ukita–Buchwald–Li conditions for CuI-catalyzed coupling reaction of 2-pyridones with aryl halides **pp 2931–2939**
Po-Shih Wang, Chih-Kai Liang and Man-kit Leung*



An effective CuI–DMCDA–K_2CO_3-catalyzed coupling reaction of 2-pyridones with aryl halides in PhMe is reported. The reaction is efficient and could be applied for polymer modification and iterative oligo-pyridone synthesis.

OTHER CONTENTS

Contributors to this issue **p I**
Instructions to contributors **pp III–VI**

*Corresponding author
Supplementary data available via ScienceDirect

Indexed/Abstracted in: AGRICOLA, Beilstein, BIOSIS Previews, CAB Abstracts, Chemical Abstracts, Chemical Engineering and Biotechnology Abstracts, Current Biotechnology Abstracts, Current Contents: Life Sciences, Current Contents: Physical, Chemical and Earth Sciences, Current Contents Search, Derwent Drug File, Ei Compendex, EMBASE/Excerpta Medica, Medline, PASCAL, Research Alert, Science Citation Index, SciSearch

ELSEVIER

ISSN 0040-4020

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2733–2742

Tetrahedron report number 708

On-microchip multiphase chemistry—a review of microreactor design principles and reagent contacting modes

George N. Doku,[a] Willem Verboom,[b] David N. Reinhoudt[b] and Albert van den Berg[a,*]

[a]*Laboratory of (Micro)Biomedical and Environmental Technology, MESA+ Research Institute, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands*
[b]*Laboratory of Supramolecular Chemistry and Technology, MESA+ Research Institute, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands*

Received 13 December 2004

Contents

1. Introduction 2733
2. Immiscible aqueous-organic liquid systems 2733
3. Gas–liquid systems 2734
4. Gas–liquid–solid systems 2736
5. Gas–gas–solid systems 2738
6. Conclusions/outlook 2739
References and notes 2739

1. Introduction

Miniaturized chemical reactors, typically on-chip microchannel reactors have become important for analytical and environmental monitoring,[1–4] as measuring devices for on-line process optimisation,[5] as catalyst screening tools,[6] for the production of micro fuel cells,[7] and especially for microorganic synthesis/production in the pharmaceutical industry where the test-rig stage in the development of a drug does not require the production of large quantities of the chemical.[8–12] The most striking advantages of such microsystems are the high portability, reduced reagent consumption, minimization of waste production, remote (on-site) applications and efficient heat dissipation owing to the high surface-area-to-volume ratios. Quite a number of the reactions executed in such systems have involved reagent phases which are immiscible with one another; these include aqueous-organic liquid,[13–16] gas–liquid,[17–37] gas–liquid–solid,[26–47] and gas–gas–solid[48–51] systems. In such systems, there exists the complexity of forcing a reactant of one phase to mix, diffuse and react with that of another, making the flow dynamics, the methods of promoting phase contacting and mixing critical.[16–37] The chemical kinetics would not only depend on the concentrations of the reacting species, but also on the mass transfer between the different phases. Whereas the design and operation of liquid–liquid and gas–liquid immiscible microreactor systems have depended mainly on the method of dispersion/phase contacting, gas–liquid–solid and gas–gas–solid systems have depended not only on the phase contacting, but also on the solid integration principles employed. In this paper, we present an overview of the critical issues in the development of on-chip multiphase chemistry systems. The microreactor design ideas employable, the methods of phase contacting and the consequent effects on the reaction yields are discussed.

2. Immiscible aqueous-organic liquid systems

In immiscible organic-aqueous systems, the two different liquids could be pumped from individual supply channels to assume a parallel (longitudinal) contact-flow in a common

Keywords: Lab-on-chip microreactors; Multiphase reactions; Immiscible liquid–liquid; Gas–liquid; Gas–liquid–solid; Gas–gas–solid; Review.
* Corresponding author. Tel.: +31 53 4892691; fax: +31 53 4892575; e-mail: a.vandenberg@el.utwente.nl

doi:10.1016/j.tet.2005.01.028

reactor channel (Fig. 1), where the phase contacting, diffusion-based dispersion and reaction occur at the longitudinal interface established.[13,14] Using a 250 μm wide, 100 μm deep and 3 cm long microchannel reactor, and a linear flow rate of 1.3 cm s^{-1} (a residence time of 2.3 s), Kitamori et al.[15] reported a specific interfacial contact area of 80 cm^{-1} and a close-to-100% conversion efficiency for the reaction between aqueous 4-nitrobenzene diazonium tetrafluoroborate (10^{-4} M) and ethyl acetate-dissolved 5-methylresorcinol (10^{-3} M), under continuous flow conditions (Scheme 1). An undesirable insoluble precipitate side product (a bisazo product), which is normally produced from the main reaction product in conventional macrosystems, was not observed for the microsystem. This was said to be due to the large specific interfacial area and short molecular diffusion distance in the microsystem, which removed the main product from the aqueous phase to the organic phase, thus preventing the undesirable side reaction. Another configuration involves multiple-pulse (segmented) injections of one liquid into a main flow of the other, where diffusion and reaction occur at the multiple transverse interfaces established (Fig. 2).[16] Mixing in immiscible liquid systems and, in particular, fluid mobilization in electro-osmotic flow-based systems are improved by changing the lipophilic properties of the non-polar species in processes such as the addition of ion pairs (e.g., quaternary ammonium salts), the formation of micelles or, optimally, the formation of oil-in-water micro-emulsions, using appropriate surfactants.[16] This is, however, possible where surfactant and any co-surfactant additives would not interfere with the chemistry to be carried out. Whichever of the above techniques are used, however, the two liquid phases could be driven into a temporary hold-up reservoir equipped with some sort of turbulence-generating mechanism to improve upon the dispersion and mass transfer.

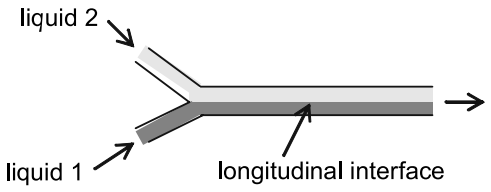
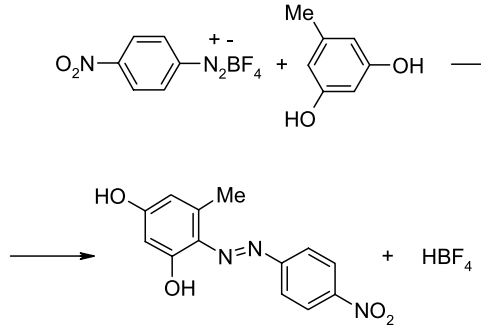


Figure 1. Cocurrent mobilization of two immiscible liquids, and established longitudinal contact interface in a microchannel.



Scheme 1.

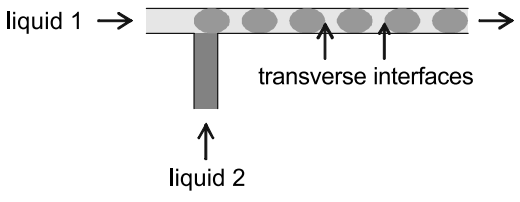


Figure 2. Segmented-pulse injection of one liquid into the main flow of another immiscible liquid, and established transverse contact interfaces in a microchannel.

3. Gas–liquid systems

Gas–liquid microreactor systems require an efficient method of dispersing the gas in the liquid to increase the interfacial contact area whilst maintaining the dispersed regime along the whole microchannel reactor within a desired time frame. These reactors have mainly been hollow microchannels equipped with gas–liquid in-feeding mechanisms. Both the gas and liquid streams could be made to flow-in cocurrently, but, whilst the gas flows continuously, the liquid flow is pulsated, which results in a single-line segmented gas–liquid distribution in the channel (Fig. 3).[17] The gas and liquid streams could be fed at high speeds in a direct counterflow configuration using a 'T' mixer, where the gas and liquid streams collide head-on and generate a single-line, segmented bubble-train into a perpendicular micro-reactor side channel (Fig. 4).[18] In another configuration described by Gañán-Calvo et al.[19–21] for the generation of monosized microbubbles dispersed in a liquid for bio-medical applications, the gas was continuously supplied through a capillary tube to form a large bubble in the vicinity of a small orifice through which a surrounding coflowing liquid stream is forced to produce a steady gas ligament which is focused through the orifix (Fig. 5). After passing through the orifice the gas ligament generated, at a constant frequency, a single-line, same-sized microbubble

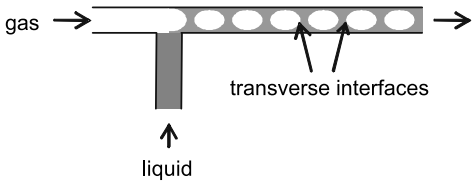


Figure 3. Cocurrent continuous mobilization of gas and pulse injection of liquid stream, leading to single-line gas microbubble train in a microchanel.

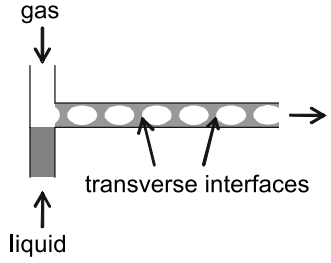


Figure 4. Counterflow mobilization of gas and liquid streams, leading to single-line gas microbubble train in a microchannel.

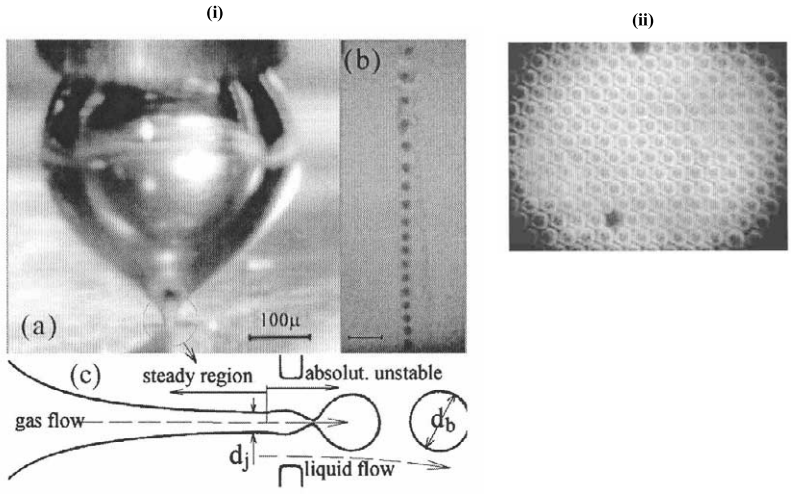


Figure 5. (i) (a) Cusplike bubble, attached to a capillary gas-feeding tube, from the cusp of which a gas ligament issues through the orifice placed in front of the capillary. (b) Stream of gas bubbles issuing from the orifice. (c) Sketch of the region about the exit orifice, showing the steady and absolutely unstable regions of the gas ligament. (ii) A 'mesocrystal foam' or lattice formed when microbubbles rise and settle (reproduced with permission from Ref. 19, pages 274501–1 and 274501–3).

train. By controlling the relative gas and liquid flow rates, the orifice diameter (30–500 μm) and liquid viscosities, the authors reported microbubble sizes ranging between 5 and 120 μm. Recent advances in gas–liquid premixers called (static) micromixers have been reported,[22–26] which have been employed for gas–liquid contacting by coupling them to microchannel reactors of internal diameters below 600 μm. Some of these micromixers are based on the principle of gas–liquid stream interdigitation (multilamination)[22–26] which, although it generates a uniform ordered array of bubbles, normally supplies only a single-line segmented microbubble train per reactor microchannel, due to the bubble size constraints (Fig. 6). Using the sulphite oxidation and carbon dioxide-sodium hydroxide reaction models, specific interfacial areas as large as 9000–50,000 m^2/m^3, with decreasing channel dimensions and

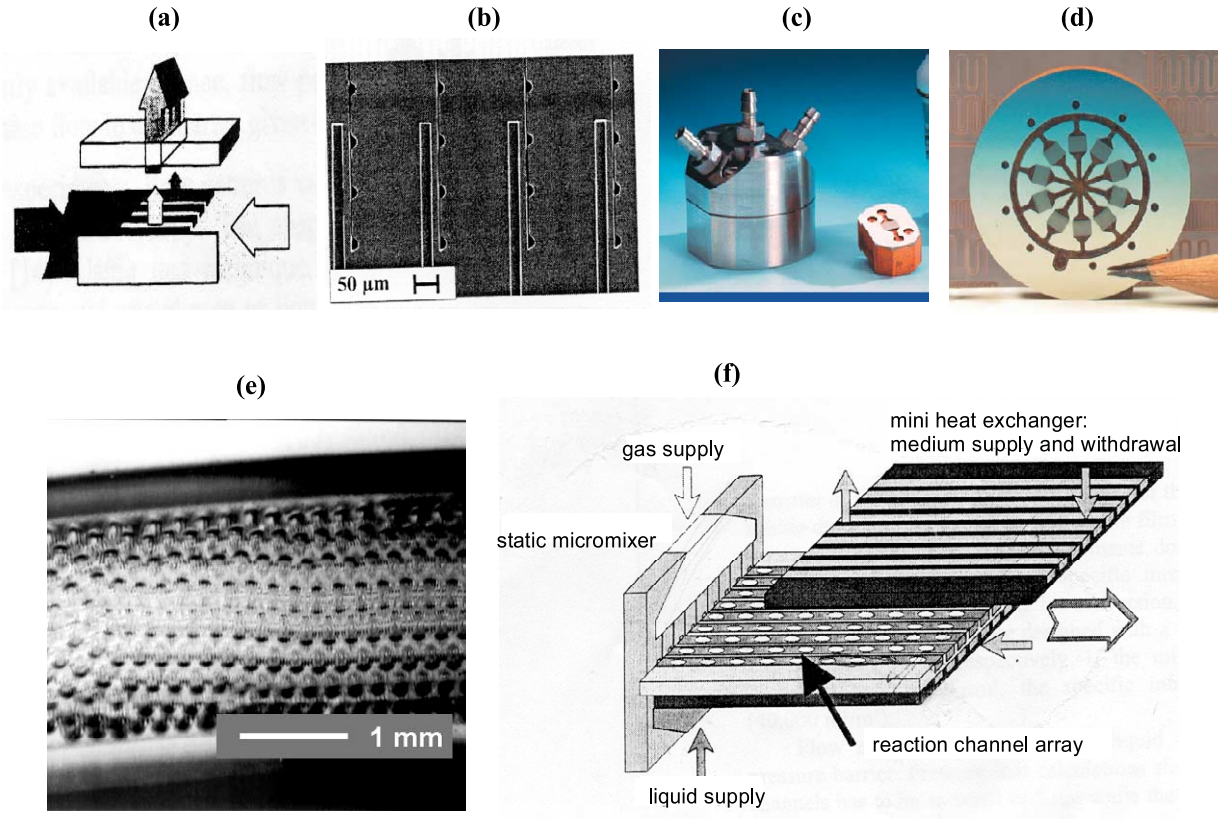
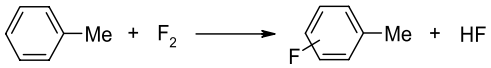


Figure 6. Static micromixer components/configuration: (a) Liga static micromixer, with arrows showing gas and liquid inflows; (b) inside design of the mixer; (c) complete unit of mixer; (d) multiple mixer configuration in one unit device; (e) uniform, ordered array of microbubbles coming out of the micromixer; (f) a micromixer coupled to a multichannel reactor (reproduced with permission from ref 23, page 1077–78, and Ref. 26, page 118).

bubble sizes down to 50 μm, have been reported,[25] and direct fluorination of toluene gave up to 20% yield of monofluorinated products, calculated from the consumption of toluene,[24,26] for the microbubble columns (Scheme 2).



Scheme 2.

Additives such as surfactants and glycerol, which increase the dispersion stability by influencing the surface tension and liquid-phase viscosity, respectively, were found to prevent coalescence of these bubbles. Gas–liquid dispersions in microfluidic structures could, however, also be stabilized by integrating the mixing and reaction channels in one microdevice, thus making chemical additives unnecessary. The characteristics of such two-phase gas–liquid flows, that is, void fractions, pressure drops and flow regimes (patterns) ranging from dispersed bubbly to churn, stratified, slug (elongated bubbles) and annular flow patterns (where channel walls are wetted by a thin liquid film surrounding a gas core), have been extensively studied and reported[27–36] for miniaturized channels of internal diameter up to 1.5 mm, and which depend on the relative in-feed rates of the gas and the liquid. The flow regimes have been found to change from microbubble columns to slugs and then to subsequent annular flow patterns (Fig. 7) as the flow conditions were changed from both high-gas and liquid flows to relatively higher gas volume flow rates, by monitoring gas pressure barriers. The turbulent and laminar behavior of two-phase gas–liquid flows in miniaturized channels have also been studied and reported,[38] which depend on the Reynold's number.[39] Falling film microreactors,[24,25,37] characterized by a thin liquid film trickling down the walls of a vertically oriented reaction channel by gravity and contacted by an upward-moving gas occupying the middle of the channel, have also been reported, that gave specific interfacial areas greater than 15,000 m^2/m^3, and a 25% monofluorinated toluene.[24–26] The bubbles generated by the micromixers, however, have sizes of the order of the channel diameters employed and would not break up into minute bubbles to enhance further dispersion in the channel. A recent development has demonstrated the use of in-channel integrated micropipette tips, prior to the reactor chamber, for online gas introduction and generation of multiple-line bubble trains (microbubble beams) in a microchannel (Fig. 8).[40] The micropipettes are erected perpendicularly to the main liquid flow and the energy of the moving liquid breaks the gas into minute-bubble sprays in the channel. The bubble sizes reported were far smaller, the bubble quantities were larger and yielded gas–liquid specific interfacial contact areas as large as 40×10^4 m^2/m^3 $\pm10\%$ (greater than achieved with single-line bubble columns reported in the literature), corresponding to bubbles as small as 5 μm in diameter. The most important operational conditions for this effective microbubble generation were small pipette internal diameter (0.3–1.0 μm), high liquid speed, reverse pipette-liquid hydrophilicity and liquid hydrophobicity. A multiple-micropipette configuration, rather than one large pipette hole, was reported as the recommended means of increasing the gas quantity requirements. If operated in conjunction with liquid recycling for the purpose of further gas enrichment, such a principle would go a long way to improve upon gas-liquid microreactor performances.

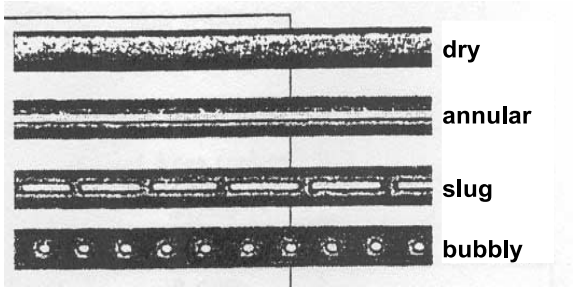


Figure 7. Microbubble columns, slugs and annular flow patterns, obtained as a function of relative gas and liquid volume flow rates (reproduced with permission from Ref. 25, page 178).

4. Gas–liquid–solid systems

In gas–liquid–solid systems, besides the method of dispersing the gas in the liquid and the maintenance of the dispersed regime along the whole reactor within a desired

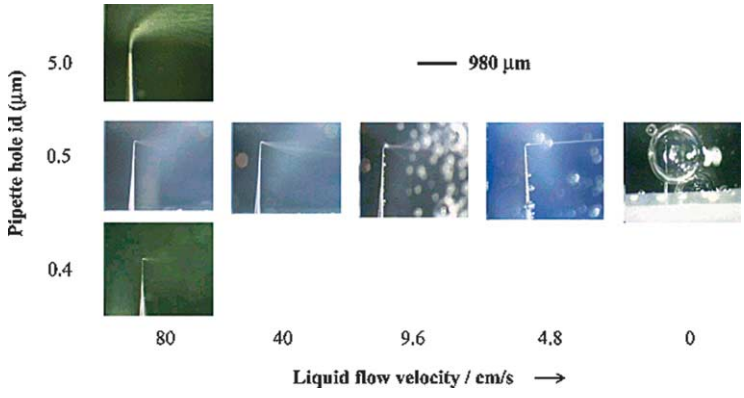
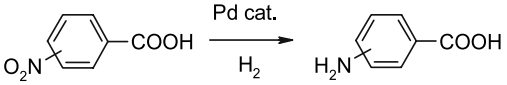


Figure 8. Pictures of a micropipet tip mounted horizontally and 2 mm deep in the channel perpendicular to liquid flow direction, and microbubble stream configurations captured with a low-magnification microscope/low-speed camera system for different pipet hole sizes (0.4, 0.5, and 5.0 μm i.d.) at a liquid velocity of 80 cm/s; and for different liquid velocitiess (40, 9.6, 4.8, and 0 cm/s) for the 0.5 μm i.d. pipet. The channel in each case is 2 mm deep, 4 mm wide, and 6 cm long. (Reproduced with permission from Ref. 40, page 3724).

time frame, the method for incorporating the solid to provide a large total solid surface area and a large net gas–liquid–solid interfacial contact area, while reducing pressure drops across the reactor channels, is also crucial for the reactor success. Only a few gas–liquid–solid microchannel reactor systems have been reported in the literature, with the solid being a catalyst in most cases, and these have relied on the microbubble train, slug and annular flow gas–liquid dispersion principles described above,[17–37] but in conjunction with monolithic,[17,23–26,40–44] packed-bed[22,45,46] or fixed-bed[45,46] solid integration principles. The mass-transfer characteristics in such multi-phase flow channel systems have been well studied and reported.[22,25,43,48–56]

Monolithic microreactors are those with the solid/catalyst immobilized on hollow channel surfaces as thin porous membranes,[17,41,42] by sputtering or surface chemistries and wash-coats, and which normally require higher in-channel pressures to aid gas diffusion through thin liquid films on the channel walls to the solid/catalyst surface. An earlier conventional monolithic device described by Hatziantoniou et al.[17] for the hydrogenation of nitrobenzoic acid consisted of 427 parallel channels (each 1.5 mm wide and ~20 cm long), created in a material made of a mixture of glass, silica, alumina and minor amounts of other oxides reinforced by asbestos. The catalyst was integrated by impregnating the channel surfaces with a $PdCl_2$ solution for 2 h, drying at 450 °C for 1 h under nitrogen and then reducing the Pd salt with hydrogen at 450 °C for 3 h, resulting in a 2.5% Pd catalyst. The gas and liquid streams were fed cocurrently, but, whilst the gas flow was continuous, the liquid was pulsated using a displacement pump, resulting in a single-line segmented bubble train flow in the channel.[17,23–26,43] Yields of over 40% were reported for the hydrogenation of the nitrobenzoic acid[17], depending on the reagent concentration, the gas–liquid feed rates, and the temperature and pressure employed (Scheme 3). The reproducibility was found to be satisfactory, the catalyst deactivation with time was negligible, and mean reaction rates in the range of 0.74–6.16 mol of H_2/s kg of catalyst were reported, depending on the operational conditions mentioned above. The reactor effectiveness was theoretically calculated to be 8.1–11.5%. Studies into smaller microchannel monolithic systems have produced gas–solid specific interfacial contact areas ranging from 9000 to 50,000 m^2/m^3, with decreasing channel dimensions and bubble widths down to 50 μm, and have been reported for the microbubble train/slug configurations,[24–26] with the annular flow configurations giving even higher specific interfacial areas.[24–26] The chemical conversion efficiencies achieved with these microchannel systems have been found to be higher than the efficiencies achieved with many other contacting mechanisms for the traditional trickle- and packed-bed reactors described in the literature.[24,25] Conventional bubble columns, for example, are characterized by specific interfacial areas in the range of only 50–600 m^2/m^3,



Scheme 3.

and even the special-type reactors designated for intensive gas–liquid contacting such as the impinging jets only yield specific areas of 2700 m^2/m^3, which are far smaller compared with the microbubble column technique.[25] As a further step in the development of microsynthesis systems, a mini-industrial system which employs a simple on-chip, single-line monolithic microreactor etched in silicon for the routine heterogeneous and high-pressure hydrogenation of organic substrates, has been developed by us,[44] which is based on in situ-generated microbubble slug or annular dispersion regimes, a Pd/Al_2O_3 catalyst and off-line electrospray-TOF-MS detection. In this system, the liquid is distributed in a main flow channel whilst a side-intersecting channel feeds the gas. The channel width (up to 200 μm) and depth (down to 5 μm), the gas-inlet angle, and the liquid velocity and gas in-flow pressure are parameters optimized for the generation of the microbubble slug and annular flow regimes and the maximization of the gas–solid interfacial contact area (m^2/m^3), whilst the inside-reactor pressure (reaction pressure), the temperature and residence time (allowed reaction time) were optimized to maximize the product yield. The catalyst deactivation, versus time and number of reaction runs, and reproducibility of the yield, were studied.

Most of the reported on-chip gas–liquid–solid microreactors are packed-bed microhydrogenation reactors[22,45,46] (channel widths 500–625 μm; Fig. 9) etched in silicon, and employing metal catalysts supported on granular porous particles (25–75 μm). This is possibly because the packed-bed principle presents the largest possible solid-catalyst surface for reaction, whilst hydrogenation reactions represent one of the typical and ubiquitous industrial processes; nearly 20% of all the reaction steps in a typical

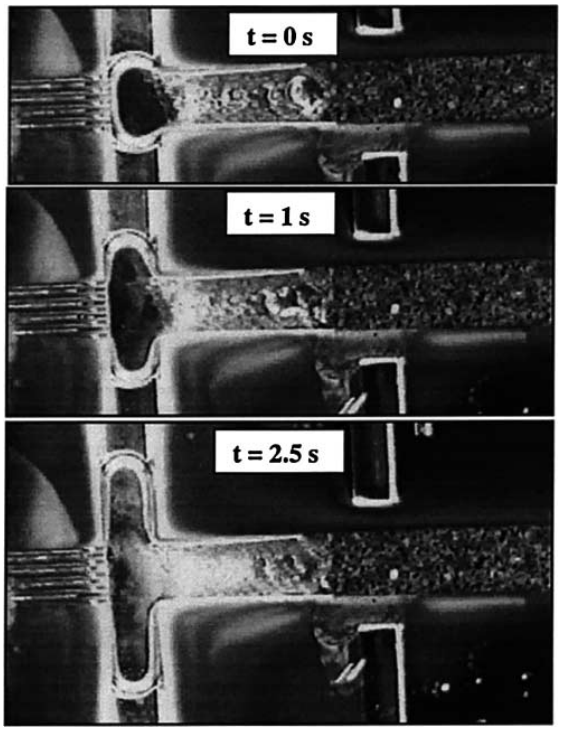
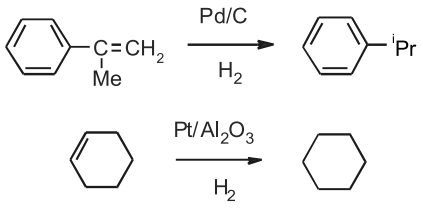


Figure 9. Packed-bed microchannel reactor, with interleaved gas–liquid supply and distribution system (reproduced with permission from Ref. 22, page 2559).

fine chemical synthesis are catalytic hydrogenation,[24] making it a good choice of study with respect to microreactor systems. The metal catalysts supported on granular porous particles are standard catalyst powders which are readily available and information on their chemical kinetics is already well known.[22,45–47] Proper scaling of the reactor and catalyst dimensions is, however, required to maintain an acceptable pressure drop; the catalyst particle sizes employed in the above systems range between 25 and 75 μm. In these systems, the gas and liquid streams were fed continuously and cocurrently by a series of interleaved (multilaminated[22–26]) inlet microchannels (25 μm wide for the gas and 50 μm wide for the liquid), and a series of rectangular posts (40 μm wide with 25 μm inter-post gaps) etched in the silicon at the outlet of the reactor served as filters to retain the catalyst particles, but the particles eventually packed at the end of the reactor and generated high-pressure drops across the channels.

Conversions of higher than 10% were reported for the hydrogenation of α-methylstyrene to cumene over a Pd/C catalyst, and the gas–liquid hydrogenation of cyclohexene over 1 wt% Pt/Al_2O_3 (Scheme 4), depending on the feed composition and flow rates.



Scheme 4.

To circumvent the problem of pressure drop, whilst still achieving the large catalyst surface areas required, a fixed-bed reactor principle that avoids packing variations associated with catalyst size distribution was suggested[45,46] (Fig. 10) for future applications, which permit reactions on the channel walls as well as in the inner volume of the reactor. This involves the erection of microposts, in a staggered configuration, on the channel bed across the whole length and breadth of the reactor channel by deep reactive ion etching into silicon for surface solid-catalyst immobilization. The posts can be made porous and modified to have different organic groups. A suitable gas–liquid feeding and distribution procedure that would be suitable for such an in-channel micropost design has yet to be established; the single-line bubble train gas–liquid distribution may not be suitable for such a micropost, fixed-bed microreactor principle, because the large gas bubble that enters the reactor would not break-up into smaller bubbles to disperse in the system.

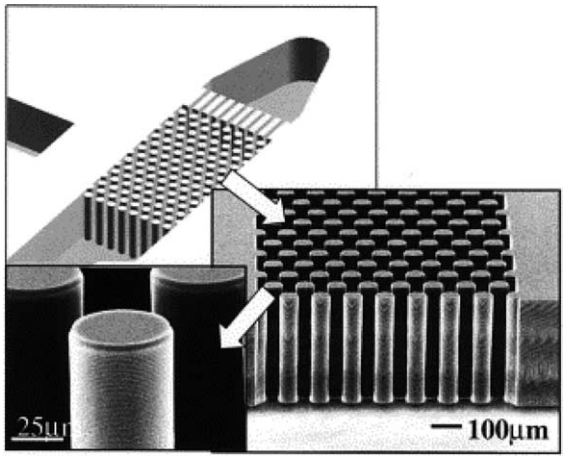


Figure 10. In-channel microposts on microreactor channel bed, produced by deep reactive ion etching into silicon, for surface-catalyst immobilization (reproduced with permission from Ref. 45, page 299).

5. Gas–gas–solid systems

Gas–gas–solid microreactor systems[57–60] are also based on the monolithic, packed-bed or fixed-bed solid integration principles, whilst the gas–gas mixing is almost completely achieved at a simple T junction[57,58] (Fig. 11), due to the inherently large radial diffusion rates of the gases in such small-volume systems. In the work by Kursawe et al.,[57] monolithic microchannel reactors made up of stacks of aluminum wafers were catalytically activated by anodic oxidation, followed by calcination in air, then soaking with toluene after a vacuum treatment and impregnating the porous alumina with $Ru(acac)_3$ dissolved in toluene for several hours at slightly elevated temperatures, and, finally, followed by calcination in air. 15 Wafers containing a total of 450 $Al/Al_2O_3/Ru$ microchannels were assembled by a mechanical packing process and were used for the difficult partial hydrogenation of benzene to cyclohexene in a gas–gas phase reaction, which yielded a 20% selectivity at 13% total conversion degree after 15 h on stream (Scheme 5). Dietzsch et al.,[58] by using a similar system but with Pd–Zn microchannels, coupled with a channel surface regeneration procedure involving oxidation and reduction cycles in order to use one microchannel for repeated catalytic runs to improve the yield, as well as adding traces of carbon monoxide to the reactor feed to improve on the selectivity, obtained a 99.9% conversion and 98% selectivity in the

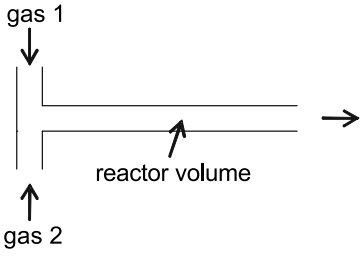
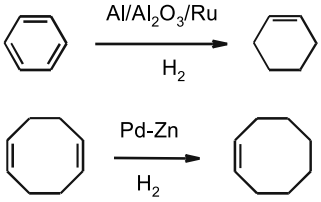


Figure 11. T-mixing of two gases in a gas–gas reactor system.



Scheme 5.

gas-phase hydrogenation of 1,5-cyclooctadiene to cyclooctene, depending on a high hydrogen/cyclooctadiene in-feed ratio (Scheme 5).

6. Conclusions/outlook

Clearly, the most difficult multiphase system to develop is the gas–liquid–solid system, with the solid being a catalyst in most cases. The most advanced solid-catalyst integration principle for such a system is the fixed-bed reactor principle[45,46] that involves the erection of microposts, in a staggered configuration, on the channel bed across the whole length and breadth of the reactor channel by deep reactive ion etching into silicon for surface solid-catalyst immobilization. It would circumvent most of the major problems associated with these microreactor systems, which include the problem of pressure drop, the achievement of large catalyst surface areas, and the maximization of heat transfer in exothermic systems. It would also permit reactions on the channel walls as well as in the inner volume of the reactor and the consequent maximization of the product yield. As pointed out earlier, a gas–liquid feeding and distribution procedure that would be suitable for such an in-channel micropost design has yet to be established and that is the challenge for future developments.

References and notes

1. Doku, G. N.; Haswell, S. J. *Anal. Chim. Acta* **1999**, *382*, 1.
2. Greenway, G. M.; Haswell, S. J.; Petsul, P. H. *Anal. Chim. Acta* **1999**, *387*, 1.
3. Reyes, D. R.; Lossifidis, D.; Auroux, P. A.; Manz, A. *Anal. Chem.* **2002**, *74*, 2623.
4. Auroux, P. A.; Lossifidis, D.; Reyes, D. R.; Manz, A. *Anal. Chem.* **2002**, *74*, 2637.
5. Ugi, I.; Almstetter, M.; Gruber, B.; Heilingbrunner, M. In *Proc. IMRET 1*; Ehrfeld, W., Ed.; Springer: Berlin, 1997; p 190.
6. Zech, T.; Honicke, D. In *Proc. IMRET 4*; Erhfeld, W., Ed.; AlChE: Atlanta, 2000; p 379.
7. Ehrfeld, W. In *Proc. IMRET 3: Industrial Prospects*; Ehrfeld, W., Ed.; Springer: Frankfurt, 1999; p 364.
8. Haswell, S. J.; Skelton, V. *Trends Anal. Chem.* **2000**, *19*, 389.
9. Greenway, G. M.; Haswell, S. J.; Morgan, D. O.; Skelton, V.; Styring, P. *Sens. Actuators B* **2000**, *63*, 153.
10. Brivio, M.; Fokkens, R. H.; Verboom, W.; Reinhoudt, D. N.; Tas, N. R.; Goedbloed, M.; van den Berg, A. *Anal. Chem.* **2002**, *74*, 3972.
11. Fletcher, P. D. I.; Haswell, S. J.; Pombo-Villar, E.; Warrington, B. H.; Watts, P.; Wong, S. Y. F.; Zhang, X. *Tetrahedron* **2002**, *58*, 4735.
12. Jähnisch, K.; Hessel, V.; Löwe, H.; Baerns, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 406.
13. Burns, J. R.; Ramshaw, C. *Trans. Inst. Chem. Eng.* **1999**, *77*, 206.
14. Anraku, R.; Hattori, A.; Asai, T.; Fukuzawa, T.; Tokeshi, M.; Kitamori, T. In Northrup, M. A., Jensen, K. F., Harrison, D. J., Eds.; Proc. μTAS 2003; Squaw Valley: California, 2003; 2003.
15. Hisamoto, H.; Saito, T.; Tokeshi, M.; Hibara, A.; Kitamori, T. *Chem. Commun.* **2001**, 2662.
16. Doku, G. N.; Haswell, S. J.; McCreedy, T.; Greenway, G. M. *Analyst* **2001**, *126*, 14.
17. Hatziantoniou, V.; Andersson, B. *Ind. Eng. Chem. Fundam.* **1984**, *23*, 82.
18. Thulasidas, T. C.; Abraham, M. A.; Cerro, R. L. *Chem. Eng. Sci.* **1995**, *50*, 183.
19. Gañán-Calvo, A. M.; Gordillo, J. M. *Phys. Rev. Lett.* **2001**, *87*, 274501–274504.
20. Gañán-Calvo, A. M. *Phys. Rev. Lett.* **1998**, *80*, 285.
21. Loscertales, G. I.; Barrero, A.; Guerrero, I.; Cortijo, R.; Marquez, M.; Gañán-Calvo, A. M. *Science* **2002**, *295*, 1695.
22. Losey, M. W.; Schmidt, M. A.; Jensen, K. F. *Ind. Eng. Chem. Res.* **2001**, *40*, 2555.
23. Ehrfeld, W.; Golbig, K.; Hessel, V.; Lowe, H.; Richter, T. *Ind. Eng. Chem. Res.* **1999**, *38*, 1075.
24. Hessel, V.; Ehrfeld, W.; Golbig, K.; Haverkamp, V.; Lowe, H.; Storz, M.; Wille, Ch.; Guber, A.; Jahnisch, K.; Baerns, M. In *Industrial Prospects*; Ehrfeld, W., Ed.; Springer: Frankfurt, 1999; p 526.
25. Hessel, V.; Ehrfeld, W.; Herweck, Th.; Haverkamp, V.; Lowe, H.; Schiewe, J.; Wille, Ch.; Kern, Th.; Lutz, N. In *Proc. IMRET 4*; Erhfeld, W., Ed.; AlChE: Atlanta, 2000; p 174.
26. Jahnisch, K.; Baerns, M.; Hessel, V.; Erhfeld, W.; Haverkamp, V.; Lowe, H.; Wille, Ch.; Guber, A. *J. Fluorine Chem.* **2000**, *105*, 117.
27. Triplett, K. A.; Ghiaasiaan, S. M.; Abdel-Khalik, S. I.; Sadowski, D. L. *Int. J. Multiphase Flow* **1999**, *25*, 377.
28. Triplett, K. A.; Ghiaasiaan, S. M.; Abdel-Khalik, S. I.; Lemouel, A.; McCord, B. N. *Int. J. Multiphase Flow* **1999**, *25*, 395.
29. Xu, J. L.; Cheng, P.; Zhao, T. S. *Int. J. Multiphase Flow* **1999**, *25*, 411.
30. Chen, W. L.; Twu, M. C.; Pan, C. *Int. J. Multiphase Flow* **2002**, *28*, 1235.
31. Hetsroni, G.; Mosyak, A.; Segal, Z.; Pogrebnyak, E. *Int. J. Multiphase Flow* **2003**, *29*, 341.
32. Akbar, M. K.; Plummer, D. A.; Ghiaasiaan, S. M. *Int. J. Multiphase Flow* **2003**, *29*, 855.
33. Chen, I. Y.; Yang, K. S.; Chang, Y. J.; Wang, C. C. *Int. J. Multiphase Flow* **2001**, *27*, 1293.
34. Koyama, S.; Lee, J.; Yonemoto, R. *Int. J. Multiphase Flow* **2004**, *30*, 291.
35. Kawahara, A.; Chung, P. M. Y.; Kawaji, M. *Int. J. Multiphase Flow* **2002**, *28*, 1411.
36. Al-Sarkhi, A.; Hanratty, T. J. *Int. J. Multiphase Flow* **2002**, *28*, 1617.
37. Mouza, A. A.; Paras, S. V.; Karabelas, A. J. *Int. J. Multiphase Flow* **2002**, *28*, 1311.
38. Yarin, L. P.; Ekilchik, L. A.; Hetsroni, G. *Int. J. Multiphase Flow* **2002**, *28*, 1589.
39. Fox, R. W.; McDonald, A. T. *Introduction to Fluid Mechanics*, 3rd ed.; Wiley: New York, 1985; p 304.
40. Doku, G. N.; Verboom, W.; Reinhoudt, D. N.; van den Berg, A. *Ind. Eng. Chem. Res.* **2003**, *42*, 3721.
41. Dittmeyer, R.; Hollein, V.; Daub, K. I. *J. Mol. Catal.* **2001**, *173*, 135.
42. AlDahhan, M. H.; Larachi, F.; Dudukovic, M. P.; Laurent, A. *Ind. Eng. Chem. Res.* **1997**, *36*, 3292.
43. Hatziantoniou, V.; Andersson, B. *Ind. Eng. Chem. Fundam.* **1982**, *21*, 451.

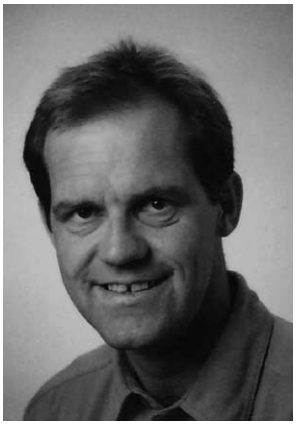
44. Doku, G. N.; Verboom, W.; Reinhoudt, D. N; van den Berg, A. Manuscript in preparation.
45. Jensen, K. F. *Chem. Eng. Sci.* **2001**, *56*, 293.
46. Losey, M. W.; Jackman, R. J.; Firebaugh, S. L.; Schmidt, M. A.; Jensen, K. F. J. *Microelectromech. Syst.* **2002**, *11*, 709.
47. Mills, P. L.; Chaudhari, R. V. *Catal. Today* **1997**, *3*, 367.
48. Brilman, D. W. F.; van Swaaij, W. P. M.; Versteeg, G. F. *Chem. Eng. Process.* **1998**, *37*, 471.
49. Thulasidas, T. C.; Abraham, M. A.; Cerro, R. L. *Chem. Eng. Sci.* **1999**, *54*, 61.
50. van Bhat, R. D.; van Swaaij, W. P. M.; Kuipers, J. A. M.; Versteeg, G. F. *Chem. Eng. Sci.* **1999**, *54*, 121.
51. van Bhat, R. D.; van Swaaij, W. P. M.; Kuipers, J. A. M.; Versteeg, G. F. *Chem. Eng. Sci.* **1999**, *54*, 137.
52. Iliuta, I.; Larachi, F.; Grandjean, B. P. A.; Wild, G. *Chem. Eng. Sci.* **1999**, *54*, 5633.
53. Brilman, D. W. F.; Goldschmidt, M. J. V.; Versteeg, G. F.; van Swaaij, W. P. M. *Chem. Eng. Sci.* **2000**, *55*, 2793.
54. Maalej, S.; Benadda, B.; Otterbein, M. *Chem. Eng. Technol.* **2001**, *24*, 1.
55. Krishna, R.; Ellenberger *J. Chem. Eng. Technol.* **2002**, *25*, 2.
56. van Male, P.; de Croon, M. H. J. M.; Tiggelaar, R. M.; van den Berg, A.; Schouten, J. C. *Int. J. Heat Mass Transfer* **2004**, *47*, 87.
57. Kursawe, A.; Dietzsch, E.; Kah, S.; Honicke, D.; Fichtner, M.; Schubert, K.; Wiebmeier, G. In *Proc. IMRET 3: Industrial Prospects*; Ehrfeld, W., Ed.; Springer: Frankfurt, 1999; p 213.
58. Dietzsch, E.; Honicke, D.; Fichtner, M.; Schubert, K.; Wiebmeier, G. In *Proc. IMRET 4*; Erhfeld, W., Ed.; AlChE: Atlanta, GA, 2000; p 89.
59. Jensen, K. F.; Hsing, I. M.; Srinivasan, R.; Schmidt, M. A.; Harold, M. P.; Lerou, J. J.; Ryley, J. F. In *Proc. IMRET 1*; Ehrfeld, W., Ed.; Springer: Berlin, 1997; p 3.
60. Bokenkamp, D.; Desai, A.; Yang, X.; Tai, Y. C.; Marzluff, M. E.; Mayo, S. L. *Anal. Chem.* **1998**, *70*, 232.

Biographical sketch

George N. Doku (1964) received his masters degree in analytical chemistry from the University of Cape Coast (Ghana). In the period 1992–1995, he was a lecturer at this university and a chemical consultant. He received his PhD under the supervision of Prof. Dr. S. J. Haswell with a thesis entitled 'analytical and synthetic reactions in micro-reactor systems' at the University of Hull (UK) in 1999. After working as a post-doc in Hull, he joined the group of Prof. Dr. Ir. A. van den Berg in the Mesa$^+$ Institute for Nanotechnology at the University of Twente (2001–2004). His present main interest is the design and application of micro reactor systems. He is the author of four basic chemistry books and quite a number of (popular) scientific publications.

Willem Verboom was born in 1954 in Goes, The Netherlands. He studied chemistry at Utrecht University, where he also received his PhD with Prof. Dr. H.J.T. Bos with a thesis entitled 'thermal and photochemical rearrangements of γ-oxo-α,β-unsaturated carboxamides and esters'. Subsequently, he joined the group of Prof. Dr. Ir. D.N. Reinhoudt at the University of Twente, where he now is an associate professor in organic chemistry. Over the years, the research interests moved form heterocyclic chemistry to supramolecular chemistry. His present research topics involve the functionalization and application of suitable molecular building blocks, in particular calixarenes and cavitands, for the development of specific receptors and larger (non-)covalent assemblies and recently, chemistry in microchips. He is the (co-)author of about 275 scientific publications. He served as a board member of the International Society of Heterocyclic Chemistry in the period 1992–1995. He was a member of the editorial board of the Dutch journal 'Recl. Trav. Chim. Pays-Bas' (1992–1996). Already for a long time he is a member of the refereeing panel of different journals of the Royal Chemical Society (UK). Currently, he is secretary of the 'Design and Synthesis' study group of the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO) (since 2001)

David N. Reinhoudt was born in 1942 in The Netherlands. He studied Chemical Technology at the Delft University of Technology and graduated (suma cum laude) in chemistry in 1969 with Professor H.C. Beijerman. In the period 1970–1975 he worked at Shell where he started the crown ether research program. In 1975, he was appointed as a part-time professor (extraordinarius) at the University of Twente followed by the appointment as a full professor in 1978. The major part of his research deals with supramolecular chemistry and technology. Nanotechnology, molecular recognition, and non-covalent combinatorial synthesis are the major fields. Application of supramolecular chemistry, for example, in 'lab-on-a-chip', in the field of electronic or optical sensor systems, catalysis, and molecular materials. Professor Reinhoudt is the scientific director of the MESA$^+$ Research Institute. Since 2002, he is the chairman of the Board of NanoNed, the Dutch Network for Nanotechnology. He is a member of the Royal Dutch Academy of Sciences, Fellow of the American Association for the Advancement of Science, and Fellow of the Institute of Physics. He is the author of more than 800 scientific publications, patents, review articles, and books. He has been honored with the Izatt-Christensen award (1995), the Simon Stevin Mastership (1998) and Knight of the Order of the Dutch Lion (2002).



Albert van den Berg received his Masters degree in applied physics from the University of Twente, The Netherlands in 1983. In 1988, he finished his thesis at the same university on the topic of chemically modified ISFETs. From 1988 to 1990, he was at the Swiss Center for Microelectronics and Microtechnology (CSEM) in Neuchâtel, Switzerland, project manager in the chemical sensor department. From 1990 to 1993, he did research on miniaturized chemical sensors and sensor systems at the IMT, University of Neuchâtel, Switzerland. From 1993 until 1999, he was research coordinator Micro Total Analysis Systems (μTAS) at MESA, University of Twente, later extended to Miniaturized Chemical Systems (MiCS). In 1998, he was appointed as full professor in the Lab-on-a-Chip group. Albert van den Berg is and has been member of numerous scientific committees (μTAS, MEMS, Micromachine Summit, Transducers, Nanotech) and served as editor of the section μTAS of Sensors and Actuators B. In 2002 he received the 'Simon Stevin Master' award from the Dutch Technical Science foundation. He has published over 100 peer reviewed papers and edited several books on Labs-on-a-Chip. His current research interests focus on analysis systems and sensors, microreactors, micro- and nanofluidics and Lab-in-a-Cell.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2743–2750

Stereoselective syntheses of tri- and tetrapeptide analogues by dynamic resolution of α-halo amides in nucleophilic substitution

Ji-yeon Chang, Eun-kyoung Shin, Hyun Jung Kim, Yangmee Kim and Yong Sun Park*

Department of Chemistry, Konkuk University, Kwangjingu Hwayangdong 1, Seoul 143-701, South Korea

Received 30 December 2004; revised 25 January 2005; accepted 25 January 2005

Abstract—Dynamic resolution of α-bromo and α-chloro acetamides in nucleophilic substitution with amine nucleophiles in the presence of TBAI has been investigated for stereoselective syntheses of tri- and tetrapeptide analogues. Mechanistic investigations suggest that primary pathway of the asymmetric induction is a dynamic kinetic resolution and real intermediate for the substitution is α-iodo acetamides. Also, application of this mild and simple methodology to stereoselective preparations of *N*-carboxyalkyl, *N*-aminoalkyl and *N*-hydroxyalkyl peptide analogues is demonstrated.

1. Introduction

Stereospecific nucleophilic substitution (S_N2) reaction of optically pure α-halo or α-sulfonyloxy acetamides with an amine nucleophile is widely used for stereoselective preparation of peptidomimetics.[1] However, most of optically pure α-halo or α-sulfonyloxy acetamides are made from natural amino acids and, consequently, this synthetic method seems to lack generality. Since various α-halo amides can be easily obtained in racemic form and configurational lability of them is readily induced, dynamic resolution of *N*-(α-haloacetyl) peptides in the substitution reaction would be more attractive strategy for asymmetric syntheses of peptide analogues. Recently, it has been shown from our group that the chiral information of adjacent amino acid residue is efficiently transferred to the new C–N bond formation at α-bromo carbon center for asymmetric syntheses of dipeptide analogues.[2] Herein we describe our recent progress to extend the scope of the methodology to tri- and tetrapeptide analogues via dynamic resolution of α-chloro or α-bromo acetamides with various amine nucleophiles. Application of this methodology to highly stereoselective N-terminal functionalization of peptides is also presented.

2. Results and discussion

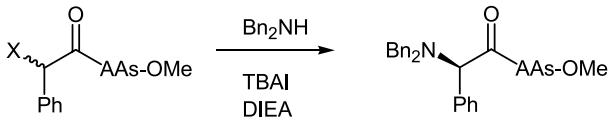
We have previously reported that the treatment of *N*-(α-bromo-α-phenylacetyl)-*L*-leucine methyl ester **1** with dibenzylamine (1.2 equiv, Bn_2NH), tetrabutylammonium iodide (1.0 equiv, TBAI) and diisopropylethylamine (1.0 equiv, DIEA) in CH_2Cl_2 at rt provided the dipeptide analogues **14** in 83% yield with 93:7 diastereomeric ratio (dr, α*R*:α*S*) as shown in Table 1, entry 1.[2a] The chiral information of *L*-leucine is transferred to the substitution at α-bromo carbon center via dynamic kinetic resolution in the nucleophilic substitution with dibenzylamine. In order to assess the effect of the additional C-terminal amino acid residues on stereoselectivity, a series of *N*-(α-bromo-α-phenylacetyl)-*L*-leucine derivatives was initially examined as shown in Table 1. No stereoselectivity was observed in the reaction of glycine-*L*-leucine derivative **2**, where the C-terminal *L*-leucine was positioned apart from the reaction center (entry 2). In all cases of *N*-(α-bromoacetyl) *L*-leucine derivatives **3**–**5** having an additional C-terminal amino acid residue in **1**, tripeptide analogues **16**, **17** and **18** were obtained with lower selectivities (87:13–80:20 drs) compared to the reaction of **1** as shown in Table 1, entries 3–5. It is also noteworthy that chirality of the additional amino acid residue affects the stereoselectivity as replacing *L*-Ala in **4** with *D*-Ala gives rise to loss of asymmetric induction (entries 4 and 5).

In the reaction of *N*-(α-bromo-α-phenylacetyl)-*L*-proline derivative **7** having an additional C-terminal glycine in **6**, the stereoselectivity was lower compared to the reaction of **6** as shown in entries 6 and 7. Under the same reaction

Keywords: Dynamic kinetic resolution; Peptidimimetics; Asymmetric syntheses; Nucleophilic substitution.
* Corresponding author. Tel.: +82 2 450 3377; fax: +82 2 3436 5382; e-mail: parkyong@konkuk.ac.kr

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.091

Table 1. Effect of additional C-terminal amino acid residues



Entry	X	AA (S.M.)[a]	% Yield[b,c] (product)	dr[d] (αR:αS)
1	Br	*L*-Leu (**1**)	83 (**14**)	93:7
2	Br	Gly-*L*-Leu (**2**)	69 (**15**)	50:50
3	Br	*L*-Leu-Gly (**3**)	69 (**16**)	81:19
4	Br	*L*-Leu-*L*-Ala (**4**)	64 (**17**)	87:13
5	Br	*L*-Leu-*D*-Ala (**5**)	75 (**18**)	80:20
6	Br	*L*-Pro (**6**)	93 (**19**)	>99:1
7	Br	*L*-Pro-Gly (**7**)	91 (**20**)	95:5
8	Cl	*L*-Pro-Gly (**8**)	84 (**20**)	95:5
9	Br	*L*-Pro-*L*-Leu (**9**)	85 (**21**)	>99:1
10	Cl	*L*-Pro-*L*-Leu (**10**)	82 (**21**)	>99:1
11	Cl	*L*-Pro-*D*-Leu (**11**)	77 (**22**)	96:4
12	Cl	*L*-Pro-*L*-Leu-*L*-Ala (**12**)	71 (**23**)	81:19
13	Cl	*L*-Pro-*L*-Leu-*D*-Ala (**13**)	68 (**24**)	76:24

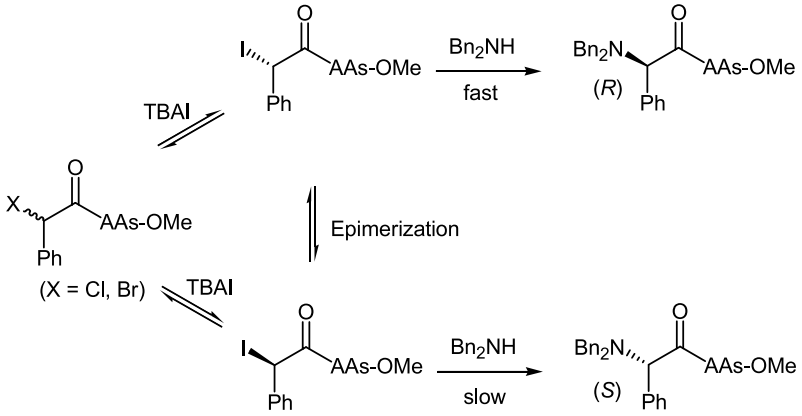
[a] Initial drs of **1**–**13** are approximately 50:50.
[b] All reactions were carried out in CH_2Cl_2 for 24 h at rt.
[c] Isolated yields.
[d] The drs are determined by ^{1}H NMR of reaction mixture using the authentic products prepared from racemic phenylglycine as a standard.

condition α-chloro acetamide **8** produced **20** in 84% yield with same selectivity as in the reaction of α-bromo acetamide **7** (entry 8). This is somewhat surprising considering the low reactivity of α-chloro acetamide and hence inefficient epimerization for dynamic resolution. This interesting fact was further supported by the reactions of α-bromo acetamide **9** and α-chloro acetamide **10**, where both reactions gave tripeptide analogue **21** with >99:1 dr in high yields (entries 9 and 10). While the additional C-terminal *L*-leucine in **6** did not affect the stereoselectivity, the selectivity was reduced with the additional C-terminal *D*-leucine (entry 11). Moreover, nucleophilic substitutions of both *N*-(α-chloroacetyl) tripeptides **12** and **13** were found to be less stereoselective compared to those of **10** and **11** (entries 12 and 13). It is also interesting to note here that chirality of third amino acid residue affects the stereoselectivity as replacing *L*-Ala in **12** with *D*-Ala gives rise to loss of asymmetric induction. The absolute configurations of major isomers of **14**–**24** were assigned as α*R* by comparison to the ^{1}H NMR of authentic epimers individually prepared from the coupling of *L*-leucine and *L*-proline derivatives and *N*,*N*-dibenzyl (*S*)- or (*R*)-phenylglycine.

To understand the reaction pathway and the origin of stereoselectivity, we carried out a series of reactions as shown in Table 2, focusing on epimerization process and transition state energy difference which may have a profound role in dictating the stereoselectivity of the substitution.[3] Two *N*-(α-bromoacetyl) dipeptides **3** and **5** were initially chosen for detailed study of their moderate stereoselectivities. When the mixture of two epimers of **3** (78:22 dr) was allowed to reach thermodynamic equilibrium in the presence of TBAI and DIEA, the epimeric ratio of recovered **3** was determined to be 52:48 (entry 1). The result indicates that α-bromo amide **3** is configurationally labile under the reaction condition and the thermodynamic stabilities of two epimers are almost same. Reaction of same population of two epimers with a deficient amount of nucleophile will give rise to a dr that reflects the intrinsic difference in activation energies between substitution reactions of each epimer.[4] In the reaction of **3** (49:51 dr) with 0.2 equiv of dibenzylamine in the absence of TBAI, the product **16** with 81:19 dr was obtained in 13% yield (entry 2). At rt, this dr of 81:19 corresponds to a difference in free energies of activation of about 0.9 kcal/mol. When **3** of 15:85 dr was treated with 1.2 equiv of dibenzylamine in the presence of both TBAI and DIEA, the reaction gave the product **16** with 81:19 dr as shown in entry 3. The dr is identical to the dr of both reactions of **3** in entry 3 (Table 1) and entry 2 (Table 2). This could be taken to suggest that the stereoselectivity depend mainly on the difference in the epimeric transition states energies and the primary pathway of the asymmetric induction is a dynamic kinetic resolution. However, with reversed diastereomeric enrichment of **3** (85:15 dr) slightly diminished dr of product **16** (73:27) was observed in the reaction of **3** (entry 4). The results indicate that the epimerization of **3** is not fast enough to get to complete thermodynamic equilibrium with respect to the substitution. As shown in entries 5–8, a series of reactions of *L*-leucine-*D*-alanine derivative **5** showed same tendency of stereochemical outcome as the reactions of *L*-leucine-glycine derivative **3**.

As expected, the reaction of α-chloro acetamide **8** does not produce the substitution product in the absence of TBAI (entry 9). In the presence of TBAB, the reactions of α-chloro acetamide **8** and α-bromo acetamide **10** gave the products **20** and **21**, respectively, with same stereoselectivities observed in the reaction with TBAI (Table 1, entries 8 and 10). However, the rate of the substitution is substantially decreased compared to the reactions with TBAI and require longer reaction time (60 h) to obtain moderate yields shown in entries 10 and 11. In addition, as shown in Table 2, entries 12 and 13, the reactions of α-bromo acetamides **3** and **4** in the presence of TBAB gave **16** and **18** with much lower stereoselectivities (55:45 and 64:36 dr) compared to the reactions with TBAI (Table 1, entries 3 and 4). The lower selectivity of the reaction of **3** than the selectivity estimated by the reaction with 0.2 equiv

Table 2. Dynamic kinetic resolution of α-bromo and α-chloro acetamides



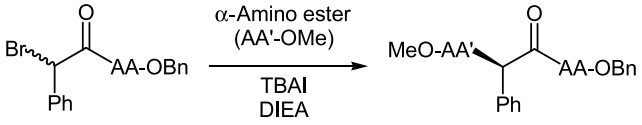
Entry	X	AA	S.M. (dr)[a]	Condition[b]	% Yield[c] (product)	dr[d] (α*R*:α*S*)
1	Br	*L*-Leu-Gly	**3** (78:22)	TBAI, DIEA	67 (**3**)	52:48
2	Br	*L*-Leu-Gly	**3** (49:51)	Bn_2NH (0.2 equiv)	13 (**16**)	81:19
3	Br	*L*-Leu-Gly	**3** (15:85)	TBAI, DIEA, Bn_2NH	74 (**16**)	81:19
4	Br	*L*-Leu-Gly	**3** (85:15)	TBAI, DIEA, Bn_2NH	83 (**16**)	73:27
5	Br	*L*-Leu-*D*-Ala	**5** (94:4)	TBAI, DIEA	85 (**18**)	53:47
6	Br	*L*-Leu-*D*-Ala	**5** (47:53)	Bn_2NH (0.2 equiv)	11 (**18**)	84:16
7	Br	*L*-Leu-*D*-Ala	**5** (19:81)	TBAI, DIEA, Bn_2NH	59 (**18**)	84:16
8	Br	*L*-Leu-*D*-Ala	**5** (83:17)	TBAI, DIEA, Bn_2NH	85 (**18**)	80:20
9	Cl	*L*-Pro-Gly	**8** (50:50)	DIEA, Bn_2NH	N.R.	—
10	Cl	*L*-Pro-Gly	**8** (50:50)	TBAB, DIEA, Bn_2NH	77 (**20**)	95:5
11	Br	*L*-Pro-*L*-Leu	**10** (48:52)	TBAB, DIEA, Bn_2NH	70 (**21**)	>99:1
12	Br	*L*-Leu-Gly	**3** (49:51)	TBAB, DIEA, Bn_2NH	48 (**16**)	55:45
13	Br	*L*-Leu-*L*-Ala	**4** (44:56)	TBAB, DIEA, Bn_2NH	51 (**18**)	64:36

[a] dr of S.M. before the reaction.
[b] All reactions were carried out in CH_2Cl_2 at rt.
[c] Isolated yields.
[d] The drs are determined by ^1H NMR of reaction mixture using the authentic products prepared from racemic phenylglycine as a standard.

of nucleophile (Table 2, entry 2) may be explained by less efficient epimerization process in the presence of TBAB. Although it is not exactly clear how iodide exerts its rate-accelerating effects on the substitution, these results in entries 9–13 can be taken to suggest that real intermediate for the substitution in the presence of TBAI is α-iodo acetamide rather than α-bromo and α-chloro acetamides.

Within the frame of a broad project aimed at asymmetric syntheses of various N-terminal functionalized peptide analogues, the scope of the methodology was examined with α-amino ester nucleophiles for the preparation of *N*-carboxyalkyl peptide analogues as shown in Table 3. Several *N*-carboxyalkyl peptides are known inhibitors of various metalloproteinases and angiotensin converting enzymes (ACE).[5] Treatment of *N*-(α-bromo-α-phenylacetyl)-*L*-leucine benzyl ester with glycine methyl ester hydrochloride (1.0 equiv), TBAI (1.0 equiv) and DIEA (2.2 equiv) in CH_2Cl_2 at rt provided the tripeptide analogue **25** in 99% yield with 86:14 dr (α*R*:α*S*) as shown in Table 3,

Table 3. Stereoselective syntheses of *N*-carboxyalkyl peptide derivatives

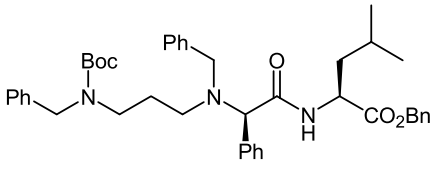


Entry[a,b]	AA′-OMe	AA-OBn	Product	Yield[c] (%)	dr[d] (α*R*:α*S*)
1	Gly-OMe	*L*-Leu-OBn	**25**	99	86:14
2	*L*-Ala-OMe	*L*-Leu-OBn	**26**	84	88:12
3	*D*-Ala-OMe	*L*-Leu-OBn	**27**	83	91:9
4	*L*-Leu-OMe	*L*-Leu-OBn	**28**	80	89:11
5	*D*-Leu-OMe	*L*-Leu-OBn	**29**	63	93:7
6	*L*-Phe-OMe	*L*-Leu-OBn	**30**	36	87:13
7	*D*-Phe-OMe	*L*-Leu-OBn	**31**	49	92:8
8	*L*-Leu-OMe	*L*-Pro-OBn	**32**	44	93:7

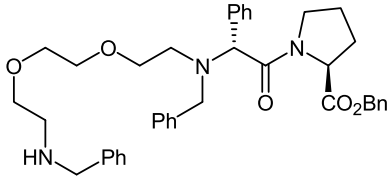
[a] Initial drs of S.M. are approximately 50:50.
[b] All reactions were carried out in CH_2Cl_2 for 24–48 h at rt.
[c] Isolated yields.
[d] The drs are determined by ^1H NMR of reaction mixture and HPLC analysis.

entry 1.[6] When two enantiomers of alanine methyl esters were used as nucleophiles, moderate double stereodifferentiation is observed as shown in entries 2 and 3. *N*-(α-Bromo-α-phenylacetyl)-*L*-leucine benzyl ester experienced matching with *D*-alanine methyl esters to afford tripeptide analogue **27** in a ratio of 91:9 (α*R*:α*S*) and mismatching with *L*-leucine amino ester to provide **26** in a 88:12 (α*R*:α*S*) ratio.[6,7] Furthermore, we found that this tendency of stereodifferentiation was also observed in both reactions of leucine and phenylalanine methyl ester nucleophiles (entries 4–7). As shown in Table 3, even mismatched cases (entries 2, 4 and 6) gave still high stereoselectivities, which allows us to have easy access to diverse *N*-carboxyalkyl peptide analogues. The substitution of *N*-(α-bromo-α-phenylacetyl)-*L*-proline benzyl ester with *L*-leucine methyl ester gave the tripeptide analogue **32** in 44% yield with 93:7 dr as shown in entry 8.

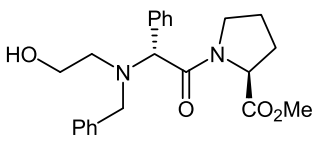
Finally, we were very pleased to demonstrate that this methodology is also efficient for the substitution with *N*-aminoxyalkyl and *N*-hydroxyalkyl amines, affording *N*-(aminoalkyl) peptides **33**–**34** and *N*-(hydroxyalkyl) peptide **35** with high selectivities and good yields as shown in Scheme 1. For example, when *N*-Boc *N*,*N*′-dibenzyl 1,3-propanediamine was used as a nucleophile for the reaction with *N*-(α-bromo-α-phenylacetyl)-*L*-leucine benzyl ester, the product **33** was formed in 63% yield with 96:4 dr. Also, treatment of *N*-(α-bromo-α-phenylacetyl)-*L*-proline benzyl ester with *N*,*N*′-dibenzyl 2,2′-(ethylenedioxy)diethyleneamine produced the mono substituted product **34** in 70% yield with 90:10 dr. Furthermore, we attempted the substitution reaction of *L*-proline methyl ester derivative with 2-(benzylamino)ethanol and found that the amino substituted product **35** was produced in 69% yield with 93:7 dr. For stereoselective preparation of various *N*,*N*-dialkyl substituted arylglycine peptide analogues, this methodology has potential advantages over *N*-alkylation of optically active arylglycine analogues in simplicity and cost. The products **33**–**35** should serve as versatile intermediates not only for the sequential asymmetric construction of non-natural oligopeptides but also for the asymmetric preparation of a variety of chiral ligands.



33 (63% yield, 96:4 dr)



34 (70% yield, 90:10 dr)



35 (69% yield, 93:7 dr)

Scheme 1.

3. Conclusion

We have shown that dynamic kinetic resolution of α-bromo and α-chloro amides in nucleophilic substitution reaction with dibenzylamine can be successfully applied towards the preparation of tri- and tetrapeptide analogues. Mechanistic investigations suggest that α-iodo acetamides is real intermediate for the nucleophilic substitutions of both α-bromo and α-chloro amides in the presence of TBAI. The methodology has also been successful for the N-terminal functionalization of peptides, affording a generalized and practical method for the asymmetric syntheses of *N*-carboxyalkyl, *N*-aminoalkyl and *N*-hydroxyalkyl peptide analogues. This mild and practical chemistry requires no special precautions and can be run on a multigram scale. The methodology of the present work should also be applicable to stereoselective syntheses of a number of related peptide analogues, now being used for the asymmetric syntheses of chiral ligands and macrocycles.

4. Experimental

4.1. General procedure for the preparation of 1–13

For α-bromo acetamides. Dipeptide or tripeptide alkyl ester (1.0 equiv), racemic α-bromo phenylacetic acid (1.0 equiv), DCC (1.0 equiv), Et_3N (2.2 equiv) and DMAP (0.2 equiv) were dissolved in CH_2Cl_2 and stirred at rt for 3 h. The precipitate was filtered off and the organic phase was washed with water. The organic phase was dried over $MgSO_4$, filtered and concentrated to provide the crude product that was purified by column chromatography on silica gel.

For α-chloro acetamides. Dipeptide or tripeptide alkyl ester (1.0 equiv), racemic α-chloro α-phenylacetyl chloride (1.0 equiv) and Et_3N (2.2 equiv) were dissolved in CH_2Cl_2 and stirred at rt for 2 h. The mixture was treated with extractive work up and the organic phase was dried over $MgSO_4$. Filtration and concentration provided the crude product that was purified by column chromatography on silica gel.

4.1.1. *N*-(α-Bromo-α-phenylacetyl)-glycine-(*S*)-leucine, methyl ester (2). A colorless oil was obtained in 55% yield. ^1H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.61–7.27 (m, 6H), 6.91–6.89 (m, 1H), 5.45, 5.39 (s, 1H), 4.60–4.55 (m, 1H), 4.05–4.00 (m, 2H), 3.71 (s, 3H), 1.62–1.47 (m, 3H), 0.90–0.89 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 168.7, 168.5, 168.4, 137.3, 129.4, 128.9, 128.8, 128.4, 128.3, 52.8, 51.3, 50.6, 44.1, 41.5, 25.2, 23.2, 22.2. HRMS calcd for $C_{17}H_{24}BrN_2O_4$ ($M^+ + 1$): 399.0919. Found: 399.0954.

4.1.2. *N*-(α-Bromo-α-phenylacetyl)-(*S*)-leucine-glycine, methyl ester (3). A colorless oil was obtained in 49% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.50–7.27 (m, 7H), 5.53, 5.50 (s, 1H), 4.75–4.73 (m, 1H), 3.98–3.94 (m, 1H), 3.77–3.71 (m, 1H), 3.67, 3.65 (m, 3H), 1.71–1.57 (m, 3H) 0.94–0.82 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 172.9, 170.3, 168.4, 137.3, 129.4, 129.2, 129.1, 128.9, 128.3, 52.6, 50.6, 49.8, 41.7, 41.5, 25.1, 23.2, 22.6. HRMS calcd for $C_{17}H_{24}BrN_2O_4$ ($M^+ + 1$): 399.0919. Found: 399.0883.

4.1.3. *N*-(α-Bromo-α-phenylacetyl)-(*S*)-leucine-(*S*)-alanine, methyl ester (4). A pale yellow oil was obtained in 66% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.48–7.27 (m, 6H), 7.02–6.81 (m, 1H), 5.44, 5.43 (s, 1H), 4.54–4.46 (m, 2H), 3.73, 3.72 (s, 3H), 1.68–1.62 (m, 3H), 1.34–1.24 (m, 3H), 0.92–0.86 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.4, 171.5, 168.2, 137.3, 129.5, 129.3, 128.8, 128.2, 128.1, 52.8, 52.5, 50.9, 48.5, 41.3, 25.3, 23.2, 22.5, 18.3. HRMS calcd for $C_{18}H_{26}BrN_2O_4$ ($M^+ + 1$): 413.1076. Found: 413.1084.

4.1.4. *N*-(α-Bromo-α-phenylacetyl)-(*S*)-leucine-(*R*)-alanine, methyl ester (5). A colorless oil was obtained in 45% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.48–7.27 (m, 6H), 7.02–6.81 (m, 1H), 5.44, 5.43 (s, 1H), 4.54–4.46 (m, 2H), 3.73, 3.72 (s, 3H), 1.68–1.62 (m, 3H), 1.34–1.24 (m, 3H), 0.92–0.86 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.4, 171.5, 168.2, 137.3, 129.5, 129.3, 128.8, 128.2, 128.1, 52.8, 52.5, 50.9, 48.5, 41.3, 25.3, 23.2, 22.5, 18.3. HRMS calcd for $C_{18}H_{26}BrN_2O_4$ ($M^+ + 1$): 413.1076. Found: 413.1071.

4.1.5. *N*-(α-Bromo-α-phenylacetyl)-(*S*)-proline-glycine, methyl ester (7). A colorless oil was obtained in 27% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.52–7.25 (m, 6H), 5.62, 5.61 (s, 1H), 4.69–4.59 (m, 1H), 4.10–3.94 (m, 2H), 3.76–3.68 (m, 4H), 3.57–3.33 (m, 1H), 2.30–1.86 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) 171.4, 170.4, 167.6, 135.9, 129.7, 129.6, 129.4, 128.7, 128.6, 61.0, 59.5, 52.7, 47.8, 41.7, 28.1, 25.4. HRMS calcd for $C_{16}H_{20}BrN_2O_4$ ($M^+ + 1$): 383.0606. Found: 383.0587.

4.1.6. *N*-(α-Chloro-α-phenylacetyl)-(*S*)-proline-glycine, methyl ester (8). A colorless oil was obtained in 55% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.50–7.25 (m, 6H), 5.63, 5.62 (s, 1H), 4.68–4.59 (m, 1H), 4.04–3.93 (m, 2H), 3.75–3.32 (m, 5H), 2.28–2.26 (m, 1H), 2.03–1.85 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 171.5, 170.4, 167.6, 135.8, 129.6, 129.4, 129.3, 128.7, 128.6, 61.0, 59.5, 52.6, 47.8, 41.5, 28.2, 25.4. HRMS calcd for $C_{16}H_{20}ClN_2O_4$ ($M^+ + 1$): 339.1122. Found: 339.1120

4.1.7. *N*-(α-Bromo-α-phenylacetyl)-(*S*)-proline-(*S*)-leucine, methyl ester (9). A colorless oil was obtained in 43% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.52–7.23 (m, 6H), 5.58, 5.56 (s, 1H), 4.70–4.62 (m, 1H), 4.52–4.43 (m, 1H), 3.72–3.40 (m, 5H), 2.37–2.34 (m, 1H), 2.04–1.46 (m, 6H), 0.97–0.79 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 170.7, 167.7, 135.7, 129.7, 129.6, 129.3, 128.7, 128.6, 61.9, 59.2, 52.6, 51.4, 47.8, 41.5, 27.4, 25.5, 25.2, 23.3, 22.2. HRMS calcd for $C_{20}H_{28}BrN_2O_4$ ($M^+ + 1$): 439.1232. Found: 439.1248

4.1.8. *N*-(α-Chloro-α-phenylacetyl)-(*S*)-proline-(*S*)-leucine, methyl ester (10). A colorless oil was obtained in 68% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.52–7.29 (m, 6H), 5.60, 5.57 (s, 1H), 4.71–4.61 (m, 1H), 4.55–4.40 (m, 1H), 3.72–3.37 (m, 5H), 2.35–2.32 (m, 1H), 2.03–1.48 (m, 6H), 0.97–0.80 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 170.7, 167.7, 136.0, 135.7, 129.6, 129.4, 129.3, 128.7, 128.5, 60.9, 58.9, 52.6, 51.4, 47.8, 41.4, 27.4, 25.5, 25.2, 23.3, 22.2. HRMS calcd for $C_{20}H_{28}BrN_2O_4$ ($M^+ + 1$): 395.1738. Found: 395.1737.

4.1.9. *N*-(α-Chloro-α-phenylacetyl)-(*S*)-proline-(*R*)-leucine, methyl ester (11). A colorless oil was obtained in 25% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.55–7.08 (m, 6H), 5.63, 5.59 (s, 1H), 4.75–4.64 (m, 2H), 3.76–3.61 (m, 3H), 3.58–3.41 (m, 2H), 2.36, 2.35 (m, 1H), 2.17–1.96 (m, 3H), 1.67–1.58 (m, 3H), 0.96–0.90 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.2, 170.6, 167.9, 135.5, 129.6, 129.5, 129.1, 128.8, 128.6, 61.0, 59.0, 52.6, 51.3, 47.8, 41.6, 27.5, 25.4, 25.2, 23.2, 22.2. HRMS calcd for $C_{20}H_{28}ClN_2O_4$ ($M^+ + 1$): 395.1738. Found: 395.1766.

4.1.10. *N*-(α-Chloro-α-phenylacetyl)-(*S*)-proline-(*S*)-leucine-(*S*)-alanine, methyl ester (12). A colorless oil was obtained in 49% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.49–7.04 (m, 7H), 5.60, 5.59 (s, 1H), 4.55–4.32 (m, 3H), 3.72–3.58 (m, 4H), 3.57–3.26 (m, 1H), 2.18–1.71 (m, 4H), 1.69–1.34 (m, 6H), 0.94–0.78 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 172.4, 171.6, 167.6, 135.7, 129.7, 129.4, 129.3, 129.1, 128.6, 61.3, 59.3, 52.7, 52.4, 48.5, 47.9, 40.8, 28.4, 25.5, 25.4, 23.3, 22.1, 18.2. HRMS calcd for $C_{23}H_{33}ClN_3O_5$ ($M^+ + 1$): 466.2109. Found: 466.2101.

4.1.11. *N*-(α-Chloro-α-phenylacetyl)-(*S*)-proline-(*S*)-leucine-(*R*)-alanine, methyl ester (13). A colorless oil was obtained in 17% yield. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.55–6.99 (m, 7H), 5.61 (s, 1H), 4.54–4.47 (m, 3H), 3.73–3.67 (m, 4H), 3.66–3.31 (m, 1H), 2.28–1.38 (m, 10H), 0.94–0.78 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.6, 171.9, 171.5, 168.0, 135.5, 129.8, 129.7, 129.6, 129.2, 128.7, 61.7, 59.3, 52.7, 52.1, 48.6, 48.0, 40.4, 28.7, 25.5, 25.3, 23.5, 21.9, 17.9. HRMS calcd for $C_{23}H_{33}ClN_3O_5$ ($M^+ + 1$): 466.2109. Found: 466.2080.

4.2. General procedure for asymmetric preparation of peptide analogues 14–35

To a solution of (α*RS*)-α-bromo or α-chloro acetamides in dry CH_2Cl_2 (ca. 0.1 M) at rt was added amine nucleophile (1.2 equiv), TBAI or TBAB (1.0 equiv) and DIEA (1.0 or 2.2 equiv). The resulting reaction mixture was stirred at rt for 24 h. The solvent in mixture was evaporated and the crude product was purified by column chromatography on silica gel.

4.2.1. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-glycine-(*S*)-leucine, methyl ester (15). A colorless oil was obtained in 69% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 8.07–8.06 (m, 1H), 7.40–7.21 (m, 15H), 7.03–7.01 (d, J=8.2 Hz, 1H) 4.64–4.52 (m, 1H), 4.44 (s, 1H), 4.13–4.08 (m, 2H), 3.84 (m, 2H), 3.67 (m, 3H), 3.27 (d, J=13.7 Hz, 2H), 1.53–1.49 (m, 2H), 1.26–1.22 (m, 1H),

0.87–0.80 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 172.8, 169.1, 138.8, 134.3, 130.7, 129.4, 129.3, 129.0, 128.7, 128.6, 128.4, 127.8, 68.4, 55.1, 52.6, 51.2, 43.7, 41.4, 25.4, 23.2, 22.2. HRMS calcd for $C_{31}H_{38}N_3O_4$ ($M^+ + 1$): 516.2862. Found: 516.2841.

4.2.2. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-leucine-glycine, methyl ester (16). A colorless oil was obtained in 69% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.80 (d, J=6.4 Hz, 1H), 7.41–7.24 (m, 15H), 6.86 (m, 1H), 4.56 (m, 1H), 4.44 (s, 1H), 3.94–3.84 (m, 4H), 3.69 (s, 3H), 3.23 (d, J=14.0 Hz, 2H), 1.78–1.59 (m, 3H), 0.95–0.88 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 172.6, 172.5, 170.4, 138.8, 133.8, 131.0, 129.4, 129.1, 129.0, 128.7, 128.6, 128.4, 127.8, 67.7, 54.9, 52.6, 51.8, 41.6, 41.4, 25.2, 23.5, 22.4. HRMS calcd for $C_{31}H_{38}N_3O_4$ ($M^+ + 1$): 516.2862. Found: 516.2887.

4.2.3. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-leucine-(*S*)-alanine, methyl ester (17). A colorless oil was obtained in 64% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.87 (d, J=7.5 Hz, 1H), 7.37–7.25 (m, 15H), 6.78 (d, J=7.3 Hz, 1H), 4.57–4.51 (m, 2H), 4.42 (s, 1H), 4.11 (d, J=13.7 Hz, 2H), 3.71 (s, 3H), 3.27 (d, J=13.7 Hz, 2H), 1.73–1.56 (m, 3H), 1.28–1.23 (m, 3H), 0.93–0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 172.3, 171.9, 138.7, 134.1, 130.8, 129.3, 129.0, 128.6, 68.0, 54.9, 52.8, 51.9, 48.4, 41.6, 25.2, 23.4, 22.3, 18.4. HRMS calcd for $C_{32}H_{40}N_3O_4$ ($M^+ + 1$): 530.3019. Found: 530.3043.

4.2.4. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-leucine-(*R*)-alanine, methyl ester (18). A colorless oil was obtained in 72% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.77 (d, J=8.2 Hz, 1H), 7.41–7.26 (m, 15H), 6.78 (d, J=7.4 Hz, 1H), 4.56–4.48 (m, 2H), 4.44 (s, 1H), 3.89–3.84 (m, 2H), 3.63 (s, 3H), 3.22 (d, J=13.6 Hz, 2H), 1.76–1.53 (m, 3H), 1.37–1.35 (m, 3H), 0.95–0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.5, 172.6, 171.9, 138.7, 133.9, 131.0, 130.6, 129.4, 129.1, 129.0, 128.9, 128.7, 128.4, 127.8, 67.8, 54.8, 52.7, 51.7, 48.5, 25.2, 23.5, 22.3, 18.5. HRMS calcd for $C_{32}H_{40}N_3O_4$ ($M^+ + 1$): 530.3019. Found: 530.3011.

4.2.5. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-proline-glycine, methyl ester (20). A colorless oil was obtained in 91% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.77–7.71 (m, 1H), 7.39–7.19 (m, 16H), 4.77–4.74 (m, 1H), 4.64 (s, 1H), 4.18–4.09 (m, 2H), 3.95–3.86 (m, 4H), 3.76 (s, 3H), 2.96 (m, 1H), 2.75 (m, 1H), 2.35 (m, 1H), 2.03–1.66 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.7, 172.2, 170.5, 140.8, 136.8, 130.4, 129.7, 129.5, 129.2, 129.1, 129.0, 128.8, 128.7, 128.4, 128.3, 127.6, 127.3, 64.5, 60.1, 55.1, 52.7, 47.3, 41.8, 27.8, 25.2. HRMS calcd for $C_{30}H_{34}N_3O_4$ ($M^+ + 1$): 500.2549. Found: 500.2538.

4.2.6. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-proline-(*S*)-leucine, methyl ester (21). A colorless oil was obtained in 85% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) 7.54 (d, J=7.6 Hz, 1H), 7.36–7.20 (m, 16H), 4.71–4.63 (m, 3H), 3.86 (s, 4H), 3.76 (s, 3H), 2.96, 2.95 (m, 1H), 2.76 (m, 1H), 2.34 (m, 1H), 1.97–1.90 (m, 2H), 1.79–1.67 (m, 4H), 1.02–0.99 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.7, 173.5, 171.7, 140.7, 136.7, 130.0, 129.6, 129.4, 129.2, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 127.6, 127.4, 64.4, 60.3, 54.9, 52.7, 51.6, 47.4, 42.0, 27.7, 25.3, 25.2, 23.4, 22.4. HRMS calcd for $C_{34}H_{42}N_3O_4$ ($M^+ + 1$): 556.3175. Found: 556.3178.

4.2.7. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-proline-(*R*)-leucine, methyl ester (22). A colorless oil was obtained in 77% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.94 (d, J=7.8 Hz, 1H), 7.41–7.22 (m, 15H), 4.80 (d, J=6.6 Hz, 1H), 4.66 (m, 2H), 3.93–3.91 (m, 4H), 3.72 (s, 3H), 2.96 (m, 1H), 2.74 (m, 1H), 2.44 (m, 1H), 1.89–1.64 (m, 6H), 1.03–1.00 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.2, 173.5, 171.4, 140.8, 136.7, 129.4, 129.2, 129.0, 128.7, 128.5, 127.3, 64.5, 60.1, 54.9, 52.6, 51.5, 47.3, 42.0, 27.1, 25.3, 25.1, 23.4, 22.4. HRMS calcd for $C_{34}H_{42}N_3O_4$ ($M^+ + 1$): 556.3175. Found: 556.3162.

4.2.8. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-proline-(*S*)-leucine-(*S*)-alanine, methyl ester (23). A colorless oil was obtained in 71% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.41–7.22 (m, 16H), 6.96 (d, J=7.1 Hz, 1H), 4.68–4.58 (m, 4H), 3.90–3.82 (m, 4H), 3.74 (s, 3H), 2.98–2.70 (m, 2H), 2.25 (m, 1H), 1.91–1.75 (m, 6H), 1.47–1.42 (m, 3H), 1.03–0.91 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.6, 173.5, 172.0, 171.9, 140.6, 136.6, 129.7, 129.5, 129.3, 129.2, 129.1, 128.9, 128.7, 128.5, 127.4, 64.2, 60.4, 55.0, 54.9, 52.8, 52.5, 48.6, 47.4, 41.6, 28.3, 25.2, 23.6, 23.3, 18.6. HRMS calcd for $C_{37}H_{47}N_4O_5$ ($M^+ + 1$): 627.3546. Found: 627.3546.

4.2.9. *N*-[(*R*)-α-Phenyl-*N*,*N*-(dibenzyl)glycinyl]-(*S*)-proline-(*S*)-leucine-(*R*)-alanine, methyl ester (24). A colorless oil was obtained in 68% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.45–7.18 (m, 17H), 4.67–4.58 (m, 4H), 3.94–3.78 (m, 4H), 3.72–3.64 (m, 3H), 2.98–2.73 (m, 2H), 2.22 (m, 1H), 1.91–1.48 (m, 9H), 1.02–0.86 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.6, 173.4, 172.1, 172.0, 140.6, 136.5, 129.9, 129.8, 129.5, 129.3, 129.1, 128.9, 128.8, 128.7, 128.5, 127.7, 127.4, 64.6, 60.8, 54.9, 52.7, 52.3, 48.6, 47.5, 41.1, 28.7, 25.4, 23.6, 23.4, 22.2, 18.6. HRMS calcd for $C_{37}H_{47}N_4O_5$ ($M^+ + 1$): 627.3546. Found: 627.3536.

4.2.10. *N*-[1-(Methoxycarbonyl)methyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (25). A colorless oil was obtained in 99% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.49 (d, J=8.6 Hz, 1H), 7.39–7.28 (m, 10H), 5.16–5.08 (m, 2H), 4.69 (m, 1H), 4.26 (s, 1H), 3.71 (s, 3H), 3.46 (m, 2H), 2.30 (br, 1H), 1.70–1.57 (m, 3H), 0.92–0.82 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.1, 172.8, 171.8, 138.8, 135.8, 129.3, 129.2, 128.9, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.7, 67.6, 52.4, 51.0, 50.8, 41.8, 25.4, 25.3, 22.2. HRMS calcd for $C_{24}H_{31}N_2O_5$ ($M^+ + 1$): 427.2233. Found: 427.2207.

4.2.11. *N*-[1-(*S*)-(Methoxycarbonyl)ethyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (26). A colorless oil was obtained in 84% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.36–7.23 (m, 10H), 5.18–5.08 (m, 2H), 4.67 (m, 1H), 4.31 (s, 1H), 3.69 (s, 3H), 3.26 (m, 1H), 2.25 (br, 1H), 1.66–1.53 (m, 3H), 1.30 (d, J=6.8 Hz, 3H), 0.90–0.85 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.3,

173.1, 172.2, 138.9, 135.8, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.3, 127.5, 77.5, 67.5, 54.6, 52.4, 51.1, 42.0, 25.3, 23.2, 22.2 19.1. HRMS calcd for $C_5H_{33}N_2O_5$ ($M^+ + 1$): 441.2389. Found: 441.2379.

4.2.12. *N*-[1-(*R*)-(Methoxycarbonyl)ethyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (27). A colorless oil was obtained in 83% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.79 (d, $J=8.6$ Hz, 1H), 7.41–7.27 (m, 10H), 5.18 (m, 2H), 4.69 (m, 1H), 4.16 (s, 1H) 3.71 (s, 3H), 3.34 (m, 1H) 2.25 (br, 1H), 1.74–1.61 (m, 3H), 1.33 (d,, $J=$ 7.3 Hz, 3H), 0.96 (d, $J=6.4$ Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.8, 172.9, 172.1, 139.2, 135.8, 129.3, 129.2, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 129.3, 128.0, 67.5, 66.7, 56.4, 52.4, 51.0, 41.8, 25.6, 23.3, 22.2, 19.9. HRMS calcd for $C_{25}H_{33}N_2O_5$ ($M^+ + 1$): 441.2389. Found: 441.2420.

4.2.13. *N*-[1-(*S*)-(Methoxycarbonyl)-3-methylbutyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (28). A colorless oil was obtained in 80% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.36–6.99 (m, 10H), 6.99 (d, $J=8.4$ Hz, 1H), 5.17 (m, 2H), 4.69 (m, 1H), 4.26 (m, 1H), 3.69 (S, 3H), 3.13 (br, 1H), 2.25 (br, 1H), 1.73–1.47 (m, 6H), 0.93–0.74 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.7, 172.9, 171.8, 138.7, 135.8, 129.3, 129.2, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 127., 67.4, 66.7, 57.6, 52.2, 51.1, 42.8, 41.9, 25.4, 25.3, 25.1, 25.0, 23.2, 22.2. HRMS calcd for $C_{28}H_{39}N_2O_5$ ($M^+ + 1$): 483.2859. Found: 483.2842.

4.2.14. *N*-[1-(*R*)-(Methoxycarbonyl)-3-methylbutyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (29). A colorless oil was obtained in 63% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.81 (d, $J=8.8$ Hz, 1H), 7.38–7.25 (m, 10H), 5.13 (m, 2H), 4.72 (m, 1H), 4.11 (s, 1H), 3.71 (s, 3H), 3.34 (m, 1H), 2.09 (br, 1H), 1.89–1.47 (m, 6H), 0.97–0.86 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) 176.0, 172.9, 172.1, 129.5, 135.8, 129.2, 129.0, 128.9, 128.8, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 67.5, 66.7, 59.8, 52.3, 50.9, 43.8, 42.2, 25.4, 25.3, 23.2, 23.1, 22.9, 22.2. HRMS calcd for $C_{28}H_{39}N_2O_5$ ($M^+ + 1$): 483.2859. Found: 483.2867.

4.2.15. *N*-[1-(*S*)-(Methoxycarbonyl)-2-phenylethyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (30). A colorless oil was obtained in 36% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.35–7.09 (m, 16H), 5.16–5.07 (m, 2H), 4.60 (m, 1H), 4.24 (s, 1H), 3.65 (s, 3H), 3.40–3.39 (m, 1H), 3.05–3.00 (m, 1H), 2.90–2.79 (m, 1H), 2.27 (br, 1H), 1.66–1.46 (m, 3H), 0.90–0.74 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.4, 172.8, 171.8, 138.1, 137.2, 135.8, 130.2, 129.6, 129.2, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 127.6, 127.3, 67.4, 65.9, 60.5, 52.3, 51.1, 41.7, 39.8, 25.3, 23.3, 22.2. HRMS calcd for $C_{31}H_{37}N_2O_5$ ($M^+ + 1$): 517.2702. Found: 517.2688.

4.2.16. *N*-[1-(*R*)-(Methoxycarbonyl)-2-phenylethyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (31). A colorless oil was obtained in 49% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.37–7.25 (m, 16H), 5.10 (q, $J=$ 12.3 Hz, 2H), 4.47–4.44 (m, 1H), 4.09 (s, 1H), 3.73 (s, 3H), 3.41–3.39 (m, 1H), 3.03–2.68 (m, 2H), 2.17 (d, $J=$ 12.6, 1H), 1.39–1.36 (m, 2H), 1.01–0.84 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.0, 172.5, 171.7, 139.4, 138.0, 136.0, 130.0, 129.8, 129.3, 129.2, 129.0, 128.9, 128.8, 128.7, 128.1, 127.9, 127.4, 67.3, 66.5, 63.0, 52.6, 50.5, 41.3, 40.6, 25.3, 23.3, 22.0. HRMS calcd for $C_{31}H_{37}N_2O_5$ ($M^+ + 1$): 517.2702. Found: 517.2683.

4.2.17. *N*-[1-(*S*)-(Methoxycarbonyl)-3-methylbutyl]-(*R*)-phenylglycine-(*S*)-proline, benzyl ester (32). A colorless oil was obtained in 44% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.37–7.24 (m, 10H), 7.15 (m, 1H), 5.19 (m, 2H), 4.50 (m, 2H), 3.69 (s, 3H), 3.55 (m, 1H), 3.08 (m, 2H), 2.85 (br, 1H), 1.99–1.43 (m, 7H), 0.83 (d, $J=6.6$ Hz, 3H), 0.65 (d, $J=6.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.9, 172.3, 171.6, 138.2, 136.2, 129.5, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 67.1, 63.5, 59.9, 56.6, 52.1, 47.2, 427, 32.0, 29.4, 29.3, 25.2, 22.1, 22.0. HRMS calcd for $C_{27}H_{35}N_2O_5$ ($M^+ + 1$): 467.2546. Found: 467.2523.

4.2.18. *N*-Benzyl-*N*-(*N*-Boc-*N*-benzyl-3-aminopropyl)-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (33). A colorless oil was obtained in 63% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.46–7.19 (m, 20H), 5.17 (m, 2H), 4.70 (m, 1H), 4.34 (m, 3H), 3.81 (m, 1H), 3.30–3.08 (m, 3H), 2.57 (m, 1H), 2.16 (m, 1H), 1.7–1.26 (m, 14H), 0.97–0.88 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.2, 171.9, 139.1, 135.9, 134.3, 131.6, 130.7, 129.2, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 80.1, 69.0, 67.4, 59.4, 51.0, 48.5, 45.1, 41.9, 28.9, 26.2, 25.4, 23.3, 22.2. HRMS calcd for $C_{43}H_{54}N_3O_5$ ($M^+ + 1$): 692.4063. Found: 692.4047.

4.2.19. *N*-Benzyl-*N*-[2-[2-(*N*-benzyl-2-aminoethoxy)-ethoxy]ethyl]-(*R*)-phenylglycine-(*S*)-leucine, benzyl ester (34). A colorless oil was obtained in 70% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.38–7.23 (m, 20H), 5.29 (m, 2H), 4.72 (m, 1H), 4.65 (m, 1H), 3.89–3.68 (m, 3H), 3.44–3.30 (m, 9H), 3.10–2.68 (m, 5H), 1.94–1.69 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) 172.6, 172.0, 141.1, 140.0, 137.4, 136.3, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.2, 128.1, 127.3, 127.2, 127.0, 71.8, 70.7, 70.2, 67.9, 66.0, 59.3, 56.7, 53.0, 52.2, 51.3, 50.0, 46.9, 29.5, 25.3. HRMS calcd for $C_{40}H_{48}N_3O_5$ ($M^+ + 1$): 650.3594. Found: 650.3582.

4.2.20. *N*-Benzyl-*N*-(2-hydroxyethyl)-(*R*)-phenylglycine-(*S*)-proline, methyl ester (35). A colorless oil was obtained in 69% yield (two diastereomers). ^{1}H NMR ($CDCl_3$, 400 MHz) 7.43–7.24 (m, 10H), 4.68 (s, 1H), 4.55 (m, 1H), 3.88 (m, 2H), 3.81 (s, 3H), 3.60 (m, 1H), 3.37 (m, 1H), 2.96 (m, 3H), 2.15–1.67 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.4, 171.8, 140.2, 13.6, 130.4, 130.0, 129.7, 129.5, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 65., 59.8, 59.3, 55.7, 52.8, 52.6, 46.8, 29.4, 25.2. HRMS calcd for $C_{23}H_{29}N_2O_4$ ($M^+ +$ 1): 397.2127. Found: 397.2125.

Acknowledgements

This paper was supported by a grant from Molecular & Cellular BioDiscovery Research Program (M1-0311-13-0003) from the Ministry of Science and Technology, Korea.

References and notes

1. (a) Yuan, Z.; Blomberg, D.; Sethson, I.; Brickmann, K.; Ekholm, K.; Johansson, B.; Nilsson, A.; Kihlberg, J. *J. Med. Chem.* **2002**, *45*, 2512. (b) Fujisawa, T.; Igeta, K.; Odake, S.; Morita, Y.; Yasuda, J.; Morikawa, T. *Bioorg. Med. Chem.* **2002**, *10*, 2569. (c) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431. (d) Haskell-Luevano, C.; Rosenquist, A.; Souers, A.; Khong, K. C.; Ellman, J. A.; Cone, R. D. *J. Med. Chem.* **1999**, *42*, 4380.
2. (a) Nam, J.; Chang, J.-Y.; Shin, E.-k.; Kim, H. J.; Kim, Y.; Jang, S.; Park, Y. S. *Tetrahedron* **2004**, *60*, 6311. (b) Nam, J.; Chang, J.-Y.; Hahm, K.-S.; Park, Y. S. *Tetrahedron Lett.* **2003**, *44*, 7727.
3. (a) Valenrod, Y.; Myung, J.; Ben, R. N. *Tetrahedron Lett.* **2004**, *45*, 2545. (b) Nam, J.; Lee, S.-k.; Park, Y. S. *Tetrahedron* **2003**, *59*, 2397. (c) Nam, J.; Lee, S.-k.; Kim, K. Y.; Park, Y. S. *Tetrahedron Lett.* **2002**, *43*, 8253. (d) Lee, S.-k.; Nam, J.; Park, Y. S. *Synlett* **2002**, 790. (e) Caddick, S.; Afonso, C. A. M.; Candeias, S. X.; Hitchcock, P. B.; Jenkins, K.; Murtagh, L.; Pardoe, D.; Santos, A. G.; Treweeke, N. R.; Weaving, R. *Tetrahedron* **2001**, *57*, 6589. (f) Lee, S.-k.; Lee, S. Y.; Park, Y. S. *Synlett* **2001**, 1941. (g) Ben, R. N.; Durst, T. *J. Org. Chem.* **1999**, *64*, 7700.
4. Seeman, J. I. *Chem. Rev.* **1983**, *83*, 83.
5. (a) Huffman, M. A.; Reider, P. J. *Tetrahedron Lett.* **1999**, *40*, 831. (b) Chapman, K. T.; Kopka, I. E.; Durette, P. L.; Esser, C. K.; Lanza, T. J.; Izquierdo-Martin, M.; Niedzwiecki, L.; Chang, B.; Harrison, R. K.; Kuo, D. W.; Lin, T.-Y.; Stein, R. L.; Hagmann, W. K. *J. Med. Chem.* **1993**, *36*, 4293–4301.
6. The absolute configurations of **25**, **26** and **27** were assigned as αR by comparison to the ^{1}H NMR of authentic epimers individually prepared from the coupling of *L*-leucine derivative and (*S*)- or (*R*)-phenylglycine derivative followed by N-alkylation with methyl α-bromo acetate or methyl (*S*)-α-bromo-α-methyl acetate. The absolute configurations of **28**–**32** are provisionally assigned by analogy to the formation of **25**, **26** and **27**.
7. In the substitution reactions of *L*-alanine methyl ester and *L*-leucine methyl ester with methyl α-bromo-α-phenyl acetate under the same condition, the (αS)-products were produced as major isomer with 65:35 and 70:30 dr, respectively. The absolute configurations of major (αS)-isomers were assigned by comparison to the ^{1}H NMR of authentic epimers individually prepared from the substitution of methyl (*R*)-α-bromo α-methyl or α-isobutyl acetate with (*S*)-phenylglycine methyl ester on the basis of inversion mechanism.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2751–2760

Synthesis of carbosilane dendrimers having peripheral mannose and mannobiose

Tomonori Mori,[a,b,*] Ken Hatano,[a] Koji Matsuoka,[a] Yasuaki Esumi,[c] Eric J. Toone[d] and Daiyo Terunuma[a,*]

[a]*Department of Functional Materials Science, Saitama University, Shimo-ohkubo, Sakura-ku, Saitama 338-8570, Japan*
[b]*Japan Association for the Advancement of Medical Equipment, 3-42-6 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[c]*The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*
[d]*Department of Chemistry, Duke University, B120 LSRC, Durham, NC 27708, USA*

Received 28 December 2004; revised 20 January 2005; accepted 24 January 2005

Abstract—The mannose monosaccharide derivative, acetylthiopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (Man), and the mannobiose derivative, acetylthiopropyl 2,4,6-tri-*O*-acetyl-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (α-1,3-Man), were synthesized respectively. These mannose derivatives were introduced into carbosilane dendrimer scaffolds of the zero and first generations. As a result, six carbosilane dendrimers were functionalized by Man and α-1,3-Man. Isothermal titration microcalorimetry was done to determine binding assay between mannose moieties of carbosilane dendrimer and concanavalin A. It was found that carbosilane dendrimers bound more efficiently to concanavalin A than free mannose (Me-α-Man) and mannobiose (Me-α-1,3-Man).

1. Introduction

Oligosaccharide chains in natural glycoconjugates which contain glycoproteins, glycolipids, and proteoglycans components of extracellular matrixes and cell surfaces play crucial roles in a variety of biological systems.[1,2] Mannose is one of the important and characteristic monosaccharides in *N*-glycans (asparagine-linked oligosaccharides).[3] A group of *N*-glycans, which contain high levels of mannose residues, is called a high-mannose type. The majority of nascent peptides in the endoplasmic reticulum (ER) are *N*-glycosylated with high-mannose type oligosaccharides.[4,5] The functions of high-mannose type oligosaccharides in the ER glycoprotein quality control have attracted recent attention.[6]

The interactions between lectins (carbohydrate-binding proteins),[7] and carbohydrates in glycoconjugates play principal roles in many cellular recognition processes.[8] At the monosaccharide level these interactions typically have weak affinities (K_D in mM). However, multivalent carbohydrates are known to greatly enhance interaction between the binding proteins (lectins) and the ligands involving carbohydrates.[9] This phenomenon, called 'the cluster effect',[10] enticed to generate a large number of functional neoglyconconjugates to achieve superior binding affinity.[11] Dendrimers, one of the typical forms to manifest the cluster effect, are targets of intensive investigation of the cluster effect, because their structures are easy to control and prepare.[12] Glycodendrimers with peripheral mannosyl group as one of the neoglycoconjugates have been shown to mimic the structure and functions of the high mannose type *N*-glycans.[13–15]

Large number of dendrimers with mannose moieties have been synthesized.[13–15] However, only one case of glycocoating carbosilane dendrimer with mannose moiety has been synthesized by Lindhorst et al.[16] as far as we know. They described the pathway to introduce mannose derivatives into carbosilane dendrimer scaffold via a hydrosilylation reaction of a protected allyl mannoside with a carbosilane containing Si–H end groups in the presence of a platinum catalyst, thus leading to an Si–C linked structure.

The carbosilane dendrimer scaffold is easy to control the number of branches at each generation and the chain length between the terminal silicon. We have been preparing

Keywords: Carbosilane dendrimer; Mannose; Mannobiose; *N*-Glycan; Glycocluster; Isothermal titration calorimetry.
* Corresponding authors. Tel./fax: +81 48 858 3535 (T.M.); tel./fax: +81 48 858 3532 (D.T.); e-mail addresses: tmori@fms.saitama-u.ac.jp; teru@fms.saitama-u.ac.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.090

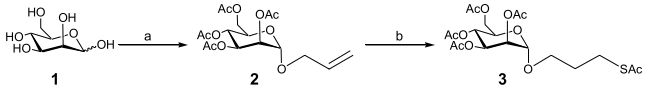
carbosilane dendrimers with peripheral functional carbohydrate moieties by a different route.[17] Our approach to vary the molecular design of carbohydrates containing carbosilane dendrimers is to control the methylene chain length of dendrimer scaffolds and the aglycon moiety length of carbohydrates. Peripheral globotriose clustered on carbosilane dendrimers were synthesized for the purpose of neutralizing Shiga-toxin producing *Escherichia coli* O157:H7.[18]

In this article, we describe syntheses of carbosilane dendrimers with peripheral mannose, and their characterization by spectrometric methods. We also determined the binding assay of concanavalin A (Con A), by the means of isothermal titration microcalorimetry (ITC).

2. Results and discussion

2.1. Preparation of mannose monosaccharide derivative (Man)

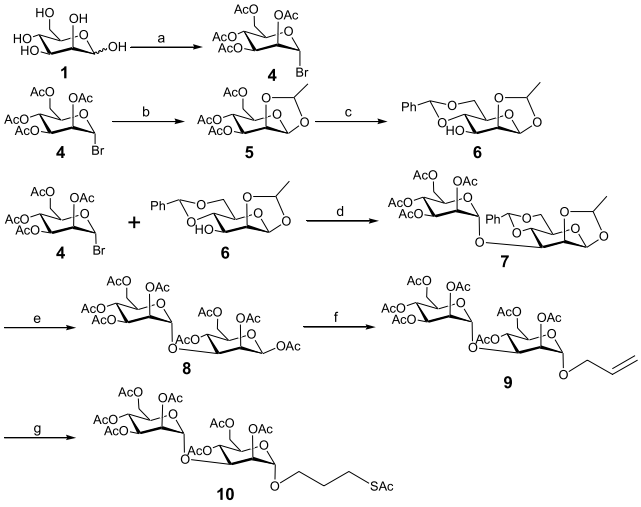
Scheme 1 summarizes the synthetic steps of mannose monosaccharide derivative, acetylthiopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (Man; **3**). The treatment of penta-*O*-acetylmannopyranose with allyl alcohol in the presence of borone trifluoride diethyl ether complex as Lewis acid produces allyl tetraacetylmannose (**2**).[17b,19] Compound **3** was synthesized by the anti-Markovnikov addition of the thio group to the allyl moiety of **2** although **3**, which is synthesized by another synthetic method of activating 2,2′-azobisisobutyronitrile (AIBN) irradiated in a photochemical reactor.[20] In this reaction, AIBN was activated by heat at 80 °C.[17d,e] Each NMR signal of **3** was assigned by following measurements: ^{1}H, ^{13}C, DEPT, HH, and HC COSY. Chemical shifts of **3** are described in Section 4.



Scheme 1. (a) $AcONa/Ac_2O$, then allyl alcohol, BF_3-OEt_2/CH_2Cl_2, 70% (2 steps); (b) AcSH, AIBN/1,4-dioxane, 73%.

2.2. Preparation of mannose disaccharide derivative (α-1,3-Man)

Mannose disaccharide derivative, 1-*O*-(3′-acetylthiopropyl)-2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-D-mannopyranosyl) D-mannopyranose (α-1,3-Man; **10**), was synthesized starting from D-mannose (Scheme 2). D-Mannose was converted to 1-bromo 2,3,4,6-tetra-*O*-acetyl mannose (**4**)[21] which will be used as a glycosyl acceptor and will also lead to a donor. Compounds **5** and **6** were synthesized by the method described in the literature:[22,23] 1,2-*O*-ethylidene protection of **4** was prepared by using $NaBH_4$ in acetonitrile at room temperature,[22] then 4,6-*O*-benzylidene protection of **5** using benzaldehyde dimethyl acetal and 1,10-camphorsulfonic acid.[23] Glycosylation of **6** with **4** in the presence in AgOTf, the reagent which was used for the formation of α-glycoside, in dichloromethane at −20 °C proceeded stereoselectively to give **7**. Compound **7**



Scheme 2. (a) HBr-AcOH, Ac_2O, quant; (b) $NaBH_4$/MeCN, 64%; (c) NaOMe/MeOH, then $PhCH(OMe)_2$, CSA/DMF, quant. (2 steps); (d) AgOTf, MS4A/CH_2Cl_2, 66%; (e) 90% CF_3COOH aq, then $AcONa/Ac_2O$, 64% (2 steps); (f) allyl alcohol, BF_3-OEt_2/CH_2Cl_2, 43%; (g) AcSH, AIBN/1,4-dioxane, 97%.

was assigned by measurements of the high resolution mass and NMR spectra to form the α-1,3-glycoside bond with both mannose moieties. Next **7** was treated with aqueous trifluoroacetic acid (90% v/v) to remove both 1,2-*O*-ethylidene and 4,6-*O*-benzylidene groups, followed by acetylation with acetic anhydride and sodium acetate to provide **8**.[24] 1-Allylation[17b,19] and thioacetylation[17d,e] of the allyl moiety were synthesized by the same method to mannose monosaccharide to give **10**.

2.3. Preparation of carbosilane dendrimders having peripheral mannose

For the introduction of mannose derivatives, we used three carbosilane dendrimer scaffolds: three-branched (Fan(0)3-Br), four-branched (Ball(0)4-Br), and six-branched (Dumbbell(1)6-Br), as described in Figure 1. Fan(0)3-Br and Ball(0)4-Br are the zero generation scaffolds which were prepared with triallylphenylsilane and tetraallylsilane by following three reaction steps: hydroxylation, mesylation, and bromination.[17b,25] On the other hand, Dumbbell(1)6-Br is the first generation carbosilane dendrimer scaffold,

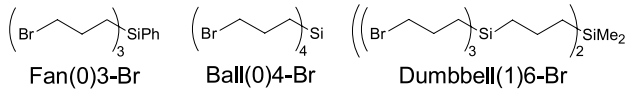
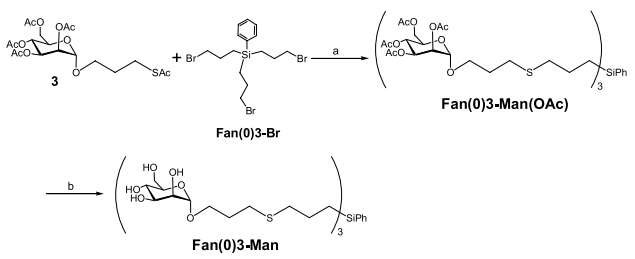


Figure 1. Carbosilane dendrimer scaffolds.



Scheme 3. (a) NaOMe/MeOH, DMF, then Ac_2O/pyridine, 66% (2 steps) and (b) NaOMe/MeOH, then 0.1 mol/l NaOH aq, 61%.

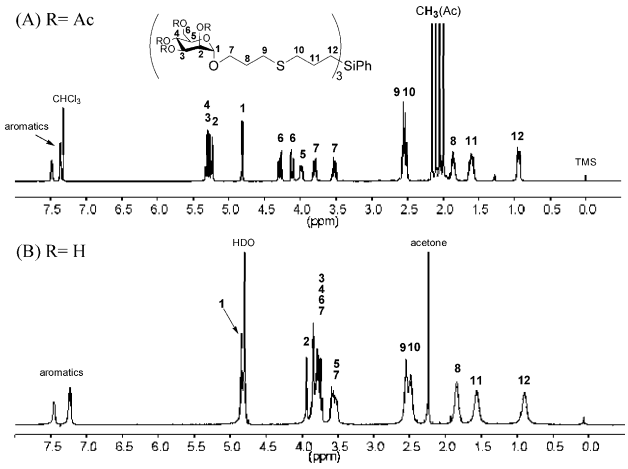


Figure 2. ^{1}H NMR spectra (400 MHz, $CDCl_3$ or D_2O): (A) Fan(0)3-Man(OAc) and (B) Fan(0)3-Man.

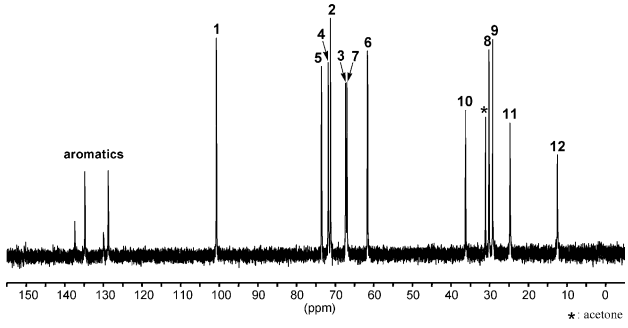


Figure 3. ^{13}C NMR spectrum (100 MHz, D_2O) of Fan(0)3-Man.

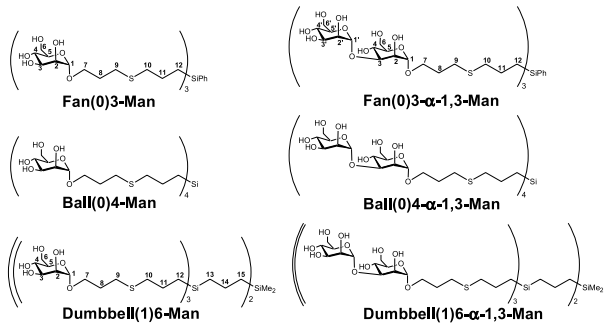


Figure 4. Carbosilane dendrimers having peripheral mannose and mannobiose moieties.

prepared with allylation of dichlorodimethylsilane followed by hydrosilylation[17d,25,26] with the first generation skeleton. The resulting reactions were the same as for the zero generation carbosilane dendrimer scaffolds.

Introduction of Man and Man-α-1,3-Man to carbosilane dendrimer scaffolds was done concurrently with deacetylation, that is, using sodium methoxide/methanol and *N*,*N*-dimethylformamide (Scheme 3). This reaction includes de-*O*- and -*S*-acetylation, followed by S_N2 replacement reaction, and then acetylation for purification by silica gel and gel permeation chromatography. After purification by means of recycling GPC, products of mannose-coated carbosilane dendrimers were obtained, and disulfide byproducts (Man-SS-Man or α-1,3-Man-SS-α-1,3-Man) was removed.

In summary, six carbosilane dendrimers were synthesized, and functionalized with acetyl-protected derivatives of mannose or mannose disaccharide (α-1,3-Man). The yields of addition of mannose monosaccharide were 62–76%, and those of α-1,3-Man were 30–35%. The difference in the yields between the mannose and mannobiose derivatives may be due to the bulkier structure of mannobioside.

All synthesized dendrimers were characterized by ^{1}H and ^{13}C NMR and high resolution mass spectrometry. From the results of high resolution mass spectrometry, the proton or sodium ion adduct peaks, $[M+H]^+$ or $[M+Na]^+$, were determined and these showed good agreement with the calculated values, within the ± 5 ppm error margins. Moreover, from the measurement of ^{1}H NMR measurements, we found the new signal at ca. 2.5 ppm (Fig. 2A) which showed that a bond was formed between the sulfur atom of saccharide moiety and the methylene carbon of the corresponding carbosilane dendrimer scaffold. Thus, these spectrometric results confirmed the structures of a carbosilane dendrimer with peripheral mannose and mannobiose.

The dendrimers with acetylated mannose moieties were deacetylated by sodium methoxide/methanol, deacetylation is saponification to yield the corresponding carbosilane dendrimers with peripheral mannose and mannose disaccharide, and then purified by gel filtration. All six types of carbosilane dendrimers functionalized by peripheral mannose moieties were synthesized and characterized by the measurements of ^{1}H and ^{13}C NMR, and high resolution mass spectrometry. Figures 2B and 3 show ^{1}H and ^{13}C NMR spectra of Fan(0)3-Man, respectively. Signals of methyl proton from the acetyl groups in mannose moiety

Table 1. ^{13}C NMR spectroscopic data (δ values) of carbosilane dendrimers functionalized peripheral mannose moieties (I)

Mannose moieties	C-1 C-1′	C-2 C-2′	C-3 C-3′	C-4 C-4′	C-5 C-5′	C-6 C-6′
Fan(0)3-Man	100.7	71.1	67.2	71.7	73.5	61.5
Ball(0)4-Man	100.6	71.0	67.2	71.6	73.4	61.5
Dumbbell(1)6-Man	100.1	70.7	66.8	71.2	72.8	60.9
Fan(0)3-α-1,3-Man	100.5	70.5	79.3	66.7	73.8	61.1
	102.9	70.8	71.0	67.1	73.4	61.4
Ball(0)4-α-1,3-Man	100.6	70.6	79.4	66.9	73.8	61.3
	103.0	70.9	71.1	67.3	73.5	61.5
Dumbbell(1)6-α-1,3-Man	100.7	70.7	79.6	67.0	74.0	61.5
	103.1	71.0	71.3	67.5	73.6	61.8

Table 2. ^{13}C NMR spectroscopic data (δ values) of carbosilane dendrimers functionalized peripheral mannose moieties (II)

Dendrimer scaffolds	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15
Fan(0)3-Man	66.9	30.1	29.1	36.2	24.6	12.4			
Ball(0)4-Man	66.9	30.1	29.1	36.3	24.8	12.5			
Dumbbell(1)6-Man	66.3	29.5	28.6	35.8	24.4	12.0	19.1	20.3	21.0
Fan(0)3-α-1,3-Man	66.1	29.8	28.9	35.9	24.1	12.1			
Ball(0)4-α-1,3-Man	66.3	29.8	29.0	36.2	24.8	12.4			
Dumbbell(1)6-α-1,3-Man	66.4	30.0	29.2	36.5	25.0	12.6	18.1	19.4	21.0

disappeared in Figure 2B, which is distinct from Figure 2A. This is in agreement with the results of ^{13}C NMR measurements. Figure 4 shows all six carbosilane dendrimers. Signals of ^{13}C NMR spectra are all assigned and partly listed in Table 1 (mannose moieties) and Table 2 (carbosilane dendrimer scaffolds). Signals from mannose moieties were assigned according to the published results.[24e,27] In Table 2, each signal displays good agreement in spite of the differences between dendrimer scaffolds and saccharides. Characteristic signals are observed on Fan(0)3- and Dumbbell(1)6-scaffolds: these were four signals of phenyl carbons at about 130 ppm on Fan(0)3 types, and the signal of methyl carbon binding core silicon atom at about −2 ppm on Dumbbell(1)6 types.

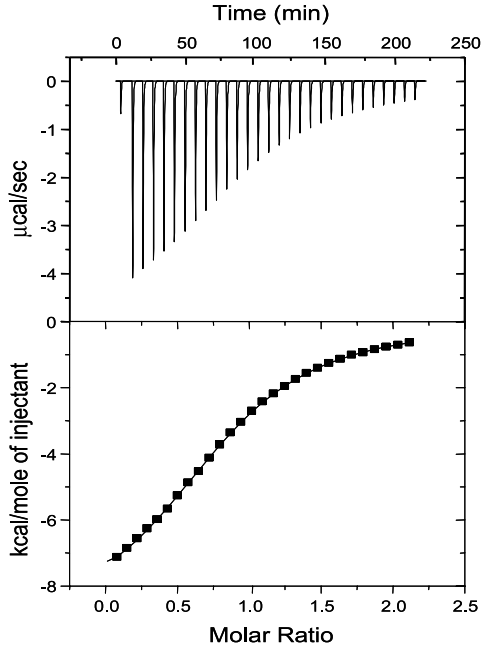


Figure 5. Calorimetric data for titration of concanavalin A, 0.21 mM, with trivalent ligand Fan(0)3-Man, 2.1 mM. Both protein and ligand were dissolved in buffer consisting of 50 mM 3,3-dimethylglutarate, 250 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$ adjusted pH 5.2. Top, raw (power vs time) data; bottom integrated heat vs molar ratio of ligand. Solid line shows best fit of data using a one-site model: $n=0.85$; $K=2.09\times10^4$; $\Delta H=-9.2$ kcal mol^{-1}. A fit to a two-site model does not provide a statistically superior fit.

2.4. Binding affinity between carbosilane dendrimers and concanavalin A

Con A is one of the most widely used lectins in biological studies. Mannose residue is recognized specifically by Con A. Recently, isothermal titration microcalorimetry (ITC) has been utilized to study protein–carbohydrate interaction.[10a] A soluble protein is titrated with aliquots of a soluble ligand in this measurement. The heat produced during ligand addition serves as a reporter signal for binding, which is yielded a binding constant, which, in turn can be related to the free energy of binding. Since this technique also directly measures binding enthalpies, an entropy of binding can be readly calculated.

The bindings of tri-, tetra-, and hexavalent ligands Fan(0)3-Man, Ball(0)4-Man, and Dumbbell(1)6-Man to dimeric Con A (pH 5.2) were evaluated by titration microcalorimetry. The titration microcalorimetry of all multivalent ligands yielded curves indicative of simple reversible binding. Figure 5 shows one of the titration curves for the carbosilane dendrimer with peripheral mannose moieties (Fan(0)3-Man) when bound to Con A in glutarate buffer (top) and the resulting one-site fit of the integrated differential power signal with respect to time (bottom). Table 3 lists the calculated binding constant and other parameters of binding between the carbosilane dendrimers and Con A. All of the carbosilane dendrimers have higher binding constant values with Con A, K, than the non-dendric mannose derivatives, Me-α-Man and Me-α-1,3-Man,[28] demonstrating cluster glycoside effect. As for monosaccharide types of the

Table 3. Binding of carbosilane dendrimers to concanavalin A

	K/M^{-1}	ΔG	ΔH	$T\Delta S$[a]
		kcal mol^{-1}		
Man	4.2×10^3	−4.9	−2.8	2.2
Me-α-Man[b]	7.6×10^3	−5.3	−6.8	−1.5
Fan(0)3-Man	2.1×10^4	−5.9	−9.2	−3.3
Ball(0)4-Man	2.2×10^4	−5.9	−5.6	0.3
Dumbbell(1)6-Man	6.0×10^4	−6.5	−3.6	3.0
Me-α-1,3-Man[b]	3.0×10^4	−6.0	−7.4	−1.4
Fan(0)3-α-1,3-Man	7.9×10^4	−6.7	−14.1	−7.4
Ball(0)4-α-1,3-Man	9.1×10^4	−6.8	−9.8	−3.0
Dumbbell(1)6-α-1,3-Man	6.1×10^4	−6.5	−4.2	2.3

[a] 298 K.
[b] See Ref. 28c.

synthesized carbosilane dendrimers, the magnitude of the effects depend on the amount of mannose in a dendrimer. In the case of three-branched dendrimer scaffolds having peripheral mannose, *K* value of the carbosilane dendrimer was higher than that of the non-carbosilane dendrimer, and other thermodynamic parameters were similar values.[28d] However, the multivalency effect was not clearly measured in the mannobiose-type of carbosilane dendrimers, because these dendrimers became highly aggregated during titration and the orientation of the saccharides could not match tightly to the binding pockets of Con A.

3. Conclusion

We synthesized six carbosilane dendrimers with peripheral mannose and mannobiose. The structures of these dendrimers were characterized by measurements of NMR and mass spectrometry. Isothermal titration microcalorimetry (ITC) was done for determining the binding assay between the carbosilane dendrimer and concanavalin A (Con A). It was found that the carbosilane dendrimers bound to Con A more frequently than to free mannose (Me-α-Man) and mannobiose (Me-α-1,3-Man), thus showing the cluster effect.

4. Experimental

4.1. Analyses and GPC

NMR spectra were recorded with a Bruker DRX-400, AM-400, and a Valian Gemini-2000 spectrometer. Fast atom bombardment (FAB) and electron spray ionization (ESI) mass spectra were obtained with a JEOL JMS-HX110A spectrometer and a JEOL JMS-T100LC spectrometer, respectively. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Isothermal titration microcalorimetry was performed using the MicroCal Omega titration microcalorimeter. High resolution mass spectrometry (HRMS) measurements were valid to ±5 ppm. Recycling preparative GPC was performed with a LC-908W (Japan Analytical Industry Co., Ltd) connected to an RI detector RI-5 (column, JAIGEL-1H-A and JAIGEL-2H-A; solvent, chloroform).

4.2. Materials

Concanavalin A (Type IV, lot No. 102K7044) was purchased from Sigma Chemical Company and dialyzed with a glutarate buffer. Protein concentration was determined by the method of Edelhoch.[29] Carbohydrate concentrations were determined by phenol-sulfuric acid method.[30] For calorimetric measurements, water was purified with a Millpore purification system that involved passage through reverse osmosis, charcoal, and two ion exchange filters to attain resistance of $>10\ \mathrm{M\Omega\ cm^{-1}}$.

4.3. Reactions

4.3.1. Acetylthiopropyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (3). D-Mannose (5.00 g, 27.8 mmol) was acetylated to yield penta-*O*-acetyl-α-D-mannose by using a mixture of sodium acetate (2.51 g, 30.62 mmol) and acetic anhydride (25.0 mL, 263 mmol). Under an argon atmosphere, penta-*O*-acetyl-α-D-mannopyranose was dissolved in dry-dichloromethane (123 mL) and allyl alcohol (9.50 mL, 139 mmol) was added, then the mixture was cooled to −5 °C. Boron trifluoride diethyl ether complex (94 mL, 742 mmol) was dropped into the solution. The reaction solution was stirred for 30 min at 0 °C, then stirred for 54 h at room temperature. After the reaction, the solution was poured into ice-water, washed with water, saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with toluene–ethyl acetate (5:1 (v/v)) as eluent to yield pure **2** (7.53 g, 70% (2 steps)).

To a stirred solution of **2** (3.65 g, 9.40 mmol) and thioacetic acid (13.4 mL, 188 mmol) in 1,4-dioxane (2.0 mL), 2,2′-azobisisobutyronitrile (AIBN; 7.72 g, 47.0 mmol) was added at 50 °C under an argon atmosphere. The mixture was stirred for 2.5 h at 80 °C, then cooled to room temperature. Cyclohexene (5.0 mL, 49.3 mmol) was added, and the mixture was stirred at room temperature for 30 min. After evaporation, silica gel chromatography of the residual syrup (toluene–ethyl acetate 10:1–5:1–3:1) yielded sulfide **3** (3.16 g, 73%): ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.33 (1H, m, H-3), 5.28 (1H, m, H-4), 5.24 (1H, dd, H-2, $J_{1,2}=1.61$ Hz, $J_{2,3}=3.21$ Hz), 4.81 (1H, H-1), 4.28 (1H, dd, H-6a, $J_{5,6a}=5.35$ Hz, $J_{6a,6b}=12.31$ Hz), 4.11 (1H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.31$ Hz), 3.98 (1H, m, H-5), 3.77 (1H, m, $OCH_2CH_2CH_2S$), 3.52 (1H, m, $OCH_2CH_2CH_2S$), 2.97 (2H, t, $OCH_2CH_2CH_2S$, $J=6.96$ Hz), 2.34 (3H, s, CH_3(SAc)), 2.16, 2.12, 2.06, 2.00 (12H, s, CH_3(OAc)), 1.91 (2H, m, $OCH_2CH_2CH_2S$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 195.2 (C, *C*=O(SAc)), 170.3, 169.7, 169.5, 169.4 (C, *C*=O(Ac)), 97.4 (CH, C-1), 69.2 (CH, C-2), 68.8 (CH, C-3), 68.3 (CH, C-5), 66.5 (CH_2, $OCH_2CH_2CH_2S$), 65.8 (CH, C-4), 62.2 (CH_2, C-6), 30.3 (CH_3, CH_3(SAc)), 28.9 (CH_2, $OCH_2CH_2CH_2S$), 25.5 (CH_2, $OCH_2CH_2CH_2S$), 20.6, 20.5, 20.41, 20.39 (CH_3, CH_3(OAc)).

4.3.2. 4,6-*O*-Benzylidene-1,2-ethylidene-β-D-manno-pyranoside (6).[23] Under an argon atmosphere, 1-bromo 2,3,4,6-tetra-*O*-acetyl mannose ((**4**)[21]; 22.9 g, 55.6 mmol) was dissolved in acetonitrile (130 mL), and sodium borohydrate (10.5 g, 278 mmol) was added, then the mixture was stirred for 22 h at room temperature. After the reaction, the solution was diluted with ethyl acetate, washed with water and brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane–ethyl acetate (5:1–3:1–2:1) yielded pure **5**[22] (11.8 g, 64%).

Under an argon atmosphere, **5** (5.73 g, 17.3 mmol) was dissolved in methanol (5.0 mL), and sodium methoxide (0.14 g, 2.60 mmol) was added, then the mixture was stirred for 1 h at room temperature. After the reaction, IR120B (H^+) resin was added to neutralize the reaction solution, and the suspension was filtered and evaporated. The residue was dissolved in *N*,*N*-dimethylformamide (15.0 mL).

Benzaldehyde dimethylacetal (3.70 mL, 24.6 mmol) and (+)-10-camphorsulfonic acid (379 mg, 1.63 mmol) was added, and the mixture was stirred over evaporation for 6 h at 30 °C. The solution was cooled to room temperature, and triethylamine (0.45 mL, 3.34 mmol) added to neutralize. The solution was evaporated, and purified by silica gel column chromatography with *n*-hexane–ethyl acetate (10:1–5:1–3:1–1:1) as eluent to yield pure **6** (5.08 g, quant. (2 steps)).

4.3.3. 4,6-*O*-Benzylidene-1,2-ethylidene-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (7). 4,6-*O*-Benzylidene-1,2-ethylidene-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-β-D-mannopyranoside (**7**). A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**4**) (298 mg, 0.72 mmol) and 4,6-*O*-benzylidene-1,2-ethylidene-β-D-mannopyranoside (**6**) (100 mg, 0.34 mmol) in anhydrous dichloromethane (8.0 mL) was stirred in the presence of activated MS4A (1.0 g) and silver trifluoromethanesulfonate (228 mg, 0.89 mmol) was added under an argon atmosphere. The reaction mixture was stirred for 2 h at −20 °C. Further, silver trifluoromethanesulfonate (113 mg, 0.44 mmol) was added to the mixture under an argon atmosphere, and the mixture was stirred for 40 min at −20 °C. Sodium carbonate (302 mg, 2.85 mmol) was added to the reaction solution, then the solution was filtered through a celite bed, diluted with chloroform, washed with saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. Then the solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with toluene–ethyl acetate (5:1) as eluent to yield pure **7** (139 mg, 66%): HRMS (ESI); calcd for $C_{29}H_{36}O_{15}Na$ $[M+Na]^+$ 647.1952, found 647.1936. $[\alpha]_D^{33}=-16.2°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 7.45–7.33 (5H, m, Ph), 5.59 (1H, s, C*H*(4,6-bndn)), 5.45 (1H, m, H-3′), 5.40 (1H, m, H-4′), 5.34 (1H, q, $J=5.35$ Hz, C*H*-(1,2-etdn)), 5.30–5.25 (2H, m, H-1, 2′), 5.19 (1H, d, $J_{1',2'}=1.61$ Hz, H-1′), 4.33–4.22 (4H, m, H-2, 3, 6a, 6′a), 4.11–4.04 (3H, m, H-4, 5′, 6′b), 3.78 (1H, m, H-6b), 3.39 (1H, m, H-5), 2.11, 2.09, 2.05, 1.99 (12H, s, Ac), 1.54 (3H, d, $J=5.35$ Hz, CH_3-(1,2-etdn)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.5, 169.8, 169.7, 169.6 (C, *C*=O (Ac)), 136.9 (C, *C* (Ph)), 128.9, 128.2, 125.9 (CH, *C*H(Ph)), 104.7 (CH, *C*H (1,2-etdn)), 101.1 (CH, *C*H (4,6-bndn)), 99.5 (CH, C-1′), 96.8 (CH, C-1), 79.6 (CH, C-3), 76.9 (CH, C-4), 75.9 (CH, C-5′), 69.3 (CH, C-4′), 68.80 (CH, C-3′), 68.77 (CH, C-2), 68.4 (CH_2, C-6), 66.4 (CH, C-2′), 65.7 (CH, C-5), 62.6 (CH_2, C-6′), 21.8 (CH_3, *C*H_3-(etdn)), 20.77, 20.75, 20.70, 20.66 (CH_3, *C*H_3-(Ac)).

4.3.4. Allyl 2,4,6-tri-*O*-acetyl-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (9). A solution of **7** (860 mg, 1.38 mmol) in 90% (v/v) aqueous trifluoroacetic acid (10 mL) was stirred for 22 h at room temperature. The solution was cooled in an ice-water bath and neutralized with sodium carbonate. Then the solution was evaporated and dried with a vacuum pump. Sodium acetate (229 mg, 2.79 mmol) and acetic anhydride (15 mL, 158 mmol) were added to the residue, and the reaction mixture was stirred for 1 h at 110 °C. To the reaction mixture was added ice-water, and the mixture was extracted with chloroform. The extract was washed with saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with *n*-hexane–ethyl acetate (1:1–1:2) as eluent to yield pure **8** (598 mg, 64% (2 steps)).

Under an argon atmosphere, **8** (4.08 g, 6.01 mmol) was dissolved in dry-dichloromethane (27 mL) and allyl alcohol (2.1 mL, 30.7 mmol) was added, then cooled to −5 °C. Boron trifluoride diethyl ether complex (8.0 mL, 63.1 mmol) was dropped into the solution. The reaction solution was stirred for 30 min at 0 °C, then stirred for 71 h at room temperature. After the reaction, the solution was poured onto ice-water, washed with water, saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with toluene–ethyl acetate (5:1–3:1–2:1–1:1–0:1) as eluent to yield pure **9** (1.73 g, 43%): ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.87 (1H, m, $OCH_2CH{=}CH_2$), 5.34–5.18 (6H, m, H-2, 3, 4, 3′, $OCH_2CH{=}CH_2$), 5.01 (1H, m, H-2′), 5.00 (1H, d, H-1′ $J_{1',2'}=1.61$ Hz), 4.88 (1H, H-1), 4.30–3.98 (8H, m, H-6, 4′, 5′, 6′, $OCH_2CH{=}CH_2$), 3.90 (1H, ddd, H-5, $J_{4,5}=10.17$ Hz, $J_{5,6a}=5.35$ Hz, $J_{5,6b}=2.68$ Hz), 2.21, 2.14, 2.13, 2.113, 2.106, 2.06, 1.99 (21H, s, CH_3(OAc)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.65, 170.62, 170.4, 170.0, 169.9, 169.8, 169.5 (C, *C*=O(Ac)), 132.8 (CH, $OCH_2CH{=}CH_2$), 118.5 (CH_2, $OCH_2CH{=}CH_2$), 98.8 (CH, C-1′), 96.5 (CH, C-1), 74.6 (CH, C-3), 70.9 (CH, C-2), 69.9 (CH, C-2′), 69.3 (CH, C-5′), 68.7 (CH, C-5), 68.5 (CH_2, $OCH_2CH{=}CH_2$), 68.2 (CH, C-3′), 67.7 (CH, C-4′), 65.9 (CH, C-4), 62.5, 62.4 (CH_2, C-6, 6′), 20.9, 20.8, 20.73, 20.71, 20.63, 20.60, 20.59 (CH_3, *C*H_3(Ac)).

4.3.5. Acetylthiopropyl 2,4,6-tri-*O*-acetyl-3-*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (10). AIBN (2.11 g, 12.8 mmol) was added to a stirred solution of **9** (1.73 g, 2.56 mmol) and thioacetic acid (3.7 mL, 52.0 mmol) in 1,4-dioxane (1.5 mL) at 50 °C under an argon atmosphere. The mixture was stirred for 3 h at 80 °C, then cooled to room temperature. Cyclohexene (1.5 mL, 14.8 mmol) was added, and the mixture was stirred for 30 min at room temperature. After evaporation, the residue was purified by silica gel column chromatography with toluene–ethyl acetate (10:1–5:1–3:1–2:1) and size exclusion chromatography (Sephadex LH-20; eluent: methanol) as eluent to yield pure **10** (1.87 g, 97%): HRMS (ESI); calcd for $C_{31}H_{44}O_{19}SNa$ $[M+Na]^+$ 775.2095, found 775.2065. $[\alpha]_D^{33}=+33.8°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.29, 5.26 (2H, m, H-3, 4), 5.23–5.19 (2H, m, H-2, 3′), 5.02 (1H, dd, H-2′, $J_{1',2'}=1.61$ Hz, $J_{2',3'}=2.14$ Hz), 5.01 (1H, d, H-1′, $J_{1',2'}=1.61$ Hz), 4.82 (1H, d, H-1, $J_{1,2}=1.60$ Hz), 4.31–4.21 (2H, m, H-6a, 6′a), 4.16 (1H, dd, H4′, $J_{3',4'}=3.75$ Hz, $J_{4',5'}=10.17$ Hz), 4.13–4.04 (3H, m, H-6b, 5′, 6′b), 3.86 (3H, m, H-5), 3.73 (1H, m, $OCH_2CH_2CH_2S$), 3.50 (1H, m, $OCH_2CH_2CH_2S$), 2.94 (2H, t, $OCH_2CH_2CH_2S$, $J=6.96$ Hz), 2.34 (3H, s, CH_3(SAc)), 2.21, 2.14, 2.13, 2.12, 2.11, 2.06, 2.00 (21H, s, CH_3(OAc)), 1.88 (2H, m, $OCH_2CH_2CH_2S$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 195.2 (C, *C*=O(SAc)), 170.5, 170.4, 170.2, 169.8, 169.7,

169.6, 169.4 (C, *C*=O(OAc)), 98.8 (CH, C-1′), 97.3 (CH, C-1), 74.8 (CH, C-3), 70.7 (CH, C-2), 69.8 (CH, C-2′), 69.2 (CH, C-5′), 68.7 (CH, C-5), 68.1 (CH, C-3′), 67.5 (CH, C-4′), 66.4 (CH_2, $OCH_2CH_2CH_2S$), 65.7 (CH, C-4), 62.4, 62.2 (CH_2, C-6, 6′), 30.4 (CH_3, CH_3(SAc)), 29.1 (CH_2, $OCH_2CH_2CH_2S$), 25.6 (CH_2, $OCH_2CH_2CH_2S$), 20.8, 20.7, 20.60, 20.56, 20.49, 20.45 (CH_3, CH_3(OAc)).

4.3.6. Introduction of mannose and mannobiose into carbosilane dendrimer scaffolds: Fan(0)3-Man(OAc). Under an argon atmosphere, a mixture of **3** (348 mg, 0.75 mmol) and a dendrimer scaffold (for example, Fan(0)-Br: 56.4 mg, 0.12 mmol) was dissolved in *N*,*N*-dimethylformamide (0.5 mL) and methanol (0.5 mL), and stirred at room temperature for 20 min. Sodium methoxide in methanol solution (1.0 M, 0.75 mL, 0.75 mmol) was added to the reaction solution and stirred at room temperature over night. Acetic acid (0.1 mL) was added to the reaction solution, and stirred at room temperature for 10 min, then evaporated in vacuo. The residue was suspended in a mixture of pyridine (0.5 mL) and acetic anhydride (1.0 mL, 10.5 mmol), and stirred at room temperature over night. The reaction mixture was evaporated in vacuo, added to ice-water and chloroform, then washed with 1 M hydrochloric acid, saturated aqueous sodium hydrogen carbonate, brine, and dried over anhydrous magnesium sulfate. The solution was filtered through a celite bed and concentrated. The residue was purified by silica gel column chromatography with hexane–ethyl acetate (1:1–1:2–0:1) as the eluent to produce pure Fan(0)3-Man(OAc): Yield 136 mg (76% (2 steps)). HRMS (ESI): Calcd for $C_{66}H_{98}O_{30}S_3SiNa$ $[M+Na]^+$ 1517.4972, found 1517.4990. $[\alpha]_D^{32}=+42.3°$ ($c=1.0$ in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 7.50–7.34 (5H, Ph), 5.34–5.24 (6H, m, H-3, 4), 5.23 (3H, dd, H-2, $J_{1,2}=1.60$ Hz, $J_{2,3}=2.14$ Hz), 4.81 (3H, d, H-1, $J_{1,2}=1.60$ Hz), 4.28 (3H, dd, H-6a, $J_{5,6a}=5.35$ Hz, $J_{6a,6b}=12.32$ Hz), 4.11 (3H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.32$ Hz), 3.98 (3H, m, H-5), 3.80 (3H, m, H-7a), 3.52 (3H, m, H-7b), 2.55 (6H, t, H-9, $J_{8,9}=6.96$ Hz), 2.53 (6H, t, H-10, $J_{10,11}=6.96$ Hz), 2.16 (9H, s, CH_3(Ac)), 2.10 (9H, s, CH_3(Ac)), 2.04 (9H, s, CH_3(Ac)), 1.99 (9H, s, CH_3(Ac)), 1.86 (6H, m, H-8), 1.60 (6H, m, H-11), 0.94 (6H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 169.8, 169.7, 169.5 (C, *C*=O(Ac)), 136.0 (C, Ph), 133.8, 129.0, 127.8 (CH, Ph), 97.4 (CH, C-1), 69.4 (CH, C-2), 68.9 (CH, C-3), 68.3 (CH, C-5), 66.4 (CH_2, C-7), 66.0 (CH, C-4), 62.2 (CH_2, C-6), 35.6 (CH_2, C-10), 28.9 (CH_2, C-8), 28.4 (CH_2, C-9), 23.8 (CH_2, C-11), 20.7, 20.6, 20.53, 20.51 (CH_3, CH_3(Ac)), 11.7 (CH_2, C-12).

Another carbosilane dendrimer with peripheral mannose or mannobiose acetate was prepared by the same method as Fan(0)3-Man. The mannobiose-bearing carbosilane dendrimers were synthesized using compound **10**.

Ball(0)4-*Man*(*OAc*). Yield 120.3 mg (66% (2 steps)). HRMS (FAB): Calcd for $C_{80}H_{125}O_{40}S_4Si$ $[M+H]^+$ 1881.6399, found 1881.6445. $[\alpha]_D^{29}=+45.1°$ ($c=1.0$ in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.32 (1H, m, H-3), 5.28 (1H, m, H-4), 5.23 (1H, dd, H-2, $J_{1,2}=1.60$ Hz, $J_{2,3}=2.14$ Hz), 4.82 (1H, d, $J_{1,2}=1.60$ Hz, H-1), 4.29 (1H, dd, H-6a, $J_{5,6a}=5.35$ Hz, $J_{6a,6b}=12.32$ Hz), 4.12 (1H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.32$ Hz), 3.99 (1H, ddd, H-5, $J_{4,5}=9.63$ Hz, $J_{5,6a}=5.35$ Hz, $J_{5,6b}=2.14$ Hz), 3.83 (1H, m, H-7a), 3.56 (1H, m, H-7b), 2.60 (2H, t, H-9, $J_{8,9}=6.96$ Hz), 2.53 (2H, t, H-10, $J_{10,11}=6.96$ Hz), 2.16 (3H, s, CH_3(Ac)), 2.11 (3H, s, CH_3(Ac)), 2.05 (3H, s, CH_3(Ac)), 2.00 (3H, s, CH_3(Ac)), 1.90 (2H, m, H-8), 1.58 (2H, m, H-11), 0.67 (2H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 169.8, 169.6, 169.5 (C, *C*=O(Ac)), 97.4 (CH, C-1), 69.3 (CH, C-2), 68.9 (CH, C-3), 68.3 (CH, C-5), 66.4 (CH_2, C-7), 65.9 (CH, C-4), 62.2 (CH_2, C-6), 35.7 (CH_2, C-10), 28.9 (CH_2, C-8), 28.4 (CH_2, C-9), 23.9 (CH_2, C-11), 20.7, 20.54, 20.48, 20.45 (CH_3, CH_3(Ac)), 11.7 (CH_2, C-12).

Dumbbell(1)6-*Man*(*OAc*). Yield 141.6 mg (62% (2 steps)). HRMS (FAB): Calcd for $C_{128}H_{205}O_{60}S_6Si_3$ $[M+H]^+$ 2978.0622, found 2978.0669. $[\alpha]_D^{29}=+40.1°$ ($c=1.0$ in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.32 (3H, m, H-3), 5.28 (3H, m, H-4), 5.23 (3H, dd, H-2, $J_{1,2}=1.61$ Hz, $J_{2,3}=2.14$ Hz), 4.82 (3H, d, $J_{1,2}=1.61$ Hz, H-1), 4.29 (3H, dd, H-6a, $J_{5,6a}=4.82$ Hz, $J_{6a,6b}=12.32$ Hz), 4.12 (3H, dd, H-6b, $J_{5,6b}=2.14$ Hz, $J_{6a,6b}=12.32$ Hz), 3.99 (3H, ddd, H-5, $J_{4,5}=9.63$ Hz, $J_{5,6a}=4.82$ Hz, $J_{5,6b}=2.14$ Hz), 3.83 (3H, m, H-7a), 3.55 (3H, m, H-7b), 2.60 (6H, t, H-9, $J_{8,9}=6.96$ Hz), 2.53 (6H, t, H-10, $J_{10,11}=6.96$ Hz), 2.16 (9H, s, CH_3(Ac)), 2.11 (9H, s, CH_3(Ac)), 2.05 (9H, s, CH_3(Ac)), 2.00 (9H, s, CH_3(Ac)), 1.90 (6H, m, H-8), 1.57 (6H, m, H-11), 1.31 (2H, m, H-14), 0.67–0.62 (8H, m, H-12, 13), 0.56 (2H, m, H-15), −0.04 (3H, s, CH_3(Si–Me)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 169.8, 169.6, 169.5 (C, *C*=O(Ac)), 97.4 (CH, C-1), 69.3 (CH, C-2), 68.9 (CH, C-3), 68.3 (CH, C-5), 66.4 (CH_2, C-7), 65.9 (CH, C-4), 62.2 (CH_2, C-6), 35.8 (CH_2, C-10), 28.9 (CH_2, C-8), 28.4 (CH_2, C-9), 24.0 (CH_2, C-11), 20.7, 20.55, 20.49, 20.46 (CH_3, CH_3(Ac)), 20.2 (CH_2, C-15), 18.1 (CH_2, C-14), 16.9 (CH_2, C-13), 11.9 (CH_2, C-12), −3.4 (CH_3, CH_3(Si–Me)).

Fan(0)3-α-1,3-*Man*(*OAc*). Yield 61.2 mg (30% (2 steps)). HRMS (ESI): Calcd for $C_{102}H_{146}O_{54}S_3SiNa$ $[M+Na]^+$ 2381.7508, found 2381.7485. $[\alpha]_D^{32}=+32.3°$ ($c=1.0$ in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 7.47–7.34 (5H, Ph), 5.29, 5.26 (6H, m, H-3, 4), 5.23–5.19 (6H, m, H-2, 3′), 5.02 (3H, m, H-2′), 4.99 (3H, H-1′), 4.82 (3H, H-1), 4.29–4.21 (6H, m, H-6a, 6′a), 4.13 (3H, dd, H-4′, $J_{3',4'}=3.75$ Hz, $J_{4',5'}=10.17$ Hz), 4.12–4.03 (9H, m, H-6b, 5′, 6′b), 3.86 (3H, ddd, H-5, $J_{4,5}=10.17$ Hz, $J_{5,6a}=5.36$ Hz, $J_{5,6b}=2.14$ Hz), 3.75 (3H, m, H-7a), 3.51 (3H, m, H-7b), 2.51 (12H, t, H-9, 10, $J_{8,9}=J_{10,11}=6.96$ Hz), 2.21, 2.14, 2.13, 2.102, 2.099, 2.05, 1.99 (63H, s, CH_3(Ac)), 1.84 (6H, m, H-8), 1.58 (6H, m, H-11), 0.92 (6H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm);170.4, 170.3, 170.2, 169.8, 169.65, 169.59, 169.4 (C, *C*=O(Ac)), 135.9 (C, Ph), 133.8, 129.1, 127.8 (CH, Ph), 98.8 (CH, C-1′), 97.2 (CH, C-1), 74.9 (CH, C-3), 70.7 (CH, C-2), 69.7 (CH, C-2′), 69.2 (CH, C-5′), 68.5 (CH, C-5), 68.0 (CH, C-3′), 67.4 (CH, C-4′), 66.3 (CH_2, C-7), 65.7 (CH, C-4), 62.3, 62.1 (CH_2, C-6, 6′), 35.6 (CH_2, C-10), 28.8 (CH_2, C-8), 28.4 (CH_2, C-9), 23.7 (CH_2, C-11), 20.7, 20.61, 20.58, 20.52, 20.46, 20.42 (CH_3, CH_3(Ac)), 11.7 (CH_2, C-12).

Ball(0)4-α-1,3-*Man*(*OAc*). Yield 81.1 mg (35% (2 steps)). HRMS (FAB): Calcd for $C_{128}H_{189}O_{72}S_4Si$ $[M+H]^+$ 3033.9780, found 3033.9751. $[\alpha]_D^{32}=+33.9°$ ($c=1.0$ in

$CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.30, 5.26 (2H, m, H-3, 4), 5.23–5.19 (2H, m, H-2, 3′), 5.02 (1H, m, H-2′), 5.00 (1H, H-1′), 4.83 (1H, H-1), 4.29–4.21 (2H, m, H-6a, 6′a), 4.14 (1H, m, H4′), 4.13–4.03 (3H, m, H-6b, 5′, 6′b), 3.88 (1H, m, H-5), 3.79 (1H, m, H-7a), 3.54 (1H, m, H-7b), 2.56 (2H, t, H-9, $J_{8,9}=6.96$ Hz), 2.51 (2H, t, H-10, $J_{10,11}=6.96$ Hz), 2.21, 2.14, 2.13, 2.11, 2.05, 1.99 (21H, s, CH_3(Ac)), 1.87 (2H, m, H-8), 1.57 (2H, m, H-11), 0.65 (2H, m, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.5, 170.4, 170.3, 169.9, 169.71, 169.65, 169.4 (C, *C*=O(Ac)), 98.8 (CH, C-1′), 97.3 (CH, C-1), 75.0 (CH, C-3), 70.8 (CH, C-2), 69.8 (CH, C-2′), 69.2 (CH, C-5′), 68.6 (CH, C-5), 68.1 (CH, C-3′), 67.4 (CH, C-4′), 66.4 (CH_2, C-7), 65.7 (CH, C-4), 62.4, 62.2 (CH_2, C-6, 6′), 35.9 (CH_2, C-10), 28.9 (CH_2, C-8), 28.6 (CH_2, C-9), 24.0 (CH_2, C-11), 20.8, 20.68, 20.65, 20.58, 20.53, 20.47 (CH_3, *C*H_3(Ac)), 11.9 (CH_2, C-12).

Dumbbell(*1*)6-α-1,3-*Man*(*OAc*). Yield 61.3 mg (31% (2 steps)). HRMS (FAB): Calcd for $C_{200}H_{301}O_{108}S_6Si_3$ $[M+H]^+$ 4706.5693, found 4706.5679. $[\alpha]_D^{33}=+33.0°$ ($c=1.0$ in $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, TMS) δ (ppm); 5.30, 5.26 (6H, m, H-3, 4), 5.23–5.19 (6H, m, H-2, 3′), 5.02 (3H, m, H-2′), 4.99 (3H, d, H-1′, $J_{1',2'}=1.61$ Hz), 4.83 (3H, H-1), 4.29–4.21 (6H, m, H-6a, 6′a), 4.14 (3H, dd, H-4′, $J_{3',4'}=3.75$ Hz, $J_{4',5'}=10.17$ Hz), 4.13–4.03 (9H, m, H-6b, 5′, 6′b), 3.87 (3H, m, H-5), 3.79 (3H, m, H-7a), 3.54 (3H, m, H-7b), 2.56 (6H, t, H-9, $J_{8,9}=6.96$ Hz), 2.51 (6H, t, H-10, $J_{10,11}=6.96$ Hz), 2.21, 2.14, 2.13, 2.11, 2.05, 1.99 (63H, s, CH_3(Ac)), 1.87 (6H, m, H-8), 1.56 (6H, m, H-11), 1.29 (2H, m, H-14), 0.65–0.60 (8H, m, H-12, 13), 0.54 (2H, m, H-15), −0.05 (3H, s, CH_3(Si–Me)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm); 170.5, 170.4, 170.3, 169.9, 169.75, 169.70, 169.5 (C, *C*=O(Ac)), 98.9 (CH, C-1′), 97.3 (CH, C-1), 75.1 (CH, C-3), 70.8 (CH, C-2), 69.8 (CH, C-2′), 69.3 (CH, C-5′), 68.6 (CH, C-5), 68.1 (CH, C-3′), 67.5 (CH, C-4′), 66.5 (CH_2, C-7), 65.8 (CH, C-4), 62.4, 62.2 (CH_2, C-6, 6′), 35.9 (CH_2, C-10), 29.0 (CH_2, C-8), 28.6 (CH_2, C-9), 24.1 (CH_2, C-11), 20.8, 20.72, 20.70, 20.62, 20.57, 20.52 (CH_3, *C*H_3(Ac)), 20.4 (CH_2, C-15), 18.2 (CH_2, C-14), 17.0 (CH_2, C-13), 12.0 (CH_2, C-12), −3.4 (CH_3, *C*H_3(Si–Me)).

4.3.7. Deprotection of carbosilane dendrimers with mannose and mannobiose: Fan(0)3-Man. A solution of sodium methoxide in methanol (12.7 mg, 235 μmol) was added to a solution of Fan(0)3-Man(OAc) (135.8 mg, 90.8 μmol) in methanol (1.5 mL) at room temperature under an argon atmosphere. The solution was stirred for 1 h, then the aqueous solution of sodium hydroxide (0.1 M) was added and was stirred at room temperature over night. After neutralizing with acetic acid, the solution was evaporated in vacuo. The residue was subjected to Sephadex G-25 size exclusion chromatography eluting with 5% (v/v) aqueous solution of acetic acid. The fractions containing carbosilane dendrimer were combined and lyophilized to yield Fan(0)3-Man as a white solid (54.8 mg (61%)): HRMS (ESI): Calcd for $C_{42}H_{74}O_{18}S_3SiNa$ $[M+Na]^+$ 1013.3704, found 1013.3696. $[\alpha]_D^{27}=+49.0°$ ($c=1.0$ in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 7.52–7.19 (5H, m, Ph), 4.84 (3H, H-1), 3.93 (3H, m, H-2), 3.89–3.64 (15H, m, H-3, 4, 6, 7a), 3.64–3.45 (6H, m, H-5, 7b), 2.55 (6H, m, H-9), 2.48 (6H, m, H-10), 1.84 (6H, m, H-8), 1.56 (6H, m, H-11), 0.89 (6H, m, H-12); ^{13}C NMR (100 MHz, D_2O) δ (ppm); 137.3 (C, Ph), 134.8, 129.8, 128.8 (CH, Ph), 100.7 (CH, C-1), 73.5 (CH, C-5), 71.7 (CH, C-4), 71.1 (CH, C-2), 67.2 (CH, C-3), 66.9 (CH_2, C-7), 61.5 (CH_2, C-6), 36.2 (CH_2, C-10), 30.1 (CH_2, C-8), 29.1 (CH_2, C-9), 24.6 (CH_2, C-11), 12.4 (CH_2, C-12).

Another carbosilane dendrimer with peripheral mannose or mannobiose acetate was deacetylated by the same method as Fan(0)3-Man. Carbosilane dendrimers with peripheral mannose or mannobiose which have no protective group of saccharide moieties were synthesized.

Ball(0)4-*Man*. Yield 64.8 mg (82%). HRMS (ESI): Calcd for $C_{48}H_{92}O_{24}S_4SiNa$ $[M+Na]^+$ 1231.4528, found 1231.4581. $[\alpha]_D^{24}=+52.7°$ ($c=1.0$ in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 4.87 (1H, H-1), 3.95 (1H, m, H-2), 3.89–3.78 (4H, m, H-3, 6a, 7a), 3.74 (1H, m, H-6b), 3.61 (2H, m, H-5, 7b), 2.65 (4H, m, H-9, 10), 1.93 (2H, m, H-8), 1.66 (2H, m, H-11), 0.76 (2H, m, H-12); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 100.6 (CH, C-1), 73.4 (CH, C-5), 71.6 (CH, C-4), 71.0 (CH, C-2), 67.2 (CH, C-3), 66.9 (CH_2, C-7), 61.5 (CH_2, C-6), 36.3 (CH_2, C-10), 30.1 (CH_2, C-8), 29.1 (CH_2, C-9), 24.8 (CH_2, C-11), 12.5 (CH_2, C-12).

Dumbbell(1)6-*Man*. Yield 33.6 mg (81%). HRMS (FAB): Calcd for $C_{80}H_{156}O_{36}S_6Si_3Na$ $[M+Na]^+$ 1991.7906, found 1991.7937. $[\alpha]_D^{30}=+46.3°$ ($c=1.0$ in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 4.90 (3H, d, $J_{1,2}=1.0$ Hz, H-1), 3.99 (3H, m, H-2), 3.92–3.75 (15H, m, H-3, 4, 6, 7a), 3.68–3.55 (6H, m, H-5, 7b), 2.66 (6H, m, H-9), 2.62 (6H, m, H-10), 1.93 (6H, m, H-8), 1.66 (6H, m, H-11), 1.48 (2H, m, H-14), 0.82–0.65 (10H, m, H-12, 13, 15), 0.06 (3H, s, Si–CH_3); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 100.1 (CH, C-1), 72.8 (CH, C-5), 71.2 (CH, C-4), 70.7 (CH, C-2), 66.8 (CH, C-3), 66.3 (CH_2, C-7), 60.9 (CH_2, C-6), 35.8 (CH_2, C-10), 29.5 (CH_2, C-8), 28.6 (CH_2, C-9), 24.4 (CH_2, C-11), 21.0 (CH_2, C-15), 20.3 (CH_2, C-14), 19.1 (CH_2, C-13), 12.0 (CH_2, C-12), −2.7 (CH_3, Si–*C*H_3).

Fan(0)3-α-1,3-*Man*. Yield 44.6 mg (quant.). HRMS (FAB): Calcd for $C_{60}H_{104}O_{33}S_3SiNa$ $[M+Na]^+$ 1499.5289, found 1499.5278. $[\alpha]_D^{22}=+78.7°$ ($c=0.87$ in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 7.52–7.25 (5H, m, Ph), 5.14 (3H, H-1′), 4.82 (3H, H-1), 4.08 (6H, m, H-2, 2′), 3.92–3.50 (36H, m, H-3, 4, 5, 6, 3′, 4′, 5′, 6′, 7), 2.52 (12H, m, H-9, 10), 1.85 (6H, m, H-8), 1.57 (6H, m, H-11), 0.91 (6H, m, H-12); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 137.1 (C, Ph), 134.6, 129.8, 128.6 (CH, Ph), 102.9 (CH, C-1′), 100.5 (CH, C-1), 79.3 (CH, C-3), 73.8 (CH, C-5), 73.4 (CH, C-5′), 71.0 (CH, C-3′), 70.8 (CH, C-2′), 70.5 (CH, C-2), 67.1 (CH, C-4′), 66.7 (CH, C-4), 66.1 (CH_2, C-7), 61.4 (CH_2, C-6′), 61.1 (CH_2, C-6), 35.9 (CH_2, C-10), 29.8 (CH_2, C-8), 28.9 (CH_2, C-9), 24.1 (CH_2, C-11), 12.1 (CH_2, C-12).

Ball(0)4-α-1,3-*Man*. Yield 75.5 mg (quant.). HRMS (ESI): Calcd for $C_{72}H_{132}O_{44}S_4SiNa$ $[M+Na]^+$ 1879.6641, found 1879.6622. $[\alpha]_D^{30}=+100.4°$ ($c=1.0$ in H_2O). ^1H NMR (200 MHz, D_2O) δ (ppm); 5.14 (H-1′), 4.92 (1H, H-1), 4.15 (2H, m, H-2, 2′), 4.08–3.60 (12H, m, H-3, 4, 5, 6, 3′, 4′, 5′, 6′, 7), 2.71 (4H, m, H-9, 10), 2.02 (2H, m, H-8), 1.73 (2H, m, H-11), 0.82 (2H, m, H-12); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 103.0 (CH, C-1′), 100.6 (CH, C-1), 79.4 (CH, C-3), 73.8 (CH, C-5), 73.5 (CH, C-5′), 71.1 (CH, C-3′), 70.9 (CH,

C-2′), 70.6 (CH, C-2), 67.3 (CH, C-4′), 66.9 (CH, C-4), 66.3 (CH_2, C-7), 61.5 (CH_2, C-6′), 61.3 (CH_2, C-6), 36.2 (CH_2, C-10), 29.8 (CH_2, C-8), 29.0 (CH_2, C-9), 24.8 (CH_2, C-11), 12.4 (CH_2, C-12).

Dumbbell(1)6-α-1,3-*Man*. Yield: 33.8 mg (90%). HRMS (ESI): Calcd for $C_{116}H_{216}O_{66}S_6Si_3Na_2/2$ $[M+2Na]^{2+}/2$ 1493.5487, found 1493.5482. $[\alpha]_D^{29}=+48.3°$ ($c=1.0$ in H_2O). ^1H NMR (400 MHz, D_2O) δ (ppm); 5.15 (3H, H-1′), 4.86 (3H, H-1), 4.09 (6H, m, H-2, 2′), 4.00–3.65 (33H, m, H-3, 4, 5, 6, 3′, 4′, 5′, 6′, 7a), 3.63 (3H, m, H-7b), 2.65 (12H, m, H-9, 10), 1.94 (6H, m, H-8), 1.65 (6H, m, H-11), 1.46 (2H, m, H-14), 0.75 (8H, m, H-12, 13), 0.69 (2H, m, H-15), 0.05 (3H, s, Si–CH_3); ^{13}C NMR (50 MHz, D_2O) δ (ppm); 103.1 (CH, C-1′), 100.7 (CH, C-1), 79.6 (CH, C-3), 74.0 (CH, C-5), 73.6 (CH, C-5′), 71.3 (CH, C-3′), 71.0 (CH, C-2′), 70.7 (CH, C-2), 67.5 (CH, C-4′), 67.0 (CH, C-4), 66.4 (CH_2, C-7), 61.8 (CH_2, C-6′), 61.5 (CH_2, C-6), 36.5 (CH_2, C-10), 30.0 (CH_2, C-8), 29.2 (CH_2, C-9), 25.0 (CH_2, C-11), 21.0 (CH_2, C-15), 19.4 (CH_2, C-14), 18.1 (CH_2, C-13), 12.6 (CH_2, C-12), −1.6 (CH_3, Si–CH_3).

4.4. Calorimetry

Isothermal titration microcalorimetry was performed using the MicroCal Omega titration microcalorimeter. Details of instrument design and data analysis are described by Wiseman et al.[31] A solution of concanavalin A (0.21 mM) in a buffer of 50 mM 3,3-dimethylglutarate, 250 mM NaCl, and 1 mM each of $CaCl_2$ and $MnCl_2$ at pH 5.2 were placed in the sample cell. Carbosilane dendrimer solutions ([mannose]=2.1 mM) in a buffer identical to that used for protein solutions were added in 10 μL increments during 30 s, with 3 min intervals between injections. Each calorimetric titration was performed at a sample cell temperature of 298 K. Protein concentrations were determined spectrometrically using an extinction coefficient of $\varepsilon_{280}=1.24$ for a 1 mg/mL of solution.

The heat evolved upon each injection was digitally recorded, and the data were integrated to generate a titration curve upon completion of the experiment. The stoichiometry of the association, n, binding constant, K, and the change in enthalpy, ΔH, were obtained from a nonlinear least-squares fit using the Origin software program. All data are presented on a valency-corrected basis.

Acknowledgements

This work was supported by a Health and Labour Sciences Research Grant for Research on Advanced Medical Technology (14-N-015) from the Ministry of Health, Labour, and Welfare, Japan.

References and notes

1. Varki, A. *Glycobiology* **1993**, *3*, 97–130.
2. *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1999.
3. Kuo, C.-C.; Takahashi, N.; Swanson, A. F.; Dzeki, Y.; Hakomori, S.-I. *J. Clin. Invest.* **1996**, *98*, 2813–2818.
4. Helenius, A.; Trombetta, E. S.; Herbert, D. N.; Simons, J. F. *Trends Cell Biol.* **1997**, *7*, 193–200.
5. Wada, I.; Kai, M.; Imai, S.; Kanoh, H. *EMBO J.* **1997**, *16*, 5420–5432.
6. Ellgaad, L.; Helenius, A. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 181–191.
7. Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
8. (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720. (b) Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 719–728.
9. (a) Lindhorst, Th. K. *Top. Curr. Chem.* **2002**, *218*, 201–235. (b) Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 696–703. (c) Jayaraman, N.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Eur. J.* **1997**, *3*, 1193–1199. (d) Roy, R. *Top. Curr. Chem.* **1997**, *187*, 241–274.
10. (a) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578. (b) Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **2000**, *17*, 543–551. (c) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755–2794.
11. (a) Kawaguchi, K.; Lee, Y. C. *Proteins Nucleic Acids Enzymes* **1980**, *25*, 707–724. (b) Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225–281. (c) Connolly, D. T.; Townsend, R. R.; Kawaguchi, K.; Bell, W. R.; Lee, Y. C. *J. Biol. Chem.* **1982**, *257*, 939–945. (d) Lee, R. T.; Lin, P.; Lee, Y. C. *Biochemistry* **1984**, *23*, 4255–4261. (e) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321–327.
12. (a) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215–3237. (b) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742–748.
13. (a) Pagé, D.; Zanini, D.; Roy, R. *Bioorg. Med. Chem.* **1996**, *4*, 1949–1961. (b) Pagé, D.; Aravind, S.; Roy, R. *Chem. Commun.* **1996**, 1913–1914. (c) Pagé, D.; Roy, R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1765–1770. (d) Pagé, D.; Roy, R. *Bioconjugete Chem.* **1997**, *8*, 714–723. (e) Pagé, D.; Roy, R. *Glycoconjugete J.* **1997**, *14*, 345–356. (f) Roy, R.; Pagé, D.; Perez, S. F.; Bencomo, V. V. *Glycoconjugete J.* **1998**, *15*, 251–263. (g) Das, S. K.; Trono, M. C.; Roy, R. *Methods Enzymol.* **2003**, *362*, 3–17.
14. (a) Lindhorst, Th. K. *Nachr. Chem. Tech. Lab.* **1996**, *44*, 1073–1079. (b) Lindhorst, Th. K.; Kieburg, C.; Krallmann-Wenzel, U. *Glycoconjugate J.* **1998**, *15*, 605–613. (c) König, B.; Fricke, T.; Waßmann, A.; Krallmann-Wensel, U.; Lindhorst, Th. K. *Tetrahedron Lett.* **1998**, *39*, 2307–2310. (d) Kötter, S.; Krallmann-Wensel, U.; Ethlers, S.; Lindhorst, Th. K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2193–2200. (e) Dubber, M.; Lindhorst, Th. K. *J. Org. Chem.* **2000**, *65*, 5275–5281. (f) Lindhorst, Th. K.; Dubber, M.; Krallmann-Wenzel, U.; Ehlers, S. *Eur. J. Org. Chem.* **2000**, 2027–2034. (g) Horst, A. K.; Kötter, S.; Lindhorst, Th. K.; Ludwig, A.; Brandt, E.; Wagener, C. *Med. Microbiol. Immunol.* **2001**, *190*, 145–149. (h) Dubber, M.; Lindhorst, Th. K. *Synthesis* **2001**, 327–330. (i) Patel, A.; Lindhorst, Th. K. *J. Org. Chem.* **2001**, *66*, 2674–2680. (j) Röckendorf, N.; Sperling, O.; Lindhorst, Th. K. *Aust. J. Chem.* **2002**, *55*, 87–93. (k) Boysen, M. M. K.; Elsner, K.; Sperling, O.; Lindhorst, Th. K. *Eur. J. Org. Chem.* **2003**, 4376–4386. (l) Röchendorf, N.; Lindhorst, Th. K. *J. Org. Chem.* **2004**, *69*, 4441–4445. (m) Köhn, M.; Benito, J. M.; Ortiz Mellet, C.; Lindhorst, Th. K.; García Fernández, J. M. *ChemBioChem* **2004**, *5*, 771–777.
15. (a) Ashton, P. R.; Hounsell, E. F.; Jayaraman, N.; Nilsen,

T. M.; Spencer, N.; Fraser Stoddart, J.; Young, M. *J. Org. Chem.* **1998**, *63*, 3429–3437. (b) Nakata, E.; Nagase, T.; Shinkai, S.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 490–495.

16. Boysen, M. M. K.; Lindhorst, Th. K. *Tetrahedron* **2003**, *59*, 3895–3898.
17. (a) Matsuoka, K.; Terabatake, M.; Saito, Y.; Hagihara, C.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2709–2713. (b) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839–7842. (c) Matsuoka, K.; Saito, Y.; Terunuma, D.; Kuzuhara, H. *Kobunshi Ronbunshu* **2000**, *57*, 691–695. (d) Matsuoka, K.; Kurosawa, H.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Carbohydr. Res.* **2000**, *329*, 765–772. (e) Matsuoka, K.; Oka, H.; Koyama, T.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **2001**, *42*, 3327–3330. (f) Matsuoka, K.; Ohtawa, T.; Hinou, H.; Koyama, T.; Esumi, Y.; Nishimura, S.-I.; Hatano, K.; Terunuma, D. *Tetrahedron Lett.* **2003**, *44*, 3617–3620.
18. Nishikawa, K.; Matsuoka, K.; Kita, E.; Okabe, N.; Mizuguchi, M.; Hino, K.; Miyazawa, S.; Yamasaki, C.; Aoki, J.; Takashima, S.; Yamakawa, Y.; Nishijima, M.; Terunuma, D.; Kuzuhara, H.; Natori, Y. *Proc. Natl Acad. Sci. U.S.A.* **2002**, *99*, 7669–7674.
19. Takano, T.; Nakatsubo, F.; Murakami, K. *Carbohydr. Res.* **1990**, *203*, 341–342.
20. Houseman, B. T.; Gawalt, E. S.; Mrksich, M. *Langmuir* **2003**, *19*, 1522–1531.
21. Ravindranathan Karcha, K. P.; Jennings, H. J. *J. Carbohydr. Chem.* **1990**, *9*, 777–781.
22. Betaneli, V. I.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285–291.
23. Zhang, J.; Kong, F. *Tetrahedron: Asymmetry* **2002**, *13*, 243–252.
24. (a) Pompipom, M. M. *Carbohydr. Res.* **1977**, *59*, 311–317. (b) Lee, E. E.; Wood, J. O. *Carbohydr. Res.* **1979**, *75*, 322–324. (c) Winnik, F. M.; Carver, J. P.; Krepinsky, J. J. *J. Org. Chem.* **1982**, *47*, 2701–2707. (d) Itoh, Y.; Tejima, S. *Chem. Pharm. Bull.* **1984**, *32*, 957–966. (e) Szurmai, Z.; Janossy, L.; Szlagyi, Z.; Vekey, K. *J. Carbohydr. Chem.* **1998**, *17*, 417–437.
25. (a) Terunuma, D.; Kato, T.; Nishio, R.; Matsuoka, K.; Kuzuhara, H.; Aoki, Y.; Nohira, H. *Chem. Lett.* **1998**, 59–60. (b) Terunuma, D.; Kato, T.; Nishio, R.; Aoki, Y.; Nohira, H.; Matsuoka, K.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 2129–2134. (c) Tsuchida, T.; Shimazaki, C.; Hatano, K.; Matsuoka, K.; Aoki, Y.; Nohira, H.; Esumi, Y.; Terunuma, D. *Kobunshi Ronbunshu* **2003**, *60*, 561–568.
26. (a) van der Made, A. W.; van Leeuwen, P. W. N. M. *J. Chem. Soc., Chem. Commun.* **1992**, 1400–1401. (b) van der Made, A. W.; van Leeuwen, P. W. N. M.; de Wilde, J. C.; Brandes, R. A. C. *Adv. Mater.* **1993**, *5*, 466–468. (c) Muzafarov, A. M.; Gorbatsevich, O. B.; Rebrov, E. A.; Ignat'eva, G. M.; Chenskaya, T. B.; Myakushev, V. D.; Bulkin, A. F.; Papkov, V. S. *Vysokomol. Soedin., Ser. A Ser. B* **1993**, *35*, 1867–1872. (d) Seyferth, D.; Son, D. Y.; Rheingold, A. L.; Ostrander, R. L. *Organometallics* **1994**, *13*, 2682–2690.
27. (a) Winnik, F. M.; Brisson, J.-R.; Carver, J. P.; Krepinsky, J. J. *Carbohydr. Res.* **1982**, *103*, 15–28. (b) Madiyalakan, R.; Chowdhary, M. S.; Rana, S. S.; Matta, K. L. *Carbohydr. Res.* **1986**, *152*, 183–194.
28. (a) Williams, B. A.; Chervenak, M. C.; Toone, E. J. *J. Biol. Chem.* **1992**, *267*, 22907–22911. (b) Chervenak, M. C.; Toone, E. J. *J. Am. Chem. Soc.* **1994**, *116*, 10533–10539. (c) Chervenak, M. C.; Toone, E. J. *Bioorg. Med. Chem.* **1996**, *4*, 1963–1977. (d) Dimick, S. M.; Powell, S. C.; McMahon, S. A.; Moothoo, D. N.; Naismith, J. H.; Toone, E. J. *J. Am. Chem. Soc.* **1999**, *121*, 10286–10296.
29. Edelhoch, H. *Biochemistry* **1967**, *6*, 1948–1954.
30. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Robers, P. A.; Smith, F. *Anal. Chem.* **1956**, *28*, 350–356.
31. Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *179*, 131–137.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2761–2766

Total syntheses of the sesquiterpenoids (+)-*trans*-dracunculifoliol and (+)-4-hydroxyoppositan-7-one

Renata M. Oballa,* Rebekah Carson, Susan Lait, Jay A. Cadieux and Joël Robichaud

Department of Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research, 16711 TransCanada Highway, Kirkland, Que., Canada H9H 3L1

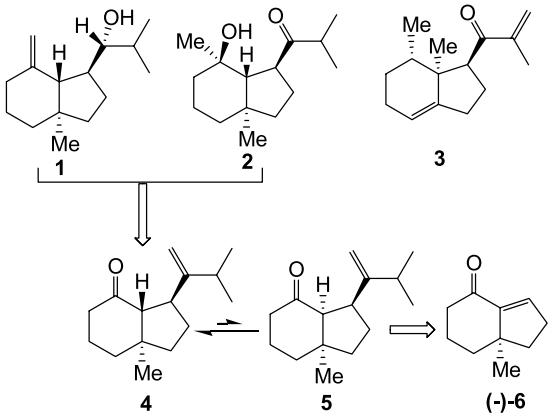
Received 2 December 2004; revised 21 January 2005; accepted 21 January 2005

Abstract—Sesquiterpenoids (+)-*trans*-dracuncuflifoliol (**1**) and (+)-4-hydroxyoppositan-7-one (**2**) were prepared stereoselectively from enantiomerically pure (7a*R*)-7a-methyl-1,2,5,6,7,7a-hexahydro-4*H*-inden-4-one ((−)-**6**), whose synthesis was described herein. Conjugate addition of the organocopper (I) reagent **10** to (−)-**6**, followed by epimerization of the ring junction, generated 3 of the 4 contiguous chiral centers of both natural products.

1. Introduction

The sesquiterpenoid (+)-*trans*-dracunculifoliol (**1**) was isolated from Vassoura oil and was previously synthesized as a racemate in eighteen linear steps with an overall yield of 1.8%.[1] A structurally related natural product, (+)-4-hydroxyoppositan-7-one (**2**) was isolated from the liverworts *Chiloscyphus pallescens*[2] and *C. rivularis*[3] and has not yet been synthesized. While the absolute stereochemistry of either **1** or **2** has not been reported, the natural product chiloscyphone (**3**), isolated from *C. polyanthus*, was elucidated by an X-ray crystallographic study.[4] One of the goals of this synthetic study is to determine if the absolute stereochemistry of **1** and **2** is as shown in Scheme 1.

Natural products **1** and **2** share a common *trans*-fused [4.3.0] bicyclo skeleton and it was envisaged that three of the four contiguous chiral centres could be created by the conjugate addition of an organocopper (I) reagent to enone (−)-**6**, to generate **5** which, after epimerization of the centre alpha to the carbonyl, gives the common intermediate **4** (Scheme 1). The stereochemical outcome of the key conjugate addition reaction was predicted to parallel those obtained in the previously reported 1,4-addition reactions to bicyclo[4.3.0]non-9-en-2-ones.[5,6] Conjugate addition of organocoper (I) reagents to enones of general structure **6** proceeds stereoselectively, with the group being introduced *trans* to the angular methyl group (i.e., **5**). In addition, a



Scheme 1.

predominant *cis*-fused ring junction is obtained.[6] However, equilibration of structurally related compounds generally dictates that the *cis*-fused isomer can be equilibrated with base to produce a mixture of epimers in which the *trans*-fused isomer, the thermodynamically more stable of the two epimers, predominates.[7]

2. Results and discussion

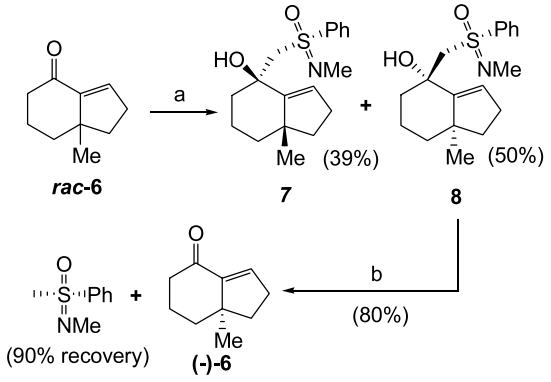
2.1. Synthesis of chiral enone (−)-6

Ohkubo et al.[8] reported an enantioselective synthesis of (+)-**6**, but the route is laborious (8 steps from a non-commercial starting material) with an overall yield of 38%.

Keywords: Natural product synthesis; Sesquiterpenoids.
* Corresponding author. Tel.: +1 514 428 3617; fax: +1 514 428 4900; e-mail: renata_oballa@merck.com

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.089

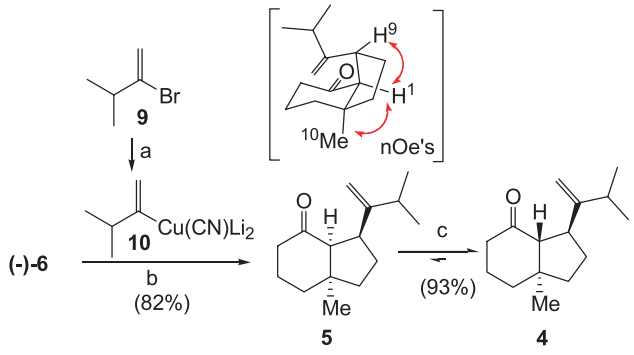
The synthesis of the chiral enone (−)-**6** was readily accomplished via the route outlined in Scheme 2. The racemic enone **6**[9] was resolved by condensation with the anion of (+)-(*S*)-*N*,*S*-dimethyl-*S*-phenylsulfoximine.[10] These conditions delivered a readily separable mixture of diastereomers **7** (39%) and **8** (50%). Sulfoximine-mediated resolution of ketones is ideally suited for bicyclic enones which exhibit diastereoface specificity towards the addition of the anion of (+)-(*S*)-*N*,*S*-dimethyl-*S*-phenylsulfoximine. Lastly, thermolysis of **8** regenerated the sulfoximine and the optically pure (−)-**6**. The absolute stereochemistry was assigned based on the comparison of the sign of rotation to that of the reported (+)-**6**,[8] in which the absolute stereochemistry is known.



Scheme 2. (a) (*S*)-(+)-PhS(O)(NMe)CH_3, *n*-BuLi, THF, −78 °C; (b) toluene, 110–120 °C.

2.2. Synthesis of keto-alkene 4

For the synthesis of the key intermediate **4**, the stereoselective conjugate addition of the organocopper (I) reagent **10** to the bicyclic enone (−)-**6** was required (Scheme 3). Reagent **10** was prepared by sequential treatment of **9**[11] with *t*-BuLi and LiCl/CuCN. The conjugate addition of reagent **10** to the bicyclic enone (−)-**6** in the presence of TMSBr provided, after hydrolysis of the resultant silyl enol ether, one single diastereomer **5** in 82% yield. It was gratifying to find that the conjugate addition had proceeded stereoselectively, as expected (vide supra). The relative configuration of **5** was confirmed by the following ^{1}H NMR nOe difference experiments. Irradiation of the signal due to Me-10 caused an enhancement of the signal for H-1 and vice versa (Scheme 3) and this confirmed the *cis*-fused nature of the ring junction. Irradiation of the signal due to H-9 caused an enhancement of the signal for H-1, thereby verifying that reagent **10** had introduced the vinyl group *trans* to the angular methyl group (Me-10), as predicted.

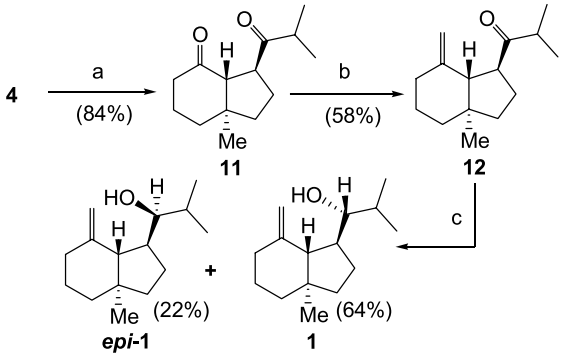


Scheme 3. (a) *t*-BuLi, THF, −78 °C; LiCl/CuCN. (b) TMSBr, THF, −78 °C; H_2O, NH_4OH–NH_4Cl. (c) NaOMe, MeOH, 55 °C.

A *trans*-fused ring junction was required for the synthesis of natural products **1** and **2**. According to previous studies[5,6] and results reported by Dana and co-workers,[7] epimerization of **5** should lead to a mixture of compounds in which the *trans*-fused epimer **4** is thermodynamically favored. In fact, treatment of **5** with NaOMe in MeOH at 55 °C resulted in a 33:1 mixture of epimers **4** and **5** (Scheme 3). These two epimers were readily separated by flash chromatography on silica gel, and the desired synthetic intermediate **4** was obtained in 93% yield.

2.3. Synthesis of (+)-*trans*-dracunculifoliol (1)

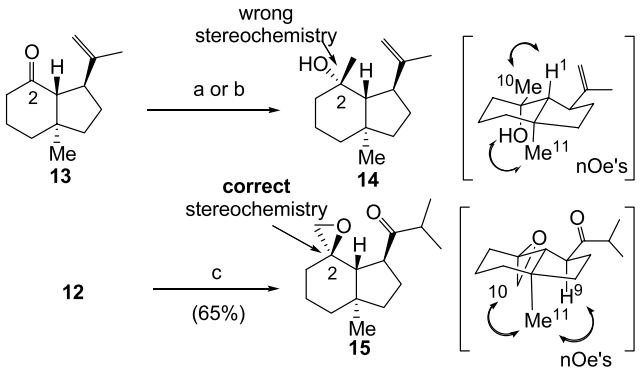
Elaboration of intermediate **4** to (+)-*trans*-dracunculifoliol (**1**) involved ozonolysis of the exocyclic alkene to afford, after treatment with triphenylphosphine, the diketone **11** (Scheme 4). A regioselective Wittig reaction was then attempted and complete selectivity was obtained to generate the desired keto alkene **12** in 58% yield. This regioselective Wittig reaction on the sterically less hindered ketone eliminated the need for a protection/deprotection strategy. Interestingly, even when 1.5 equiv of ylide were used, no *bis*-olefinated product was detected. Reduction of **12** with Super Hydride® afforded a mixture of **1** and *epi*-**1** in a 3:1 ratio.[12] The ^{1}H NMR and ^{13}C NMR data derived from the synthetic **1** are identical with those of the natural product **1**.[1] The absolute configuration of the natural product was confirmed by the fact that the sign of the specific optical rotation of the synthetic material ($[\alpha]^{20}_D = +54.4$ (*c* 0.8, $CHCl_3$)) is the same as that reported for the natural product ($[\alpha]^{20}_D = +19$ (*c* 0.4, $CHCl_3$), 85% pure by GC[1]). Thus, the synthesis of **1** was accomplished in a concise (5 steps) and enantioselective manner. The overall yield of **1** from (−)-**6** was 24%.



Scheme 4. (a) O_3, CH_2Cl_2, −78 °C; PPh_3, rt; (b) Ph_3PCH_3Br, *n*-BuLi, THF, 0 °C; (c) $LiEt_3BH$, THF, −78 °C to rt.

2.4. Synthesis of (+)-4-hydroxyoppositan-7-one (2)

Elaboration to (+)-4-hydroxyoppositan-7-one (**2**) required the formation of a quaternary center at C-2. The first attempt involved the addition of a methyl group to the ketone of the

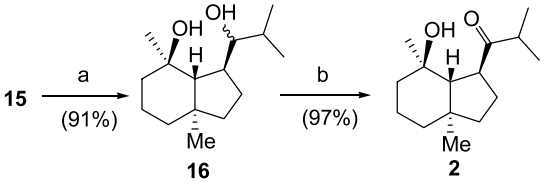


Scheme 5. (a) MeMgBr, TMSBr, Et_2O, −78 to 0 °C; (b) MAD, MeLi, PhMe, −78 to −25 °C; (c) *m*-CPBA, CH_2Cl_2, 0 °C.

model compound **13** (Scheme 5). Reaction of **13** with MeMgBr provided the tertiary alcohol **14** in 55% yield with exclusively one stereoisomer at C-2. The following ^{1}H NMR nOe difference experiments confirmed that the methyl group added from the less sterically hindered face of the molecule to provide the undesired stereochemistry at C-2 (axial OH). Irradiation of the signal due to Me-11 caused an enhancement of the signal for the tertiary alcohol proton (Scheme 5) and irradiation of the signal due to the newly introduced Me-10 caused an enhancement of the signal for H-1, thereby verifying that the Grignard reagent had delivered the methyl group *trans* to the axial angular methyl group (Me-11). Even treatment with MAD (methyl aluminum-*bis*-(2,6-di-*tert*-butyl-4-methylphenoxide)), a Lewis acid known to complex with the less hindered face of carbonyls, gave exclusively **14** upon addition of MeLi.

Knowing that the face opposite to the angular axial methyl group is more accessible, we decided to add the equatorial alcohol functionality of **2** to the alkene of **12** from the less hindered face. This transformation firstly required the epoxidation of **12** with *m*-CPBA which did, indeed, provide the desired epoxide **15** in which the stereochemistry was confirmed by the following ^{1}H NMR nOe difference experiments (Scheme 5). Irradiation of the signal due to Me-11 caused an enhancement of the signals for H-9 and the epoxide protons on C-10 and vice versa.

The reductive opening of the epoxide **15** was accomplished with $LiEt_3BH$ to generate the desired tertiary alcohol with concomitant reduction of the ketone to provide the diol **16** in 91% yield (Scheme 6). The secondary alcohol of **16** was oxidized to the ketone with TPAP, thereby completing the first total synthesis of (+)-4-hydroxyoppositan-7-one (**2**) in 7 steps and 25% overall yield from (−)-**6**. The ^{1}H NMR and ^{13}C NMR data derived from the synthetic **2** are identical with those of natural **2**.[2] The absolute configuration of the



Scheme 6. (a) $LiEt_3BH$, THF, rt; (b) TPAP, NMO, 4A mol. sieves, CH_2Cl_2, rt.

natural product was confirmed by the fact that the sign of the specific optical rotation of the synthetic material ($[\alpha]^{20}_D = +73.6$ (*c* 0.65, $CHCl_3$)) is the same as that reported for the natural product ($[\alpha]^{20}_D = +76.1$ (*c* 0.28, $CHCl_3$)[2]).

3. Conclusion

The work described in this paper culminated in the first total synthesis of (+)-4-hydroxyoppositan-7-one (**2**) and the first enantiopure synthesis of (+)-*trans*-dracunculifoliol (**1**). These syntheses started with the enantiomerically pure bicyclic enone (−)-**6**, whose efficient synthesis is described herein. The key step used to generate the advanced common intermediate **4** involved a stereoselective conjugate addition of the organocopper (I) reagent **10** to enone (−)-**6**, followed by equilibration of the ring junction to the thermodynamically more stable *trans*-fused isomer **4**. This sequence efficiently generated 3 of the 4 contiguous chiral centers required in the natural products. Intermediate **4** was elaborated in a straightforward manner to the keto alkene **12** which was then used to complete the syntheses of both natural products **1** and **2**. The absolute stereochemistries of **1** and **2** were also confirmed by these syntheses.

4. Experimental

4.1. General

All substrates and reagents were obtained commercially and used without further purification unless otherwise noted. All glassware was dried in an oven overnight and flame dried under dry nitrogen. Reactions were carried out with continuous stirring under a positive pressure of nitrogen except where noted. Flash chromatography was carried out with silica gel 60, 230–400 mesh. Anhydrous solvents were purchased from Aldrich and used without further purification. TSMBr was distilled from CaH_2 immediately before use and LiCl was flame dried under vacuum. Proton (^{1}H NMR) and carbon (^{13}C NMR) magnetic resonance spectra were recorded on a Bruker AMX spectrometer at 300 and 75.3 MHz, respectively, unless otherwise noted. All spectra were recorded in $CDCl_3$ using residual solvent ($CHCl_3$) as internal standard. Signal multiplicity was designated according to the following abbreviations: s=singlet, d=doublet, dd=doublet of doublets, ddd=doublet of doublet of doublets, t=triplet, q=quartet, dt=doublet of triplets, dq=doublet of quartets, m=multiplet, br s=broad singlet, br d=broad doublet. Elemental analyses were provided by Oneida Research Services Inc., Whitesboro, NY. High resolution mass spectra (HRMS-FAB$^+$) were obtained at the Biomedical Mass Spectrometry Unit, McGill University, Montreal, Quebec, Canada. Rotations were measured on a Perkin Elmer polarimeter (model #241).

4.1.1. (4*S*,7a*R*)-7a-Methyl-4-[(*N*-methyl-*S*-phenylsulfonimidoyl)methyl]-2,4,5,6,7,7a-hexahydro-1*H*-inden-4-ol (8). To a cold (−30 °C) solution of (+)-(*S*)-*N*,*S*-dimethyl-*S*-phenylsulfoximine (13.6 mL, 15.8 g, 93.3 mmol, 2 equiv) in dry THF (230 mL) was slowly added *n*-butyllithium (37.3 mL, 2.5 M in hexanes,

93.3 mmol, 2 equiv). The reaction was then warmed to room temperature and stirred for 15 min. Once formation of the sulfoximine ylide was complete, the mixture was cooled to −78 °C and a solution of 6-methylbicyclo[4.3.0]non-9-en-2-one (*rac*-**6**)[9] (7.0 g, 47 mmol, 1 equiv) in THF (50 mL) was added via cannula, rinsing with THF (2×5 mL). The reaction was allowed to stir at −78 °C for 1 h, then poured into a 1:2 mixture of aqueous saturated ammonium chloride and diethyl ether (200 mL). The aqueous phase was extracted with diethyl ether (4×200 mL) and the combined organic extracts were washed with brine (150 mL) and dried over sodium sulfate. Gradient flash chromatography of the crude residue (90/10→80/20 hexane/ethyl acetate) afforded, in order of elution, diastereomers **8** (7.1 g, 47 or 50% yield based on recovered starting material) and **7** (5.4 g, 36 or 39% yield based on recovered starting material).

Compound **8**. $[\alpha]^{20}_{D}=+21.5$ (*c* 1.185, $CHCl_3$); ^{1}H NMR: δ 7.87 (2H, d), 7.58 (3H, m), 5.83 (1H, br s), 3.38 (2H, q), 2.65 (3H, s), 2.19 (2H, m), 1.20–1.75 (9H, m), 0.80 (3H, s); ^{13}C NMR: δ 150.9, 138.2, 133.0, 129.3, 129.2, 128.8, 125.7, 72.8, 62.2, 46.2, 43.9, 40.7, 39.5, 31.4, 28.6, 24.4, 22.4, 20.7; HRMS (FAB+) *m*/*z* Calcd for $C_{18}H_{25}NO_2S$ 320.1606, found: 320.1685. Anal. Calcd for $C_{18}H_{25}NO_2S$: C, 67.67; H, 7.89; N, 4.38, found: C, 67.64; H, 7.51; N, 4.33.

4.1.2. (4*R*,7a*S*)-7a-Methyl-4-[(*N*-methyl-*S*-phenylsulfonimidoyl)methyl]-2,4,5,6,7,7a-hexahydro-1*H*-inden-4-ol (7). $[\alpha]^{20}_{D}=+36.3$ (*c* 1.075, $CHCl_3$); ^{1}H NMR: δ 7.81 (2H, d), 7.62 (3H, m), 5.87 (1H, br s), 3.35 (2H, br s), 2.61 (3H, s), 2.20–2.45 (2H, m), 1.20–1.85 (9H, m), 0.98 (3H, s). ^{13}C NMR: δ 149.4, 138.9, 132.9, 129.3, 128.9, 128.8, 126.4, 72.1, 62.7, 60.2, 46.4, 43.6, 41.5, 40.9, 28.8, 28.7, 24.1, 20.0; HRMS (FAB+) *m*/*z* Calcd for $C_{18}H_{25}NO_2S$ 320.1606, found: 320.1685. Anal. Calcd for $C_{18}H_{25}NO_2S$: C, 67.67; H, 7.89; N, 4.38, found: C, 67.10; H, 7.70; N, 4.32.

4.1.3. (7a*R*)-7a-Methyl-1,2,5,6,7,7a-hexahydro-4*H*-inden-4-one ((−)-6). A solution of **8** (10.1 g, 31.6 mmol, 1 equiv) in toluene (200 mL) was refluxed overnight (oil bath temperature 110–120 °C). Since it was difficult to remove the toluene by rotary evaporation without also evaporating the product (−)-**6**, the reaction mixture was loaded directly onto a silica gel column and flushed with hexane to elute the toluene. This was followed by gradient elution (100/0→90/10 hexane/diethyl ether) to afford (−)-**6** (3.7 g, 79% yield). $[\alpha]^{20}_{D}=-114.5$ (*c* 1.135, $CHCl_3$). ^{1}H NMR identical to that reported for *rac*-**6**.[9] In order to recover the (+)-(*S*)-*N*,*S*-dimethyl-*S*-phenylsulfoximine, the column was eluted with ethyl acetate/acetone (50/50) to afford the sulfoximine (3.6 g, 90% recovery) in an unchanged state of optical purity.

4.1.4. (3*S*,3a*S*,7a*R*)-3-(1-Isopropylvinyl)-7a-methyloctahydro-4*H*-inden-4-one (5). To a cold (−78 °C), yellow solution of *t*-butyllithium (1.7 M in pentane, 31 mL, 53 mmol, 6 equiv) in THF (200 mL) was slowly added 2-bromo-3-methylbut-1-ene (**9**)[11] (4.0 g, 27 mmol, 3 equiv) in THF (50 mL) over 30 min. To the resultant clear, cold (−78 °C) solution was added, via cannula, a solution of LiCl (2.3 g, 53 mmol, 6 equiv) and CuCN (2.6 g, 29 mmol, 3.2 equiv) in THF (80 mL) followed by TMSBr (4.5 mL, 34 mmol, 3.9 equiv). This was immediately followed by the addition of a solution of (−)-**6** (1.3 g, 8.8 mmol, 1 equiv) in THF (10 mL), via cannula. The orange solution was stirred at −78 °C for 4 h followed by quenching with aqueous HCl (1%, 200 mL). After warming to room temperature, the mixture was poured into diethyl ether (400 mL) and sat. aq. NH_4Cl/NH_4OH (pH 8, 400 mL) and stirred vigorously overnight. The deep blue aqueous phase was extracted with diethyl ether (4×150 mL) and the combined organic extracts were washed with water (2×150 mL) and brine (200 mL), then dried over $MgSO_4$. Upon concentration, the residual oil was subjected to flash column chromatography (95/5 hexane/ethyl acetate) to yield **5** (1.4 g, 70% yield or 82% based on recovered starting material) followed by recovered starting material (−)-**6** (190 mg). ^{1}H NMR: δ 4.91 (1H, s), 4.84 (1H, s), 3.07 (1H, dt, $J=10.9$, 7.3 Hz), 2.59 (1H, d, $J=10.6$ Hz), 2.11–2.28 (3H, m), 1.97–2.08 (1H, m), 1.85–1.93 (1H, m), 1.65–1.84 (4H, m), 1.45–1.54 (2H, m, 5d), 1.12 (3H, s), 0.98 (6H, dd, $J=12.4$, 6.8 Hz); ^{13}C NMR (100.4 MHz): δ 213.9, 156.9, 106.0, 62.7, 47.2, 46.2, 41.2, 39.4, 34.8, 33.6, 29.4, 29.0, 23.0, 21.4, 20.8.

4.1.5. (3*S*,3a*R*,7a*R*)-3-(1-Isopropylvinyl)-7a-methyloctahydro-4*H*-inden-4-one (4). A solution of **5** (0.8 g, 3.5 mmol, 1 equiv) and NaOMe (6 mL, 0.5 M in MeOH, 3 mmol, 0.9 equiv) was stirred for 1 week at 55 °C. The reaction was quenched with 30 mL of water, and the residual methanol was removed by rotary evaporation. Diethyl ether (60 mL) and sat. aq. $NaHCO_3$ (60 mL) were added and the aqueous phase was extracted with diethyl ether (4×60 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (125 mL), brine (125 mL) and dried over $MgSO_4$. The ^{1}H NMR ratio of the crude material indicated a 33:1 ratio of epimers **4** and **5**. The mixture was subjected to flash column chromatography (gradiant elution of 95/5→90/10 hexane/ethyl acetate) to yield **4** (0.72 g, 93% yield). $[\alpha]^{20}_{D}=+53.4$ (*c* 0.825, $CHCl_3$); ^{1}H NMR: δ 4.70 (1H, s), 4.58 (1H, s), 2.83 (1H, dt, $J=10.9$, 6.6 Hz), 2.56 (1H, d, $J=11.1$ Hz), 2.21–2.32 (3H, m), 1.75–2.13 (4H, m), 1.51–1.69 (3H, m), 1.36–1.47 (1H, m), 1.03 (6H, dd, $J=6.8$, 5.5 Hz), 0.74 (3H, s); ^{13}C NMR: δ 210.5, 160.0, 104.1, 64.8, 49.1, 41.4, 39.9, 39.2, 38.5, 33.5, 29.6, 24.2, 22.4, 18.5; HRMS (FAB+) *m*/*z* Calcd for $C_{15}H_{24}O$ 221.1827, found: 221.1904. Anal. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98; found: C, 81.04; H, 10.40.

4.1.6. (3*S*,3a*R*,7a*R*)-3-Isobutyryl-7a-methyloctahydro-4*H*-inden-4-one (11). A solution of **4** (0.7 g, 3.3 mmol, 1 equiv) in CH_2Cl_2 (73 mL) was cooled to −78 °C. O_3 was bubbled through the solution until it turned pale blue in colour (5–10 min). N_2 was then bubbled through the solution to remove the excess O_3, rendering the reaction mixture colourless. Triphenylphosphine (1.8 g, 6.8 mmol, 2.1 equiv) was added and the mixture was allowed to warm to room temperature. The reaction was stirred for 3 h, followed by removal of the solvent by rotary evaporation. The crude mixture was flash chromatographed (gradient elution of 95/5→90/10 hexane/ethyl acetate) to afford the diketone **11** (0.6 g, 84% yield), a colourless oil. $[\alpha]^{20}_{D}=+58.8$ (*c* 0.585, $CHCl_3$); ^{1}H NMR: δ 3.35 (1H, dt, $J=10.5$, 5.7 Hz), 2.83 (1H, d, $J=10.7$ Hz), 2.76 (1H, septet, $J=6.9$ Hz), 2.24 (2H, m), 1.80–2.06 (4H, m), 1.52–1.71 (4H, m), 1.09 (6H, t, $J=7.2$ Hz), 0.73 (3H, s); ^{13}C

NMR: δ 209.5, 216.8, 62.6, 48.7, 44.0, 41.0, 40.5, 39.8, 37.6, 26.1, 23.7, 18.4, 17.9, 17.8; HRMS (FAB+) *m/z* Calcd for $C_{14}H_{22}O$ 223.1620, found: 223.1697. Anal. Calcd for $C_{14}H_{22}O$: C, 75.63; H, 9.97; found: C, 75.03; H, 9.30.

4.1.7. 2-Methyl-1-[(1*S*,3a*R*,7a*S*)-3a-methyl-7-methylene-octahydro-1*H*-inden-1-yl]propan-1-one (12). To a cold (0 °C), stirred solution of bromomethyltriphenylphosphine (0.36 g, 1.0 mmol, 1 equiv) in THF (5.7 mL) was added dropwise *n*-BuLi (2.5 M in hexanes, 0.4 mL, 1.0 mmol, 1 equiv). The resultant bright yellow solution was stirred at 0 °C for 45 min, followed by the addition of a solution of diketone **11** (0.23 g, 1.0 mmol, 1 equiv) in THF (6 mL). The reaction mixture was allowed to warm slowly to room temperature for 1 h. At this time another 0.25 equiv of ylide was prepared using the above procedure. Since all the starting material had not been consumed after 1 h, the 0.25 equiv of prepared ylide was added to the reaction mixture. After an additional h, the reaction was quenched with sat. aq. NH_4Cl (25 mL). The aqueous phase was extracted with diethyl ether (4×40 mL) and the combined organic extracts were washed with brine (3×40 mL) and then dried over $MgSO_4$. After removal of the solvent by rotary evaporation, the crude mixture was flash chromatographed (gradient elution of 95/5→90/10 hexane/diethyl ether) to afford product **12** (0.13 g, 58% yield). $[\alpha]^{20}_D$ = +100.7 (*c* 3.1, $CHCl_3$); ^{1}H NMR: δ 4.68 (1H, s), 4.31 (1H, s), 3.08 (1H, dt, *J*=11.2, 6.3 Hz), 2.71 (1H, septet, *J*=6.9 Hz), 2.35 (1H, d, *J*=11.3 Hz), 2.22 (1H, ddd, *J*=13.5, 2.9, 1.5 Hz), 1.89–2.10 (2H, m), 1.30–1.78 (7H, m), 1.07 (6H, dd, *J*=6.9, 3.0 Hz), 0.65 (3H, s); ^{13}C NMR: δ 217.2, 147.8, 104.8, 55.5, 47.5, 44.1, 40.4, 39.4, 38.8, 35.3, 26.5, 23.3, 18.3, 18.3, 17.9; HRMS (FAB+) *m/z* Calcd for $C_{15}H_{24}O$ 221.1827, found: 221.1904. Anal. Calcd for $C_{15}H_{24}O$: C, 81.76; H, 10.98; found: C, 81.30; H, 10.85.

4.1.8. 2-Methyl-1-[(1*S*,3a*R*,7a*S*)-3a-methyl-7-methylene-octahydro-1*H*-inden-1-yl]propan-1-ol (1). To a cold (−78 °C), stirred solution of **12** (26 mg, 0.12 mmol, 1 equiv) in THF (1.5 mL) was added Super Hydride® (1 M in THF, 0.3 mL, 2.5 equiv). The mixture was allowed to warm to 0 °C and stirred for 1.5 h followed by the addition of another aliquot of Super Hydride® (1 M in THF, 0.3 mL, 2.5 equiv). The mixture was warmed to rt and stirred for 1.5 h. Water was added and the product was extracted with diethyl ether (2×20 mL), washed with brine (1×10 mL) and dried over $MgSO_4$. After removal of the solvent by rotary evaporation, the crude mixture was purified by flash chromatography (90/10 hexane/diethyl ether) to afford the desired natural product **1** (16.8 mg, 64% yield) followed by the corresponding diastereomer at C-12, *epi*-**1** (5.7 mg, 22% yield).

Compound **1**. $[\alpha]^{20}_D$ = +54.4 (*c* 0.80, $CHCl_3$); natural **1**: $[\alpha]^{20}_D$ = +19 (*c* 0.4, $CHCl_3$) 85% pure by GC.[1] ^{1}H NMR (500 MHz): δ 4.86 (1H, d, *J*=1.3 Hz), 4.71 (1H, d, *J*=1.4 Hz), 3.22 (1H, dd, *J*=9.9, 2.4 Hz), 2.27 (1H, dd, *J*=13.0, 4.9 Hz), 2.21 (1H, dq, *J*=10.3, 5.0 Hz), 1.96 (1H, dt, *J*=13.0, 5.6 Hz), 1.5–1.86 (8H, m), 1.22–1.35 (3H, m), 0.97 (3H, d, *J*=6.9 Hz), 0.88 (3H, d, *J*=6.8 Hz), 0.64 (3H, s); ^{13}C NMR: δ 151.2, 105.7, 82.9, 58.6, 45.4, 39.4, 39.19, 39.16, 36.6, 31.2, 25.6, 24.0, 20.4, 18.0, 14.6; HRMS (FAB+) *m/z* Calcd for $C_{15}H_{26}O$ 223.1984, found: 223.2063. Anal. Calcd for $C_{15}H_{26}O$: C, 81.02; H, 11.79; found: C, 81.94; H, 12.20.

Compound epi-**1**. $[\alpha]^{20}_D$ = +49.4 (*c* 0.32, $CHCl_3$); ^{1}H NMR (400 MHz): δ 4.76 (1H, d, *J*=1.5 Hz), 4.39 (1H, d, *J*=1.6 Hz), 3.36 (1H, d, *J*=7.9 Hz), 2.24 (2H, dq, *J*=9.4, 1.7 Hz), 1.97 (1H, d, *J*=11.7 Hz), 1.91 (1H, dt, *J*=13.1, 6.0 Hz),1.48–1.69 (7H, m), 1.21–1.35 (3H, m), 0.97 (3H, d, *J*=6.6 Hz), 0.90 (3H, d, *J*=6.7 Hz), 0.64 (3H, s); ^{13}C NMR: δ 148.5, 104.3, 54.1, 44.1, 39.5, 39.2, 38.5, 36.2, 33.3, 23.9, 19.9, 19.5, 19.2, 17.8; HRMS (FAB+) *m/z* Calcd for $C_{15}H_{26}O$ 223.1984, found: 223.2024. Anal. Calcd for $C_{15}H_{26}O$: C, 81.02; H, 11.79; found: C, 81.01; H, 12.16.

4.1.9. (3*S*,3a*R*,7a*R*)-3-Isopropenyl-7a-methyloctahydro-4*H*-inden-4-one (13). Synthesized as reported by Piers and Boulet.[13]

4.1.10. (3*S*,3a*R*,4*S*,7a*R*)-3-Isopropenyl-4,7a-dimethyl-octahydro-1*H*-inden-4-ol (14). To a cold (−78 °C), stirred solution of MeMgBr (1.4 M in 3:1 toluene:THF, 1.1 mL, 1.5 mmol, 9 equiv) in diethyl ether (2 mL) was added, via cannula, a solution of **13** (32.3 mg, 0.17 mmol) and TMSBr (100 μl, 0.76 mmol, 4.5 equiv) in diethyl ether (1 mL). The mixture was stirred at −78 °C for 30 min, −43 °C for 3 h then 0 °C for 2 h. The reaction was quenched with water (1 mL) and treated with aqueous HCl (10%, 0.5 mL) for 1 h at room temperature. Water (10 mL) was added and the product was extracted with diethyl ether (3×5 mL). The combined organic extracts were washed with brine (10 mL) and dried over $MgSO_4$. After rotary evaporation of solvents, the product was flash chromatographed over silica (95/5 hexane/ethyl acetate) to yield **14** (19.5 mg, 55% yield). ^{1}H NMR (500 MHz): δ 4.82 (1H, s), 4.63 (1H, s), 2.80 (1H, dt, *J*=11.4, 5.5 Hz), 1.89–1.98 (1H, m), 1.78 (1H, tq, *J*=13.7, 3.9 Hz), 1.69 (4H, br s), 1.59 (1H, br d, *J*=14.1 Hz), 1.45 (1H, br d, *J*=13.9 Hz), 1.34–1.40 (2H, m), 1.29 (1H, td, *J*=13.8, 4.8 Hz), 1.26 (1H, d, *J*=11.9 Hz), 1.17 (3H, s), 1.08–1.20 (2H, m), 1.05 (1H, s), 1.02 (3H, s). ^{13}C NMR (100.4 MHz): δ 149.9, 110.8, 72.6, 55.3, 44.1, 42.1, 41.4, 41.2, 39.3, 29.6, 28.0, 19.9, 19.0, 17.7.

4.1.11. 2-Methyl-1-[(3*S*,3a*R*,4*R*,7a*R*)-7a-methyloctahydrospiro[indene-4,2′-oxiran]-3-yl]propan-1-one (15). To a cold (0 °C), stirred solution of **12** (23 mg, 0.11 mmol, 1 equiv) in CH_2Cl_2 (2 mL) was added *m*-chloroperbenzoic acid (*m*-CPBA, 70% pure, 31 mg, 0.13 mmol, 1.2 equiv) and the reaction was allowed to stir at 0 °C for 30 min. The reaction was then warmed to room temperature and an additional aliquot of *m*-CPBA (44 mg, 1.7 equiv) was added and the mixture was stirred for 45 min. Excess *m*-CPBA was destroyed by the addition of sat. aq. $Na_2S_2O_3$ (1 mL). The aqueous layer was extracted with diethyl ether (2×25 mL) and the combined organic extracts were washed with sat. aq. $NaHCO_3$ (2×25 mL) and then dried over $MgSO_4$. After removal of solvents by rotary evaporation, the residue was flash chromatographed on silica gel (95/5 hexane/ethyl acetate) to yield epoxide **15** (18.5 mg, 75% yield). $[\alpha]^{20}_D$ = +63.2 (*c* 0.85, $CHCl_3$); ^{1}H NMR: δ 2.80 (1H, br s), 2.66 (1H, dt, *J*=11.1, 5.6 Hz), 2.56 (2H, m), 2.26 (1H, d, *J*=11.4 Hz), 1.94 (1H, m), 1.47–1.83 (7H, m), 1.22–1.32 (2H, m), 1.02 (6H, t, *J*=7.4 Hz), 0.82 (3H, s); ^{13}C NMR: δ 217.1, 59.2, 54.5, 49.8, 45.5, 45.4, 41.2, 40.1, 37.9,

34.4, 27.2, 22.2, 18.4, 18.0, 17.5; HRMS (FAB+) *m/z* Calcd for $C_{15}H_{24}O_2$ 237.1776, found: 237.1854.

4.1.12. (3*S*,3a*R*,4*R*,7a*R*)-3-(1-Hydroxy-2-methylpropyl)-4,7a-dimethyloctahydro-1*H*-inden-4-ol (16). To a stirred solution of **15** (16 mg, 0.07 mmol, 1 equiv) in THF (1.5 mL) was added Super Hydride® (1 M in THF, 0.3 mL, 4 equiv) and the mixture was stirred at rt for 40 min. An additional aliquot of Super Hydride® (1 M in THF, 0.3 mL, 4 equiv) was added and the reaction mixture was stirred for 2 h at rt. The reaction was then cooled to 0 °C and quenched with 10% HCl (~5 mL). The solution was neutralized with sat. aq. $NaHCO_3$. The mixture was extracted with ethyl acetate (3×25 mL) and the combined organic extracts were washed with brine (1×25 mL) and dried over $MgSO_4$. After rotary evaporation of solvents, the residue was flash chromatographed on silica gel (gradient elution of 90/10→80/20 hexane/ethyl acetate) to afford the diol **16** (14.7 mg, 91% yield) as a white powder. ^{1}H NMR: δ 3.84 (2H, br s), 3.30 (1H, dd, J=9.5, 1.7 Hz), 2.04 (1H, m), 1.68–1.91 (3H, m), 1.27–1.62 (8H, m), 1.23 (3H, s), 1.01–1.22 (1H, m), 0.99 (3H, d, J=6.9 Hz), 0.86 (3H, d, J=7.0 Hz), 0.85 (3H, s).

4.1.13. 1-[(1*S*,3a*R*,7*R*,7a*R*)-7-Hydroxy-3a,7-dimethyloctahydro-1*H*-inden-1-yl]-2-methylpropan-1-one (2). To a stirred mixture of the diol **16** (12.5 mg, 0.05 mmol, 1.0 equiv), 4-methylmorpholine-*N*-oxide, NMO (9 mg, 0.08 mmol, 1.5 equiv) and powdered 4 Å molecular sieves (20 mg) in CH_2Cl_2 (1.5 mL) was added tetrapropylammoniumperruthenate, TPAP (4.6 mg, 0.01 mmol, 0.25 equiv). The reaction was allowed to stir at rt for 3 h. The reaction mixture was then filtered through a short column of silica. After rotary evaporation of the solvents, the residue was flash chromatographed on silica gel (80/20 hexane/ethyl acetate) to yield the desired natural product **2** (12.1 mg, 97% yield). $[\alpha]^{20}_D$=+73.6 (*c* 0.65, $CHCl_3$); natural **2**: $[\alpha]^{20}_D$=+76.1 (*c* 0.28, $CHCl_3$)[2] and +84 (*c* 0.31, $CHCl_3$)[3]; ^{1}H NMR: δ 3.07 (1H, dt, J=11.4, 5.8 Hz), 2.75 (1H, septet, J=6.9 Hz), 2.00 (1H, m), 1.86 (1H, d, J=11.7 Hz), 1.79 (2H, br d), 1.63 (2H, dd, J=12.6, 2.5 Hz), 1.31–1.58 (4H, m), 1.13–1.26 (2H, m), 1.09 (9H, s and d), 0.86 (3H, s); ^{13}C NMR: δ 220.5, 72.4, 60.6, 46.1, 43.7, 43.6, 42.1, 41.7, 38.5, 27.0, 21.6, 21.3, 20.1, 18.3, 18.1; HRMS (FAB M+H+Na) *m/z* Calcd for $C_{15}H_{26}O_2$ 261.1833, found: 261.1830. Anal. Calcd for $C_{15}H_{26}O_2$: C, 75.58; H, 10.99; found: C, 74.93; H, 10.75.

References and notes

1. Weyerstahl, P.; Marschall, H.; Schneider, K. *Liebigs Ann. Org. Bioorg. Chem.* **1995**, *2*, 231–240.
2. Harrison, L. J.; Asakawa, Y. *Phytochemistry* **1991**, 3806–3807. In this report, (+)-4-hydroxyoppositan-7-one (**2**) is referred to as ent-(5*R*,6*S*,9*R*)-4α-hydroxyoppositan-4-one.
3. Wu, C.; Gunatilaka, A. A. L.; McCabe, F. L.; Johnson, R. K.; Spjut, R. W.; Kingston, D. G. I. *J. Nat. Prod.* **1997**, *60*, 1281–1286.
4. Tori, M.; Hasebe, T.; Asakawa, Y.; Ogawa, K.; Yoshimura, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2303–2305.
5. Piers, E.; Oballa, R. M. *Tetrahedron Lett.* **1995**, *36*, 5857–5860.
6. Piers, E.; Oballa, R. M. *J. Org. Chem.* **1996**, *61*, 8439–8447.
7. Cicero, B. L.; Weisbuch, F.; Dana, G. *J. Org. Chem.* **1981**, *46*, 914.
8. Ohkubo, T.; Akino, H.; Asaoka, M.; Takei, H. *Tetrahedron Lett.* **1995**, *36*, 3365–3368.
9. Helquist, P.; Bal, S. A.; Marfat, A. *J. Org. Chem.* **1982**, *47*, 5045–5050.
10. Johnson, C. R.; Zeller, J. R. *J. Am. Chem. Soc.* **1982**, *104*, 4021–4023.
11. Hara, S.; Dojo, H.; Takinami, S.; Suzuki, A. *Tetrahedron Lett.* **1983**, *24*, 731–734.
12. A 2.5:1 ratio of **1** and *epi*-**1** was obtained from the $LiAlH_4$ reduction of *rac*-**12** as reported in Ref. 1.
13. Piers, E.; Boulet, S. L. *Tetrahedron Lett.* **1997**, *38*, 8815–8818.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2767–2778

Free radical synthesis of benzofused tricyclic β-lactams by intramolecular cyclization of 2-azetidinone-tethered haloarenes

Benito Alcaide,[a,*] Pedro Almendros,[b] Alberto Rodríguez-Vicente[a] and M. Pilar Ruiz[a]

[a]*Departamento de Química Orgánica I, Facultad de Química, Universidad Complutense de Madrid, 28040-Madrid, Spain*
[b]*Instituto de Química Orgánica General, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*

Received 6 December 2004; revised 14 January 2005; accepted 20 January 2005

Abstract—*o*-Halogenophenyl- and *o*-halogenobenzyl-4-alkenyl-β-lactams can be prepared both in the racemic form and in optically pure form using the ketene–imine cyclization. These 2-azetidinone-tethered haloarenes were used for the regio- and stereoselective preparation of benzofused tricyclic β-lactams including benzocarbapenems and benzocarbacephems via intramolecular aryl radical cyclisation.

1. Introduction

Since the discovery of penicillin, 2-azetidinone-based heterocycles have been one of the main classes of drugs used for the treatment of bacterial infections.[1] The extensive use of common β-lactam antibiotics such as penicillins and cephalosporins in medicine has resulted in an increasing number of resistant strains of bacteria through mutation and β-lactamase gene transfer.[2] In order to oppose the destructive action of β-lactamases, one strategy consists of modifying the structure of the β-lactam antibiotic, aiming to render it insensitive to the β-lactamase attack. A second approach uses a reagent, typically a β-lactam derivative, which incapacitates the β-lactamase, in synergy with the β-lactam antibiotic. Among others, benzocarbapenems and benzocarbacephems have been designed as suicide inactivators of β-lactamases. The preparation of benzocarbacephems has received more attention,[3] while the synthesis of benzocarbapenems is a less explored area. The first synthesis of these benzofused β-lactams was reported by Wakselman by using a copper-promoted intramolecular aryl substitution of 4-(*o*-bromophenyl)methyl-2-azetidinones.[4] A more recent contribution by Gilchrist described the preparation of benzocarbapenems by reduction and cyclization of 2-substituted indoles.[5] Both syntheses are racemic.

In connection with our current research interest in the preparation and synthetic utility of β-lactams,[6] here we examine the feasibility and efficiency of an approach (racemic and asymmetric) to benzofused tricyclic β-lactams including benzocarbapenems as well as benzocarbacephems, through intramolecular aryl radical cyclization of 2-azetidinone-tethered haloarenes.[7]

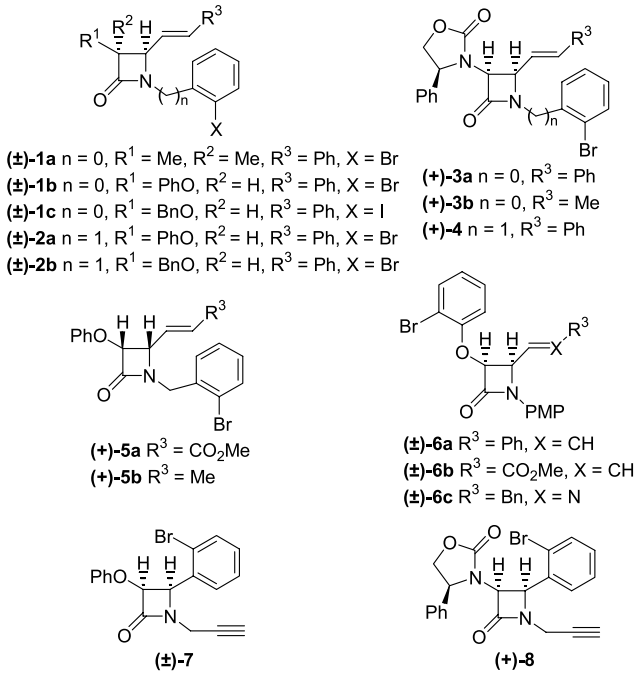
2. Results and discussion

Starting cyclisation substrates, alkenyl- and alkynyl-β-lactam-tethered haloarenes **1**–**8** (Scheme 1), were prepared both in the racemic form and in optically pure form using the ketene–imine cycloaddition as the key step.[8] 2-Azetidinones **1**–**4** were obtained from the corresponding imine[9] through Staudinger reaction with the appropriate acid chloride in the presence of Et_3N (Scheme 1, Table 1). Racemic compounds **1** bearing a *N*-(*o*-halophenyl) moiety were obtained as *cis*/*trans* mixtures with low *cis*-selectivity,[10] the *cis*-isomers being easily separated by fractional recrystallization of the mixtures. In contrast, racemic compounds **2** were obtained as single *cis*-diastereomers. Enantiomerically pure β-lactams (+)-**3a**, (+)-**3b** and (+)-**4** were prepared by reaction of the corresponding imines with the ketene derived from the Evans and Sjögren chiral oxazolidinone.[11] β-Lactams **3** and **4** were obtained exclusively as their *cis*-diastereoisomers with good to excellent stereoselectivity.[12]

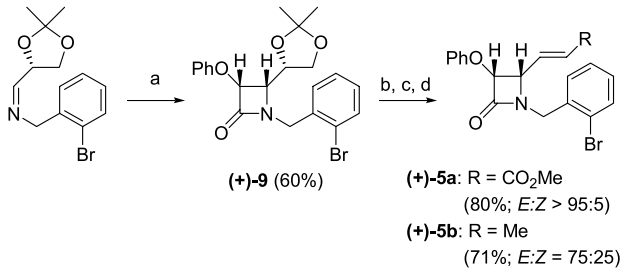
Enantiopure β-lactam (+)-**9** was obtained as a single *cis*-enantiomer from the *o*-bromobenzyl imine of (*R*)-2,3-*O*-isopropylideneglyceraldehyde, through Staudinger reaction with phenoxyacetyl chloride in the presence of Et_3N. Sequential acidic acetonide hydrolysis and oxidative cleavage of the resulting diol, followed by Wittig olefination

Keywords: Lactams; Nitrogen heterocycles; Radical reactions; Polycycles; Cycloaddition.
* Corresponding author. Tel.: +34 913 944 314; fax: +34 913944103; e-mail: alcaideb@quim.ucm.es

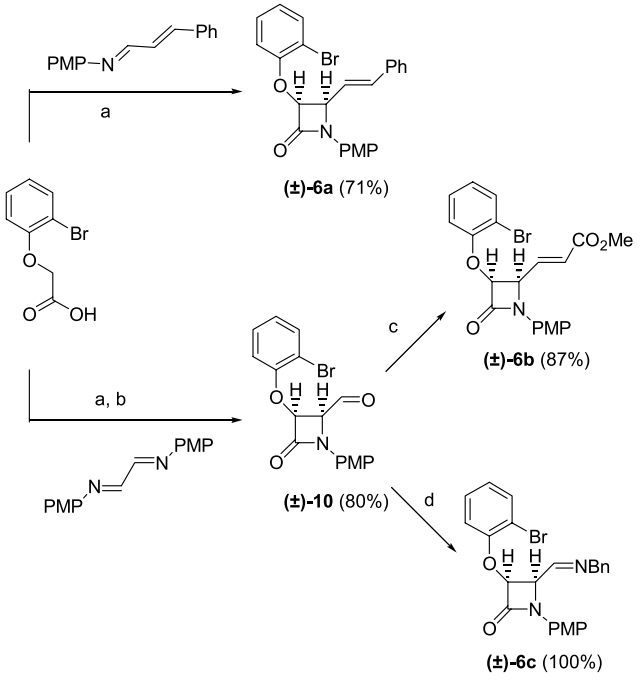
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.082



Scheme 1. Starting β-lactam-tethered haloarenes **1**–**8**.



Scheme 2. Reagents and conditions: (a) $PhOCH_2COCl$, Et_3N, dichloromethane, rt, 12 h; (b) PTSA, THF/H_2O, reflux, 3 h; (c) $NaIO_4$, $NaHCO_3$, dichloromethane, rt, 2 h; (d) for **5a**: $Ph_3P{=}CHCO_2Me$, THF, reflux, 3 h. For **5b**: $Ph_3(Et)PI$, LiBu, THF, rt, 16 h.



Scheme 3. Reagents and conditions: (a) $PhOP(O)Cl_2$, Et_3N, dichloromethane, rt, 16 h; (b) 5% aqueous HCl; (c) $Ph_3P{=}CHCO_2Me$, THF, reflux, 3 h.; (d) $BnNH_2$, $MgSO_4$, dichloromethane, rt, 16 h.

of the corresponding 4-oxoazetidine-2-carbaldehyde afforded the enantiopure alkenes (+)-**5a** and (+)-**5b** (Scheme 2). The preparation of cyclization precursors **6a**–**c** bearing the haloaryl moiety at C3 is shown in Scheme 3. Styryl-2-azetidinone **6a** was obtained with total *cis*-selectivity via direct Staudinger reaction of the *E*-cinnamaldehyde/*p*-anisidine derived imine and the ketene obtained from *o*-bromophenoxy acetic acid. Racemic β-lactam aldehyde **10** was obtained as a single *cis*-diastereoisomer, following our one-pot method from *N*,*N*-di-(*p*-methoxyphenyl)glyoxal diimine.[13] Alkene **6b** was achieved through Wittig olefination of aldehyde **10**, while imine **6c** was prepared by condensation with benzylamine in the presence of magnesium sulphate.

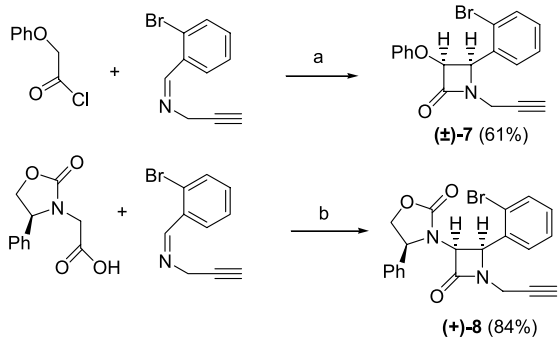
Alkynyl-2-azetidinones **7** and (+)-**8** were obtained with complete stereoselectivity in good yields, through the ketene–imine cycloaddition of the imine obtained from *o*-bromobenzaldehyde and propargylamine on reacting with the corresponding ketene (Scheme 4).

Having obtained the monocyclic precursors, the next stage was set to carry out the key radical cyclisation step. The stereoselective synthesis of complex heterocycles and carbocycles by radical cyclisation has now been established as an efficient methodology in organic chemistry.[14] This wide research has been fostered by its operational simplicity and its tolerance to substrate functionalization. Furthermore, by a combination of stereoelectronic and molecular orbital

Table 1. Preparation of β-lactams **1**–**4**[a]

Product	R^1	R^2	R^3	X	n	Yield (%)[b]	*cis*/*trans* ratio[c]
1a	Me	Me	Ph	Br	0	40	
1b	PhO	H	Ph	Br	0	74	62:38
1c	BnO	H	Ph	I	0	70	64:36
2a	PhO	H	Ph	Br	1	40	100:0
2b	BnO	H	Ph	Br	1	60	100:0
(+)-**3a**	(*S*)-Ox[d]	H	Ph	Br	0	61	100:0
(+)-**3b**	(*S*)-Ox	H	Me	Br	0	69	90:10
(+)-**4**	(*S*)-Ox	H	Ph	Br	1	50	100:0

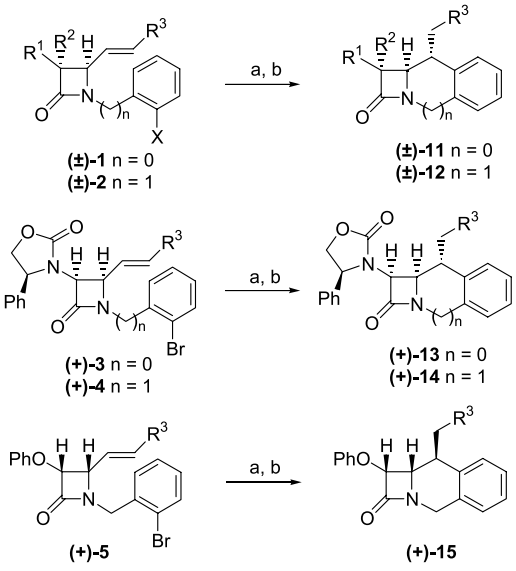
[a] Compounds **1** and **2** are racemic.
[b] Yield of pure, isolated product (or mixture of isomers, when applicable) with correct analytical and spectral data.
[c] The ratio was determined by integration of well-resolved signals in the ^1H NMR spectra of the crude reaction mixtures before purification.
[d] (*S*)-Ox= (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl.



Scheme 4. Reagents and conditions: (a) $PhOCH_2COCl$, Et_3N, dichloromethane, rt, 12 h; (b) $PhOP(O)Cl_2$, Et_3N, dichloromethane, rt, 16 h.

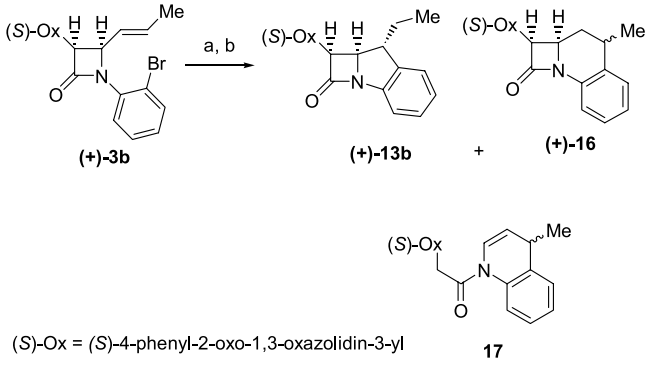
effects, radical cyclisations occur, in general, with high degrees of both regio- and stereo-control.

Haloaryl β-lactams **1**–**5** were reacted with tributyltin hydride and AIBN in benzene at reflux to give the expected benzocarbapenems **11** and **13** and benzocarbacephems **12**, **14** and **15** in good yields as single diastereomers after chromatographic purification (Scheme 5, Table 2). These intramolecular radical reactions were carried out under



Scheme 5. Reagents and conditions: (a) Bu_3SnH (1.2 equiv), AIBN (0.1 equiv), benzene, reflux, 1–2 h. (b) 10% aqueous KF, 30 min.

standard dilution conditions, and did not require the use of high dilution techniques. Removal of the organotin halides by a solution of KF in water is essential for an appropriate chromatographic purification of compounds **11**–**15**.[15] With the exception of the reaction of (+)-**3b**, neither cyclisation products different from **11**–**15** nor reduction products were detected in the ^1H NMR spectra of the crude reaction mixtures. The full stereoselectivity of the radical cyclisation is particularly attractive, being independent of the substitution at C3 or N1 on the β-lactam ring. In addition, 2-azetidinones bearing styryl or carboxymethyl substituents at C4 underwent 5(or 6)-*exo-trig* radical cyclization to benzocarbapenems and benzocarbacephems **11**–**15** in a totally regioselective fashion, as expected when the radical acceptor has a radical-stabilizing moiety at the β-position. The radical reaction of the crotonaldehyde-imine derived β-lactam (+)-**3b**, lacking a radical-stabilizing moiety, deserves special mention. Haloalkenyl β-lactam (+)-**3b** formed, along with benzocarbapenem (+)-**13b** (major product, 30%), benzocarbacephem (+)-**16** (minor product, 10%) and 1,4-dihydroquinoline **17** (relative proportions 3:1:2.5, respectively) (Scheme 6). Compounds (+)-**13b**, (+)-**16**, and **17** were obtained as single diastereoisomers, and thus it is clear that 6-*endo* cyclisation competes with 5-*exo* process when an unactivated double bond is used as the radical acceptor. However, this result is in sharp contrast with the radical reaction of compound (+)-**5b**, being obtained exclusively the 6-*exo* cyclisation product. In this case, independently of the substituent at the acceptor double bond, the 7-*endo* mode of cyclisation is not competitive.[16] Formation of compounds **11**, **13**, **16**, and **17** may be



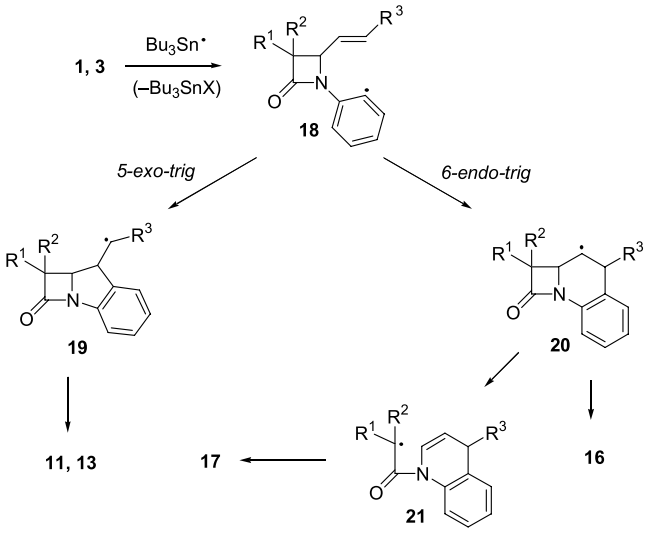
Scheme 6. Reagents and conditions: (a) Bu_3SnH (1.2 equiv), AIBN (0.1 equiv), benzene, reflux, 1.5 h. (b) 10% aqueous KF, 30 min.

Table 2. Preparation of fused tricyclic β-lactams **11**–**15**

Substrate[a]	R^1	R^2	R^3	X	n	Product[a]	Yield (%)[b]
1a	Me	Me	Ph	Br	0	**11a**	65
1b	PhO	H	Ph	Br	0	**11b**	66
cis-**1c**	BnO	H	Ph	I	0	*cis*-**11c**	60
trans-**1c**	BnO	H	Ph	I	0	*trans*-**11c**	65
2a	PhO	H	Ph	Br	1	**12a**	50
2b	BnO	H	Ph	Br	1	**12b**	61
(+)-**3a**			Ph		0	(+)-**13a**	70
(+)-**3b**			Me		0	(+)-**13b**	30
(+)-**4**			Ph		1	(+)-**14**	57
(+)-**5a**			CO_2Me			(+)-**15a**	64
(+)-**5b**			Me			(+)-**15b**	47

[a] Compounds **1**, **2**, **11** and **12** are racemic.
[b] Yield of pure, isolated product with correct analytical and spectral data.

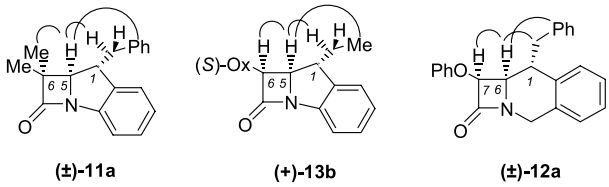
rationalized as shown in Scheme 7. Bromine abstraction by a stannyl radical followed by either 5-*exo*- or 6-*endo* cyclisation of radical **18** would form radicals **19** and **20**, respectively, depending on which of the two olefinic carbons is attacked. These radicals would lead to benzocarbapenems **11** and **13** or benzocarbacephem **16**, respectively, after hydrogen abstraction from tributyltin hydride. Formation of compound **17** may be explained by an homolytic C3–C4 bond cleavage in the 2-azetidinone nucleus of intermediate **20** to form radical intermediate **21**. This interesting process, which is an example of a radical C3–C4 bond breakage in the β-lactam ring,[17] is closely related to the cyclobutylcarbinyl radical cleavage, a useful methodology for the synthesis of medium size rings.[18] In our case, the driving force of the cleavage may be the stability of the captodative radical **21** together with the strain in the β-lactam ring.



Scheme 7.

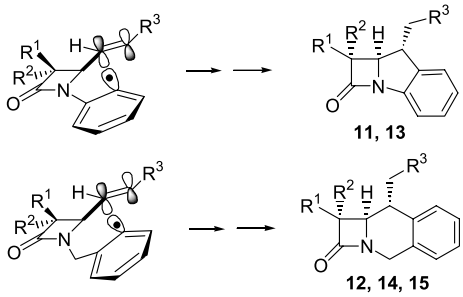
The relative stereochemistry of the 4-membered ring was established from the values of $J_{5,6}$ (benzocarbapenems) or $J_{6,7}$ (benzocarbacephems) vicinal proton couplings and it is transferred unaltered from the starting 2-azetidinone to the cyclized products. The stereochemistry of the new stereocenter at C1 in compounds **11**–**15** was derived from our previous results on stannylcarbapenams and stannylcarbacephams,[19] as well as by qualitative homonuclear NOE difference spectra on representative compounds. As an example, irradiation of the H5 hydrogen in compound **11a** resulted in a 5% increment on the proton signal of the methylene group at lower field (2.67 ppm), and a 5% increment on the phenyl group, and on the methyl group at lower field (1.18 ppm). Irradiation of the H5 hydrogen in compound (+)-**13b** gave a 3% increment both on the most shielded proton of the methylene group (1.43 ppm), and on the methyl group corresponding to the ethyl substituent at C1. Similar figures were observed on performing NOE experiments in tricycle **12a** (Scheme 8). In this way, *anti* stereochemistries H1/H5 (benzocarbapenems) or H1/H6 (benzocarbacephems) were assigned.

The complete selectivity observed in the formation of



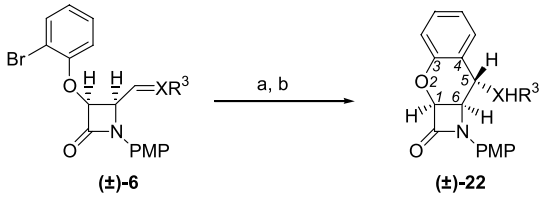
Scheme 8. Selected NOE effects and stereochemistry of compounds **11a**, (+)-**13b** and **12a**.

benzocarbapenems **11** and **13** and benzocarbacephems **12**, **14** and **15** must be due to the preference of the radical intermediates for the conformation depicted in Scheme 9 for these cyclisations.



Scheme 9.

Next, we decided to explore the extension of the above radical intramolecular cyclisation of *N*-haloaryl-β-lactams to 2-azetidinones bearing the proradical center at C3. The treatment of haloarenes **6a**–**c** under similar conditions for the preparation of benzocarbapenems and benzocarbacephems **11**–**15** gave the fused tricyclic β-lactams **22a**–**c** (Scheme 10, Table 3). Compounds **22a** and **22b** were obtained as mixtures of diastereomers, which are epimers at the newly formed C5 stereocenter, while the amino derivative **22c** could be prepared as a single isomer. The relative stereochemistry of the new chiral center of compounds **22** at C5 was determined by the value of coupling constants of H5–H6 protons (J=0–1.2 Hz for the major *anti*-isomers; J=3.6–4.2 Hz for the minor *syn*-isomers).[20]



Scheme 10. Reagents and conditions: (a) Bu_3SnH (1.2 equiv), AIBN (0.1 equiv), benzene, reflux, 1 h. (b) 10% aqueous KF, 30 min.

The slight variation in the diastereomeric ratio in the formation of compounds **22a** and **22b**, could be explained taking into account the higher bulkiness of the phenyl group in comparison to the carboxymethyl moiety. However, steric reasons can not be used to explain the nearly total stereoselectivity of the azaderivative **22c**.

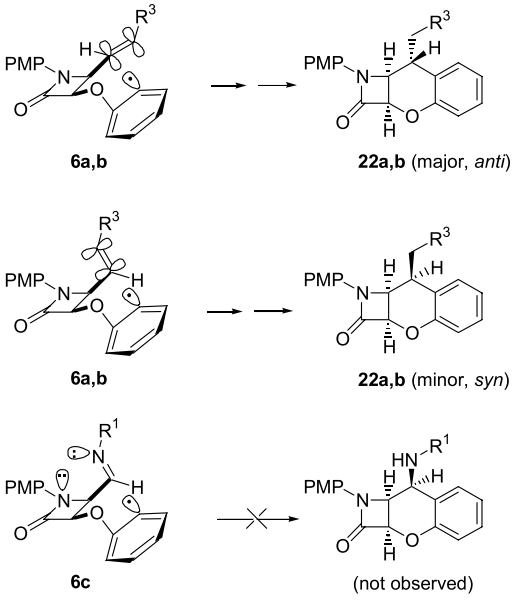
These cyclisations may be understood in terms of the

Table 3. Preparation of fused tricyclic β-lactams **22**[a]

Substrate[b]	R^3	X	dr[c]	Product[b]	Yield (%)[d]
6a	Ph	CH	85:15	**22a**	69
6b	CO_2Me	CH	70:30	**22b**	56
6c	Bn	N	>95:5	**22c**	60

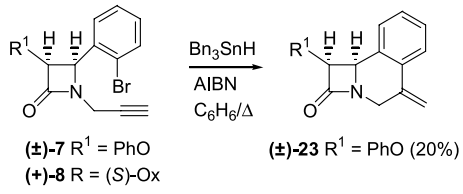
[a] PMP=4-$MeOC_6H_4$.
[b] Compounds **6** and **22** are racemic.
[c] The ratio was determined by integration of well-resolved signals in the ^1H NMR spectra of the crude reaction mixtures before purification.
[d] Yield of pure product (mixture of isomers).

possible conformations presented in Scheme 11. The highest overlap between the aryl radical and the π* orbital of the acceptor double bond is for the conformations giving rise to the major *anti*-isomers. For **22c**, the possible electronic repulsion between the unshared electronic pairs of the nitrogen atoms can be responsible of the destabilization of the conformation giving rise to the minor *syn*-isomer.



Scheme 11.

2-Azetidinones **7** and (+)-**8** were selected as monocyclic precursors with the aim of using a terminal alkyne as a proradical center in the synthesis of benzofused tricyclic β-lactams. Unfortunately, tricycle **23** was isolated in a poor 20% yield from the alkynyl haloarene **7**, while the radical reaction of (+)-**8** gave a complex mixture (Scheme 12).



Scheme 12. Reagents and conditions: (a) Bu_3SnH (1.2 equiv), AIBN (0.1 equiv), benzene, reflux. (b) 10% aqueous KF, 30 min.

In conclusion, easily available 2-azetidinone-tethered haloarenes have proved to be appropriate substrates for the regio- and stereoselective intramolecular aryl radical cyclisation leading to different enantiopure or racemic benzocarbapenems, benzocarbacephems, as well as other fused tricyclic β-lactams. A new radical C3–C4 bond cleavage of the β-lactam ring has been also observed. Efforts to develop this methodology for the preparation of more elaborate benzocarbapenems and benzocarbacephems are currently underway in our research group.

3. Experimental

3.1. General methods

Melting points were taken using a Gallenkamp apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 781 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-300, Varian VRX-300S or Bruker AC-200. NMR spectra were recorded in $CDCl_3$ solutions, except otherwise stated. Chemical shifts are given in ppm relative to TMS (^1H, 0.0 ppm), or $CDCl_3$ (^{13}C, 76.9 ppm). Mass spectra were recorded on a Hewlett–Packard 5989A spectrometer. Microanalyses were performed in the UCM Microanalysis Service (Facultad de Farmacia, UCM, Madrid). Optical rotations were measured using a Perkin–Elmer 241 polarimeter. Specific rotation $[\alpha]_D$ is given in deg cm^2 g^{-1} at 25 °C, and the concentration (*c*) is expressed in g per 100 mL. All commercially available compounds were used without further purification. THF was distilled from Na-benzophenone. Benzene, dichloromethane and triethylamine were distilled from CaH_2. Flame-dried glassware and standard Schlenk techniques were used for moisture sensitive reactions. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). Identification of products was made by TLC (Kiesegel 60F-254). UV light (λ=254 nm), and a vanillin solution in sulfuric acid and 95% EtOH (1 g vanillin, 5 mL H_2SO_4, 150 mL EtOH) was used to develop the plates.

3.2. Materials

The following chemicals were prepared according to previously reported procedures: *N*,*N*-di-(*p*-methoxy-phenyl) glyoxaldiimine,[21] 2,3-*O*-(isopropylidene)-D-glyceraldehyde,[22] (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl-acetic acid,[23] (2-bromophenoxy)acetic acid.[24]

3.3. General procedures for the synthesis of cyclisation substrates 1–4, 6a, and 7–9

Method A. A mixture of aldehyde (10 mmol), 2-halogenoaniline (10 mmol) and a catalytic amount of $ZnCl_2$/α-phenylethylamine complex (0.1 mmol) in benzene (50 mL) was heated at reflux (2–4 h) on a Dean–Stark apparatus. Then, the mixture was filtered and the solvent

was removed under reduced pressure. To a cooled (0 °C) solution of the imine in anhydrous dichloromethane (50 mL), Et_3N (4.16 mL, 30 mmol), the corresponding acid or acid chloride (15 mmol), [and $PhOP(O)Cl_2$, only when (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl-acetic acid and 2-(*o*-bromophenyl)acetic acid are used, 2.25 mL, 15 mmol] were successively added under argon. The resulting mixture was allowed to warm to room temperature, and was stirred for 16 h. The crude mixture was diluted with dichloromethane (100 mL) and washed with saturated $NaHCO_3$ (2×20 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure.

Method B. A solution of the corresponding aldehyde (10 mmol), and amine (10 mmol) in dichloromethane (50 mL) was stirred overnight at room temperature over $MgSO_4$ (100 mmol). Then, the $MgSO_4$ was filtered off, washed with an additional 15 mL of dichloromethane, and the resulting solution cooled at 0 °C under argon. Et_3N (4.16 mL, 30 mmol), the corresponding acid or acid chloride (15 mmol), [and $PhOP(O)Cl_2$, only when (*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl-acetic acid and 2-(*o*-bromophenyl)-acetic acid are used, 2.25 mL, 15 mmol] were successively added under argon. The resulting mixture was allowed to warm to room temperature, and was stirred for 16 h. The crude mixture was diluted with dichloromethane (100 mL) and washed with saturated $NaHCO_3$ (2×20 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure.

3.3.1. (±)-1-(2-Bromophenyl)-3,3-dimethyl-4-(*E*)-styryl-azetidin-2-one (1a). *Method A*. From 1.32 g (10 mmol) of *trans*-cinnamaldehyde, 1.67 g (10 mmol) of *o*-bromoaniline, and 1.59 g (15 mmol) of 2-methylpropanoyl chloride, 840 mg (40%) of compound **1a** was obtained as a pale yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 2:1). ^{1}H NMR: δ 1.30 (s, 3H), 1.52 (s, 3H), 4.79 (d, 1H, J=8.7 Hz), 6.17 (dd, 1H, J=8.7, 15.9 Hz), 6.64 (d, 1H, J=15.9 Hz), 7.30 (m, 9H). ^{13}C NMR: δ 172.2, 135.8, 134.4, 133.7, 128.6, 128.2, 128.1, 128.0, 128.0, 127.4, 126.6, 124.3, 118.2, 68.7, 55.5, 22.6, 17.9. IR ($CHCl_3$, cm^{-1}): ν 1755, 1480. MS (EI), *m*/*z*: 357 (M^++2, 7), 355 (M^+, 7), 286 (41), 284 (36), 158 (100), 143 (94%). Anal. Calcd for $C_{19}H_{18}NOBr$: C, 64.06; H, 5.09; N, 3.93. Found: C, 64.18; H, 5.06; N, 3.91.

3.3.2. (±)-*cis*-1-(2-Bromophenyl)-3-phenoxy-4-(*E*)-styryl-azetidin-2-one (1b). *Method A*. From 790 mg (6.0 mmol) of *trans*-cinnamaldehyde, 1.0 g (6.0 mmol) of *o*-bromoaniline, and 1.53 g (9.0 mmol) of phenoxyacetyl chloride, 1.87 g (74%) of compound **1b** was obtained as a mixture of isomers *cis*/*trans* (62:38). The *cis* isomer was isolated by recrystallization as a colourless solid. Mp 130–132 °C (hexanes/ethyl acetate). ^{1}H NMR: δ 5.50 (dd, 1H, J=4.8, 9.0 Hz), 5.59 (d, 1H, J=4.8 Hz), 6.32 (dd, 1H, J=9.0, 15.9 Hz), 6.74 (d, 1H, J=15.9 Hz), 7.30 (m, 14H). ^{13}C NMR: δ 164.4, 157.2, 137.7, 135.7, 133.7, 129.5, 128.7, 128.7, 128.5, 128.3, 128.1, 127.7, 126.7, 122.3, 121.7, 117.6, 115.6, 81.8, 64.0. IR ($CHCl_3$, cm^{-1}): ν 1750, 1480. MS (EI), *m*/*z*: 421 (M^++2, 1), 419 (M^+, 1), 328 (15), 286 (36), 246 (45), 222 (23), 128 (100%). Anal. Calcd for $C_{23}H_{18}NO_2Br$: C, 65.73; H, 4.32; N, 3.33. Found: C, 65.63; H, 4.34; N, 3.35.

3.3.3. (±)-3-Benzyloxy-1-(2-iodophenyl)-4-(*E*)-styryl-azetidin-2-one (*cis*- and *trans*-1c). *Method A*. From 1.44 g (11.0 mmol) of *trans*-cinnamaldehyde, 2.39 g (11.0 mmol) of *o*-iodooaniline, and 3.04 g (16.5 mmol) of benzyloxyacetyl chloride, 3.70 g (70%) of compound **1c** was obtained as a mixture of isomers *cis*/*trans* (64:36). The *cis* isomer was isolated by recrystallization, while the *trans* isomer was purified by flash chromatography (hexanes/ethyl acetate, 2:1).

2-Azetidinone cis-**1c**. Colourless solid. Mp 139–141 °C (hexanes/ethyl acetate). ^{1}H NMR: δ 4.68 (AB system, 2H, J=8.7 Hz), 4.97 (d, 1H, J=4.5 Hz), 5.13 (dd, 1H, J=4.5, 9.3 Hz), 6.34 (dd, 1H, J=9.3, 15.9 Hz), 6.61 (d, 1H, J=15.9 Hz), 6.90 (m, 1H), 7.20 (m, 12H), 7.75 (dd, 1H, J=1.2, 7.8 Hz). ^{13}C NMR: δ 165.6, 139.9, 137.3, 137.2, 136.5, 135.8, 129.3, 129.0, 128.6, 128.4, 128.4, 128.3, 128.3, 128.1, 127.8, 122.6, 93.7, 82.9, 73.0, 63.8. IR ($CHCl_3$, cm^{-1}): ν 1750, 1470. MS (EI), *m*/*z*: 390 (M^+−91, 6), 298 (12), 245 (10), 115 (32), 91 (100%). Anal. Calcd for $C_{24}H_{20}NO_2I$: C, 59.78; H, 4.21; N, 2.93. Found: C, 59.89; H, 4.19; N, 2.91.

2-Azetidinone trans-**1c**. Colourless oil. ^{1}H NMR: δ 4.57 (d, 1H, J=2.1 Hz), 4.64 (d, 1H, J=12.0 Hz), 4.85 (dd, 1H, J=2.1, 9.0 Hz), 4.88 (d, 1H, J=12 Hz), 5.96 (dd, 1H, J=9.0, 16.2 Hz), 6.44 (d, 1H, J=16.2 Hz), 6.9 (m, 1H), 7.25 (m, 12H), 7.75 (d, 1H). ^{13}C NMR: δ 164.7, 140.4, 137.6, 137.0, 135.6, 129.5, 129.1, 128.7, 128.6, 128.5, 127.7, 127.1, 126.8, 124.0, 122.8, 93.7, 87.3, 83.1, 73.2, 65.7. IR ($CHCl_3$, cm^{-1}): ν 1760, 1600. Anal. Calcd for $C_{24}H_{20}NO_2I$: C, 59.99; H, 4.16; N, 2.90. Found: C, 59.89; H, 4.19; N, 2.91.

3.3.4. (±)-*cis*-1-(2-Bromobenzyl)-3-phenoxy-4-(*E*)-styryl-azetidin-2-one (2a). *Method B*. From 260 mg (2.0 mmol) of *trans*-cinnamaldehyde, 370 mg (2.0 mmol) of *o*-bromobenzylamine, and 450 mg (3.0 mmol) of phenoxyacetyl chloride, 340 mg (40%) of compound **2a** was obtained as a pale yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 1:1). ^{1}H NMR: δ 4.26 (d, 1H, J=15 Hz), 4.35 (dd, 1H, J=4.5, 9.0 Hz), 4.7 (d, 1H, J=15 Hz), 5.27 (d, 1H, J=4.5 Hz), 6.05 (dd, 1H, J=9.0, 15.6 Hz), 6.48 (d, 1H, J=15.6 Hz), 7.15 (m, 14H, Ar). ^{13}C NMR: δ 165.3, 157.2, 137.1, 135.9, 134.3, 133.0, 131.1, 129.7, 129.7, 129.4, 129.2, 128.5, 128.2, 127.8, 126.6, 122.1, 115.6, 82.0, 61.0, 44.7. IR ($CHCl_3$, cm^{-1}): ν 1760, 1600, 1500. Anal. Calcd for $C_{24}H_{20}NO_2Br$: C, 66.37; H, 4.64; N, 3.22. Found: C, 66.46; H, 4.66; N, 3.20.

3.3.5. (±)-*cis*-3-Benzyloxy-1-(2-bromobenzyl)-4(*E*)-styryl-azetidin-2-one (2b). *Method B*. From 260 mg (2.0 mmol) of *trans*-cinnamaldehyde, 370 mg (2.0 mmol) of *o*-bromobenzylamine, and 470 mg (3.0 mmol) of benzyloxyacetyl chloride, 540 mg (60%) of compound **2b** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR: δ 4.11 (dd, 1H, J=4.5, 9.0 Hz), 4.21 (d, 1H, J=15.0 Hz), 4.56 (AB system, 2H, J=11.4 Hz), 4.64 (d, 1H, J=15.0 Hz), 4.74 (d, 1H, J=4.5 Hz), 6.09 (dd, 1H, J=9.0, 15.6 Hz), 6.44 (d, 1H, J=15.6 Hz), 7.20 (m, 14H). ^{13}C NMR: δ 166.8, 136.7, 136.6, 136.2, 134.7, 133.1, 131.1,

129.7, 128.7, 128.5, 128.4, 128.3, 128.2, 127.8, 126.8, 123.9, 123.2, 83.5, 72.9, 61.1, 44.5. IR ($CHCl_3$, cm^{-1}): ν 1755, 1600. Anal. Calcd for $C_{25}H_{22}NO_2Br$: C, 66.97; H, 4.95; N, 3.12. Found: C, 66.86; H, 4.98; N, 3.10.

3.3.6. (+)-(3*S*,4*R*)-1-(2-Bromophenyl)-3-[(*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl]-4-(*E*)-styryl-azetidin-2-one, (+)-3a. *Method A*. From 260 mg (2.0 mmol) of *trans*-cinnamaldehyde, 330 mg (2.0 mmol) of *o*-bromoaniline, 660 mg (3.0 mmol) of (*S*)-(2-oxo-4-phenyl-oxazolidin-3-yl)-acetic acid, and 440 μL (3.0 mmol) of phenyl dichlorophosphate, 600 mg (61%) of compound (+)-**3a** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 169–171 °C (hexanes/ethyl acetate). $[\alpha]_D = +59.0$ (*c* 1.0, $CHCl_3$). ^1H NMR: δ 4.15 (dd, 1H, $J=7.5$, 8.7 Hz.), 4.61 (t, 1H, $J=8.7$ Hz), 4.64 (d, 1H, $J=5.1$ Hz), 4.87 (t, 1H, $J=8.7$ Hz), 5.24 (dd, 1H, $J=5.1$, 9.3 Hz), 6.12 (dd, 1H, $J=9.3$, 15.9 Hz), 6.63 (d, 1H, $J=15.9$ Hz), 7.02 (m, 1H), 7.35 (m, 13H). ^{13}C NMR: δ 163.0, 158.0, 138.0, 137.0, 135.7, 134.2, 133.5, 129.7, 128.8, 128.8, 128.7, 128.5, 128.3, 127.9, 127.0, 127.0, 122.6, 117.7, 71.1, 64.2, 62.8, 60.5. IR ($CHCl_3$, cm^{-1}): ν 1760, 1740. MS (EI), *m*/*z*: 490 ($M^+ +2$, 3), 488 (M^+, 3), 286 (100), 284 (70%). Anal. Calcd for $C_{26}H_{21}N_2O_3Br$: C, 63.81; H, 4.33; N, 5.72. Found: C, 63.93; H, 4.31; N, 5.75.

3.3.7. (+)-(3*S*,4*R*)-1-(2-Bromophenyl)-3-[(*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl]-4-[(*E*)-1-propenyl]-azetidin-2-one, (+)-3b. *Method A*. From 130 mg (1.95 mmol) of 2-butenal, 326 mg (1.95 mmol) of *o*-bromoaniline, 630 mg (2.85 mmol) of (*S*)-(2-oxo-4-phenyl-oxazolidin-3-yl)-acetic acid, and 420 μL (2.85 mmol) of phenyl dichlorophosphate, 570 mg (69%) of compound (+)-**3b** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 201–203 °C (hexanes/ethyl acetate). $[\alpha]_D = +97.2$ (*c* 0.6, $CHCl_3$). ^1H NMR: δ 1.65 (dd, 3H, $J=1.8$, 6.9 Hz), 4.26 (dd, 1H, $J=7.5$, 8.7 Hz), 4.65 (d, 1H, $J=5.4$ Hz), 4.73 (t, 1H, $J=8.7$ Hz), 4.96 (dd, 1H, $J=7.5$, 8.7 Hz), 5.07 (dd, 1H, $J=5.4$, 9.3 Hz), 5.25 (m, 1H), 5.80 (m, 1H), 7.25 (m, 9H). ^{13}C NMR: δ 163.0, 137.2, 135.5, 134.2, 133.5, 129.7, 129.6, 128.7, 128.5, 128.2, 128.0, 127.8, 124.3, 117.7, 71.1, 63.8, 62.3, 60.2, 18.3. IR ($CHCl_3$, cm^{-1}): ν 1752, 1748. MS (EI), *m*/*z*: 428 ($M^+ +2$, 3), 426 (M^+, 3), 229 (20), 224 (68), 184 (70), 144 (57%). Anal. Calcd for $C_{21}H_{19}N_2O_3Br$: C, 59.03; H, 4.48; N, 6.56. Found: C, 58.93; H, 4.51; N, 6.53.

3.3.8. (+)-(3*S*,4*R*)-1-(2-Bromobenzyl)-3-[(*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl]-4-(*E*)-styryl-azetidin-2-one, (+)-4. *Method B*. From 260 mg (2.0 mmol) of *trans*-cinnamaldehyde, 370 mg (2.0 mmol) of *o*-bromobenzylamine, 660 mg (3.0 mmol) of (*S*)-(2-oxo-4-phenyl-oxazolidin-3-yl)-acetic acid, and 440 μL (3.0 mmol) of phenyl dichlorophosphate, 510 mg (50%) of compound (+)-**4** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 193–195 °C (hexanes/ethyl acetate). $[\alpha]_D = +35.9$ (*c* 2.0, $CHCl_3$). ^1H NMR: δ 4.10 (dd, 1H, $J=7.5$, 9.0 Hz), 4.20 (dd, 1H, $J=4.8$, 9.0 Hz), 4.28 (d, 1H, $J=15.6$ Hz), 4.47 (d, 1H, $J=4.8$ Hz), 4.55 (m, 2H), 4.80 (dd, 1H, $J=7.5$, 9.0 Hz), 5.84 (dd, 1H, $J=9.0$, 16.2 Hz), 6.43 (d, 1H, $J=16.2$ Hz), 7.20 (m, 14H). ^{13}C NMR: δ 170.0, 166.8, 152.2, 137.0, 135.7, 134.4, 132.8, 130.9, 129.6, 129.5, 129.4, 129.4, 128.6, 128.4, 127.8, 126.8, 123.6, 123.1, 70.8, 62.8, 61.6, 60.1, 45.2. IR ($CHCl_3$, cm^{-1}): ν 1760, 1750. Anal. Calcd for $C_{27}H_{23}N_2O_3Br$: C, 64.42; H, 4.61; N, 5.56. Found: C, 64.53; H, 4.63; N, 5.53.

3.3.9. (±)-*cis*-3-(2-Bromophenoxy)-1-(4-methoxyphenyl)-4(*E*)-styryl-azetidin-2-one (6a). *Method B*. From 130 mg (1.0 mmol) of *trans*-cinnamaldehyde, 120 mg (1.0 mmol) of *p*-anisidine, 350 mg (1.5 mmol) of (*o*-bromo)phenoxyacetic acid, and 220 μL (1.5 mmol) of phenyl dichlorophosphate, 320 mg (71%) of compound **6a** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 80–82 °C (hexanes/ethyl acetate). ^1H NMR: δ 3.76 (s, 3H), 4.99 (dd, 1H, $J=4.8$, 8.7 Hz), 5.48 (d, 1H, $J=4.8$ Hz), 6.45 (dd, 1H, $J=8.7$, 16.2 Hz), 6.86 (m, 4H), 7.30 (m, 10H). ^{13}C NMR: δ 162.0, 156.7, 154.1, 137.2, 136.0, 133.6, 131.0, 128.8, 128.7, 128.5, 127.0, 123.6, 123.1, 118.9, 115.7, 114.5, 112.4, 82.1, 60.9, 55.6. IR ($CHCl_3$, cm^{-1}): ν 1750. Anal. Calcd for $C_{24}H_{20}NO_3Br$: C, 64.01; H, 4.48; N, 3.11. Found: C, 64.12; H, 4.45; N, 3.09.

3.3.10. (±)-*cis*-4-(2-Bromophenyl)-3-phenoxy-1-prop-2-ynyl-azetidin-2-one (7). *Method B*. From 370 mg (2.0 mmol) of *o*-bromobenzaldehyde, 100 mg (2.0 mmol) of propargylamine, and 510 mg (3.0 mmol) of phenoxyacetyl chloride, 450 mg (61%) of compound **7** was obtained as a pale orange oil after purification by flash chromatography (hexanes/ethyl acetate, 1:1). ^1H NMR: δ 2.28 (t, 1H, $J=2.5$ Hz), 3.77 (dd, 1H, $J=2.5$, 17.7 Hz), 4.47 (dd, 1H, $J=2.7$, 17.7 Hz), 5.50 (d, 1H, $J=4.7$ Hz), 5.56 (d, 1H, $J=4.7$ Hz), 7.15 (m, 9H). ^{13}C NMR: δ 165.6, 156.9, 132.9, 132.4, 130.0, 129.3, 128.9, 127.3, 124.1, 122.4, 115.9, 82.5, 75.6, 73.7, 60.8, 30.3. IR ($CHCl_3$, cm^{-1}): ν 3310, 1755. Anal. Calcd for $C_{18}H_{14}NO_2Br$: C, 60.69; H, 3.96; N, 3.93. Found: C, 60.60; H, 3.93; N, 3.96.

3.3.11. (+)-(3*S*,4*R*)-4-(2-Bromophenyl)-3-[(*S*)-4-phenyl-2-oxo-1,3-oxazolidin-3-yl]-1-prop-2-ynyl-azetidin-2-one, (+)-8. *Method B*. From 180 mg (1.0 mmol) of *o*-bromobenzaldehyde, 60 mg (1.0 mmol) of propargylamine, 330 mg (1.5 mmol) of (*S*)-(2-oxo-4-phenyl-oxazolidin-3-yl)-acetic acid, and 220 μL (1.5 mmol) of phenyl dichlorophosphate, 360 mg (84%) of compound (+)-**8** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 1:1). $[\alpha]_D = +145$ (*c* 1.0, $CHCl_3$). ^1H NMR: δ 2.17 (t, 1H, $J=2.7$ Hz), 3.77 (dd, 1H, $J=2.7$, 17.7 Hz), 3.92 (dd, 1H, $J=7.5$, 8.7 Hz), 4.22 (t, 1H, $J=8.7$ Hz), 4.35 (d, 1H, $J=4.8$ Hz), 4.53 (dd, 1H, $J=2.7$, 17.7 Hz), 4.66 (dd, 1H, $J=7.5$, 8.7 Hz), 5.15 (d, 1H, $J=4.8$ Hz), 7.40 (m, 9H). ^{13}C NMR: δ 164.0, 156.3, 136.5, 132.6, 132.2, 129.9, 129.7, 129.6, 128.0, 127.4, 127.3, 122.7, 75.9, 73.5, 70.3, 63.1, 61.8, 60.2, 30.9. IR ($CHCl_3$, cm^{-1}): ν 3300, 1750. Anal. Calcd for $C_{21}H_{17}N_2O_3Br$: C, 59.31; H, 4.03; N, 6.59. Found: C, 59.24; H, 4.10; N, 6.48.

3.3.12. (+)-(3*R*,4*S*)-1-(2-Bromobenzyl)-4-(2,2-dimethyl-[1,3]dioxolan-4-yl)-3-phenoxy-azetidin-2-one, (+)-9. *Method B*. From 980 mg (7.6 mmol) of 2,3-*O*-isopropylidene-D-glyceraldehyde, 1.42 g (7.6 mmol) of *o*-bromobenzylamine, and 1.70 g (11.4 mmol) of phenoxyacetyl chloride, 2.0 g (60%) of compound (+)-**9** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Mp 95–97 °C (hexanes/ethyl

acetate). $[\alpha]_D = +56.7$ (*c* 1.0, $CHCl_3$). ^1H NMR: δ 1.25 (s, 3H), 1.27 (s, 3H), 3.53 (dd, 1H, $J=6.0$, 8.7 Hz), 3.61 (dd, 1H, $J=5.1$, 8.7 Hz), 4.05 (dd, 1H, $J=5.4$ Hz), 4.39 (m, 2H), 4.86 (d, 1H, $J=15.3$ Hz), 5.14 (d, 1H, $J=5.4$ Hz), 7.20 (m, 9H). ^{13}C NMR: δ 165.8, 157.3, 134.3, 133.0, 131.5, 129.6, 129.5, 127.3, 123.7, 122.5, 115.7, 109.7, 79.8, 76.9, 66.8, 59.9, 45.7, 26.5, 25.0. IR ($CHCl_3$, cm^{-1}): ν 1770. Anal. Calcd for $C_{21}H_{22}NO_4Br$: C, 58.34; H, 5.13; N, 3.24. Found: C, 58.24; H, 5.19; N, 3.26.

3.4. Synthesis of other cyclisation substrates

3.4.1. (+)-(3*R*,4*S*)-1-(2-Bromobenzyl)-4-(*E*)-(2-methoxycarbonylethenyl)-3-phenoxy-azetidin-2-one, (+)-5a. To a solution of the corresponding acetonide β-lactam (+)-**9** (233 mg, 0.54 mmol) in THF/water (1:1, 12 mL) was added solid *p*-TsOH·H_2O (124 mg, 0.65 mmol) in a single portion. The resulting clear solution was heated under reflux for 3 h. The reaction mixture was allowed to cool to room temperature, and then was neutralized with solid $NaHCO_3$. The mixture was extracted with ethyl acetate, the organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product (colourless oil) was used for next step without any further purification. Saturated aqueous sodium hydrogen carbonate (0.05 mL) was added to a solution of the corresponding diol (196 mg, 0.5 mmol) in dichloromethane (10 mL), maintaining the temperature below 25 °C. Solid sodium periodate (214 mg, 1.0 mmol) was added over a 10 min period with vigorous stirring and the reaction was allowed to proceed for 2 h, while the temperature was maintained below 25 °C. The solid was removed by filtration, the filtrate was dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford the crude aldehyde as a colourless oil (178 mg, 0.5 mmol) which was directly used without further purification. To a stirred solution of the aldehyde in THF (10 mL) at 0 °C a solution of methyl (triphenylphosphoranylidene)acetate (202 mg, 0.6 mmol) in THF (2 mL) was slowly added and the mixture was heated at reflux under an argon atmosphere for 3 h, before being concentrated under reduced pressure. Flash chromatography of the residue eluting with hexanes/ethyl acetate 3:1 mixture gave compound (+)-**5a** (185 mg, 82% overall yield from **9**) as a colourless solid. Mp 96–98 °C (hexanes/ethyl acetate). $[\alpha]_D = +7.4$ (*c* 1.0, $CHCl_3$). ^1H NMR: δ 3.62 (s, 3H), 4.25 (d, 1H, $J=15.3$ Hz), 4.31 (dd, 1H, $J=5.1$, 7.8 Hz), 4.72 (d, 1H, $J=15.3$ Hz), 5.26 (d, 1H, $J=5.1$ Hz), 5.91 (d, 1H, $J=15.9$ Hz), 6.73 (dd, 1H, $J=7.8$, 15.9 Hz), 6.88 (m, 3H), 7.20 (m, 5H), 7.50 (m, 1H). ^{13}C NMR: δ 165.4, 165.0, 157.2, 140.5, 134.0, 133.3, 131.5, 130.2, 129.7, 128.1, 126.8, 124.1, 122.6, 115.7, 82.3, 59.1, 51.9, 45.2. IR ($CHCl_3$, cm^{-1}): ν 1750, 1710. Anal. Calcd for $C_{20}H_{18}NO_4Br$: C, 57.71; H, 4.36; N, 3.36. Found: C, 57.81; H, 4.33; N, 3.38.

3.4.2. (+)-(3*R*,4*S*)-1-(2-Bromobenzyl)-3-phenoxy-4-[1-(*E*)-propenyl]-azetidin-2-one, (+)-5b. The starting aldehyde obtained from β-lactam (+)-**9** as described in Section 3.4.1 was directly used without further purification. To a stirred suspension of ethyltriphenylphosphonium iodide (292 mg, 0.7 mmol) in THF (5 mL) under an argon atmosphere, BuLi (1.6 M in hexanes, 0.6 mmol) was added dropwise. After the addition was finished, the reaction mixture was stirred for further 30 min at room temperature. Then, a solution of crude aldehyde (178 mg, 0.5 mmol) in THF (5 mL) was added dropwise, and the reaction mixture was stirred for 16 h at room temperature. The crude mixture was diluted with brine and extracted with ethyl acetate. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Compound (+)-**5b** (143 mg, 77%) was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 5:1) as a mixture of isomers *E*/*Z* (75:25). A fraction containing analytically pure *E*-isomer could be isolated. $[\alpha]_D = +45.0$ (*c* 1.0, $CHCl_3$). ^1H NMR: δ 1.49 (dd, 3H, $J=1.8$, 7.0 Hz), 4.24 (d, 1H, $J=17.8$ Hz), 4.60 (dd, 1H, $J=4.5$, 10.2 Hz), 4.77 (d, 1H, $J=17.8$ Hz), 5.28 (d, 1H, $J=4.5$ Hz), 5.40 (m, 1H), 5.75 (m, 1H), 7.30 (m, 9H). IR ($CHCl_3$, cm^{-1}): ν 1750. Anal. Calcd for $C_{19}H_{18}NO_2Br$: C, 61.30; H, 4.87; N, 3.76. Found: C, 61.19; H, 4.84; N, 3.78.

3.4.3. (±)-*cis*-3-(2-Bromophenoxy)-4-[(*E*)-(2-methoxycarbonylethenyl)]-1-(4-methoxyphenyl)-azetidin-2-one (6b). To a stirred solution of aldehyde **10** (150 mg, 0.40 mmol) in THF (8 mL) at 0 °C a solution of methyl (triphenylphosphoranylidene)acetate (162 mg, 0.48 mmol) in THF (2 mL) was slowly added and the mixture was heated at reflux under an argon atmosphere for 3 h, before being concentrated under reduced pressure. Flash chromatography of the residue eluting with hexanes/ethyl acetate 1:1 mixture gave compound **6b** (150 mg, 87%) as a colourless oil. ^1H NMR: δ 3.66 (s, 3H), 3.72 (s, 3H), 4.91 (dd, 1H, $J=5.4$, 7.5 Hz), 5.40 (d, 1H, $J=5.4$ Hz), 6.14 (d, 1H, $J=16.5$ Hz), 6.81 (d, 2H, $J=9.0$ Hz), 6.86 (m, 1H), 7.04 (dd, 1H, $J=7.5$, 16.5 Hz), 7.22 (m, 4H), 7.45 (d, 1H, $J=7.8$ Hz). ^{13}C NMR: δ 165.5, 162.0, 157.0, 154.5, 151.6, 140.6, 133.7, 131.3, 128.8, 126.8, 124.1, 118.7, 116.2, 114.7, 82.4, 58.4, 55.6, 52.0. IR ($CHCl_3$, cm^{-1}): ν 1760, 1715. Anal. Calcd for $C_{20}H_{18}NO_5Br$: C, 55.57; H, 4.20; N, 3.24. Found: C, 55.68; H, 4.24; N, 3.21.

3.4.4. Synthesis of β-lactam imine (±)-6c. A solution of benzylamine (0.03 mL, 0.27 mmol) in dichloromethane (1 mL) was added dropwise to a stirred suspension of the 4-oxoazetidine-2-carbaldehyde **10** (100 mg, 0.27 mmol) and magnesium sulfate (320 mg, 2,7 mmol) in dichloromethane (5 mL) at room temperature. After stirring 16 h at room temperature, the mixture was filtered and the solvent was removed under reduced pressure to give 120 mg (100%) of compound **6c** which was directly used without further purification. Colourless oil. ^1H NMR: δ 3.80 (s, 3H), 4.70 (m, 2H), 4.90 (dd, 1H, $J=5.0$, 6.0 Hz), 5.50 (d, 1H, $J=5.0$ Hz), 6.90 (d, 2H, $J=9.0$ Hz), 6.95 (m, 1H), 7.25 (m, 6H), 7.40 (d, 2H, $J=9.0$ Hz), 7.55 (d, 1H), 8.0 (d, 1H, $J=4.0$ Hz). ^{13}C NMR: δ 161.3, 160.8, 156.8, 153.6, 137.7, 133.5, 130.8, 128.7, 128.6, 128.2, 127.3, 123.8, 118.5, 115.4, 114.5, 112.2, 81.6, 65.2, 60.6, 55.5. IR ($CHCl_3$, cm^{-1}): ν 1755, 1670.

3.5. General procedure for the radical cyclization reaction. Synthesis of tricyclic β-lactams 11, 12, 13a, 14, 15, 23, and 24

A solution of the corresponding *o*-halogenophenyl-β-lactam **1–7** (1 mmol), Bu_3SnH (1.2 mmol), and AIBN (0.1 mmol) in dry benzene (20 mL) was refluxed under argon

atmosphere until complete disappearance of the starting substrate (TLC, 1.5–3 h). The resulting crude reaction mixture was treated with 10% aqueous solution of KF (20 mL) for 30 min. The organic layer was separated, dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography of the residue eluting with hexanes/ethyl acetate mixtures gave analytically pure tricyclic β-lactam.

3.5.1. Tricyclic β-lactam (±)-11a. From 300 mg (0.84 mmol) of compound **1a**, 149 mg (65%) of compound **11a** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 123–125 °C (hexanes/ethyl acetate). ^{1}H NMR: δ 0.79 (s, 3H), 1.18 (s, 3H), 2.72 (dd, 1H, J=10.8, 13.5 Hz), 3.44 (dd, 1H, J=4.8, 13.5 Hz), 3.66 (m, 1H), 3.79 (d, 1H, J=8.1 Hz), 7.20 (m, 9H). ^{13}C NMR: δ 179.6, 141.6, 140.4, 139.1, 128.8, 128.8, 128.8, 126.7, 125.0, 124.5, 116.8, 69.7, 52.8, 45.9, 39.9, 23.1, 18.1. IR ($CHCl_3$, cm^{-1}): ν 1760, 1600. MS (EI), *m/z*: 277 (M^+, 11), 208 (25), 186 (96), 158 (59), 91 (100%). Anal. Calcd for $C_{19}H_{19}NO$: C, 82.28; H, 6.90; N, 5.05. Found: C, 82.12; H, 6.72; N, 5.28.

3.5.2. Tricyclic β-lactam (±)-11b. From 300 mg (0.71 mmol) of compound **1b**, 160 mg (66%) of compound **11b** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp >130 °C (decomp.) (hexanes/ethyl acetate). ^{1}H NMR: δ 2.92 (dd, 1H, J=8.4, 13.8 Hz), 3.30 (dd, 1H, $J_=$6.6, 13.8 Hz), 4.14 (m, 1H), 4.38 (dd, 1H, J=4.5, 7.8 Hz), 5.46 (d, 1H, J=4.5 Hz), 7.05 (m, 14H). ^{13}C NMR: δ 173.2, 157.2, 142.1, 141.1, 138.6, 129.8, 129.2, 128.8, 128.3, 126.8, 126.0, 125.2, 122.5, 117.9, 115.6, 80.7, 64.2, 43.0, 39.8. IR ($CHCl_3$, cm^{-1}): ν 1740, 1670. MS (EI), *m/z*: 341 (M^+, 6), 319 (19), 250 (8), 235 (44), 105 (76), 91 (100%). Anal. Calcd for $C_{23}H_{19}NO_2$: C, 80.92; H, 5.61; N, 4.10. Found: C, 80.77; H, 5.53; N, 4.05.

3.5.3. Tricyclic β-lactam *cis*-(±)-11c. From 400 mg (0.92 mmol) of compound *cis*-**1c**, 170 mg (60%) of compound *cis*-**11c** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 1:1). This compound decomposed quickly on standing in solution at room temperature. ^{1}H NMR: δ 2.80 (dd, 1H, J=9.9, 13.8 Hz), 3.42 (dd, 1H, J=4.5, 13.8 Hz), 4.12 (m, 1H), 4.19 (dd, 1H, J=4.5, 4.8 Hz), 4.25 (AB system, 2H, J=12.0 Hz), 4.78 (d, 1H, J=4.8 Hz), 7.20 (m, 14H). IR ($CHCl_3$, cm^{-1}): ν 1775, 1650.

3.5.4. Tricyclic β-lactam *trans*-(±)-11c. From 200 mg (0.46 mmol) of compound *trans*-**1c**, 90 mg (62%) of compound *trans*-**11c** was obtained as a white solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 87–89 °C (hexanes/ethyl acetate). ^{1}H NMR: δ 2.69 (dd, 1H, J=10.5, 13.8 Hz), 3.29 (dd, 1H, J=5.7, 13.8 Hz), 3.63 (m, 1H), 3.99 (m, 3H), 4.35 (d, 1H, J=2.4 Hz), 7.0–7.3 (m, 14H). ^{13}C NMR: δ 171.9, 141.0, 139.1, 138.8, 136.7, 129.3, 129.1, 128.6, 128.2, 127.9, 127.0, 125.6, 124.7, 116.6, 87.2, 71.9, 67.0, 48.0, 40.1, 25.3. IR ($CHCl_3$, cm^{-1}): ν 1770, 1650, 1600. MS (EI), *m/z*: 298 (27), 264 (5), 91 (100). Anal. Calcd for $C_{24}H_{21}NO_2$: C, 81.10; H, 5.96; N, 3.94. Found: C, 80.98; H, 6.03; N, 3.86.

3.5.5. Tricyclic β-lactam (±)-12a. From 200 mg (0.46 mmol) of compound **2a**, 80 mg (50%) of compound **12a** was obtained as a pale yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 1:1). ^{1}H NMR: δ 3.03 (dd, 1H, J=7.5, 14.7 Hz), 3.12 (dd, 1H, J=5.1, 14.7 Hz), 3.68 (m, 1H), 3.82 (dd, 1H, J=4.5, 8.1 Hz), 4.06 (d, 1H, J=15.6 Hz), 4.72 (d, 1H, J=15.6 Hz), 5.28 (dd, 1H, J=1.5, 4.5 Hz), 7.15 (m, 14H). ^{13}C NMR: δ 167.2, 157.6, 139.3, 136.7, 131.8, 129.7, 129.2, 128.7, 128.4, 127.5, 126.9, 126.7, 126.5, 122.4, 115.9, 81.8, 54.7, 40.9, 38.9, 36.3. IR ($CHCl_3$, cm^{-1}): ν 1760, 1600. Anal. Calcd for $C_{24}H_{21}NO_2$: C, 81.10; H, 5.96; N, 3.94. Found: C, 81.22; H, 5.94; N, 3.94.

3.5.6. Tricyclic β-lactam (±)-12b. From 220 mg (0.49 mmol) of compound **2b**, 110 mg (61%) of compound **12b** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^{1}H NMR: δ 3.00 (br s, 2H), 3.56 (br s, 2H), 3.97 (d, 1H, J=18.3 Hz), 4.45 (d, 1H, J=11.7 Hz), 4.64 (d, 1H, J=18.3 Hz), 4.64 (br s, 1H), 4.72 (d, 1H, J=11.7 Hz), 7.15 (m, 14H). ^{13}C NMR: δ 168.5, 139.5, 137.1, 136.8, 131.6, 129.1, 128.7, 128.6, 128.5, 127.8, 127.2, 126.7, 126.6, 126.4, 126.2, 82.4, 72.7, 54.7, 40.4, 38.9, 35.9. IR ($CHCl_3$, cm^{-1}): ν 1750, 1640. Anal. Calcd for $C_{25}H_{23}NO_2$: C, 81.27; H, 6.27; N, 3.79. Found: C, 81.40; H, 6.24; N, 3.81.

3.5.7. Tricyclic β-lactam (+)-13a. From 100 mg (0.20 mmol) of compound (+)-**3a**, 66 mg (70%) of compound (+)-**13a** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 168–170 °C (hexanes/ethyl acetate). $[\alpha]_D$=+110.0 (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 2.57 (dd, 1H, J=11.1, 13.2 Hz), 3.05 (t, 1H, J=7.8 Hz), 3.45 (dd, 1H, J=3.9, 13.2 Hz), 3.86 (dd, 1H, J=7.8, 8.7 Hz), 4.07 (m, 3H), 4.23 (t, 1H, J=8.7 Hz), 7.20 (m, 14H). ^{13}C NMR: δ 169.6, 157.3, 141.9, 140.8, 140.4, 136.6, 129.6, 129.5, 129.3, 129.2, 128.3, 127.3, 126.8, 125.7, 124.7, 117.4, 70.9, 65.5, 59.7, 59.5, 46.3, 39.5. IR ($CHCl_3$, cm^{-1}): ν 1795, 1755, 1600. MS (EI), *m/z*: 410 (M^+, 7), 319 (81), 207 (52), 130 (84), 104 (62), 91 (100%). Anal. Calcd for $C_{26}H_{22}N_2O_3$: C, 76.08; H, 5.40; N, 6.82. Found: C, 76.19; H, 5.43; N, 6.78.

3.5.8. Tricyclic β-lactam (+)-14. From 250 mg (0.50 mmol) of compound (+)-**4**, 120 mg (57%) of compound (+)-**14** was obtained as a colourless solid after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Mp 178–180 °C (hexanes/ethyl acetate). $[\alpha]_D$=+97.4 (*c* 0.5, $CHCl_3$). ^{1}H NMR: δ 2.65 (dd, 1H, J=9.3, 13.8 Hz), 3.26 (br s, 1H), 3.41 (dd, 1H, $J_=$4.5, 13.8 Hz), 3.53 (br s, 1H), 3.65 (dd, 1H, J=4.5, 8.7 Hz), 3.91 (dd, 1H, J=5.7, 8.7 Hz), 4.04 (d, 1H, J=4.5 Hz), 4.06 (d, 1H, J=17.1 Hz), 4.28 (t, 1H, J=8.7 Hz), 4.70 (d, 1H, J=17.1 Hz), 7.20 (m, 14H). ^{13}C NMR: δ 162.9, 157.5, 140.2, 138.0, 135.6, 131.2, 129.6, 129.5, 129.3, 129.1, 128.3, 127.3, 127.2, 126.9, 126.8, 126.6, 70.9, 62.6, 59.3, 56.8, 41.0, 40.4, 36.2. IR ($CHCl_3$, cm^{-1}): ν 1760, 1750. Anal. Calcd for $C_{27}H_{24}N_2O_3$: C, 76.39; H, 5.70; N, 6.60. Found: C, 76.51; H, 5.68; N, 6.57.

3.5.9. Tricyclic β-lactam (+)-15a. From 70 mg (0.17 mmol) of compound (+)-**5a**, 40 mg (64%) of compound (+)-**15a** was obtained as a colourless oil after

purification by flash chromatography (hexanes/ethyl acetate, 4:1). $[\alpha]_D = +56.0$ (*c* 0.5, $CHCl_3$). ^1H NMR: δ 2.76 (dd, 1H, $J=6.0$, 16.5 Hz), 2.85 (dd, 1H, $J=5.1$, 16.5 Hz), 3.57 (s, 3H), 3.62 (m, 1H), 4.04 (dd, 1H, $J=4.2$, 9.3 Hz), 4.21 (dd, 1H, $J=1.8$, 16.8 Hz), 4.78 (d, 1H, $J=16.8$ Hz), 5.43 (dd, 1H, $J=1.8$, 4.2 Hz), 7.10 (m, 9H). ^{13}C NMR: δ 172.2, 166.8, 157.5, 135.1, 131.1, 129.7, 127.7, 127.1, 127.0, 126.6, 122.5, 115.7, 81.5, 54.0, 52.0, 40.8, 35.2, 33.3. IR ($CHCl_3$, cm^{-1}): ν 1775. Anal. Calcd for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15. Found: C, 71.09; H, 5.72; N, 4.12.

3.5.10. Tricyclic β-lactam (+)-15b. From 200 mg (0.54 mmol) of compound (+)-**5b**, 80 mg (47%) of compound (+)-**15b** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). $[\alpha]_D = +47.0$ (*c* 1.0, $CHCl_3$). ^1H NMR: δ 0.89 (t, 3H, $J=7.5$ Hz), 1.90 (m, 2H), 3.23 (m, 1H), 3.74 (dd, 1H, $J=4.2$, 8.9 Hz), 4.13 (d, 1H, $J=17.0$ Hz), 4.73 (d, 1H, $J=17.0$ Hz), 5.38 (dd, 1H, $J=1.6$, 4.2 Hz), 7.25 (m, 9H). ^{13}C NMR: δ 166.8, 157.8, 135.9, 131.5, 129.7, 127.7, 127.4, 126.9, 126.5, 122.4, 115.8, 81.9, 54.1, 40.6, 35.7, 24.0, 10.9. IR ($CHCl_3$, cm^{-1}): ν 1775. Anal. Calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.91; H, 6.49; N, 4.75.

3.5.11. Tricyclic β-lactam (±)-22a. From 220 mg (0.49 mmol) of compound **6a**, 120 mg (69%) of compound **22a** was obtained as a mixture (85:15) of epimers. The major isomer could be isolated after purification by flash chromatography (hexanes/ethyl acetate, 1:1). Colourless solid. Mp 155–157 °C (hexanes/ethyl acetate). ^1H NMR: δ 2.87 (d, 2H, $J=8.1$ Hz), 3.43 (t, 1H, $J=8.7$ Hz), 3.69 (s, 3H), 4.46 (dd, 1H, $J=1.2$, 5.1 Hz), 5.33 (d, 1H, $J=5.1$ Hz), 6.95 (m, 13H). ^{13}C NMR: δ 162.1, 156.5, 152.2, 138.5, 130.4, 129.7, 129.0, 128.9, 128.6, 126.8, 125.8, 123.5, 118.6, 118.5, 114.5, 79.6, 58.2, 55.4, 40.3, 39.9. IR ($CHCl_3$, cm^{-1}): ν 1755, 1590. Anal. Calcd for $C_{24}H_{21}NO_3$: C, 77.61; H, 5.70; N, 3.77. Found: C, 77.73; H, 5.67; N, 3.75.

3.5.12. Tricyclic β-lactam (±)-22b. From 100 mg (0.23 mmol) of compound **6b**, 50 mg (56%) of compound **22b** was obtained as a mixture (70:30) of epimers. The major isomer could be isolated after purification by flash chromatography (hexanes/ethyl acetate, 3:1). Colourless oil. ^1H NMR: δ 2.49 (dd, 1H, $J=5.1$, 17.1 Hz), 2.70 (dd, 1H, $J=10.2$, 17.1 Hz), 3.68 (s, 3H), 3.71 (s, 3H), 3.78 (dd, 1H, $J=5.1$, 9.9 Hz), 4.59 and 5.30 (d, each 1H, $J=4.5$ Hz), 6.81 (d, 2H, $J=9.0$ Hz), 6.93 (m, 3H), 7.15 (m, 1H), 7.35 (d, 2H, $J=9.0$ Hz). ^{13}C NMR: δ 172.1, 161.8, 156.6, 152.4, 130.0, 129.8, 129.4, 125.1, 124.0, 118.9, 118.6, 114.6, 79.5, 58.5, 55.5, 52.0, 37.1, 34.3. IR ($CHCl_3$, cm^{-1}): ν 1760, 1750. Anal. Calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 67.87; H, 5.45; N, 3.98.

3.5.13. Tricyclic β-lactam (±)-22c. From 70 mg (0.14 mmol) of compound **6c**, 30 mg (60%) of compound **22c** was obtained as a pale yellow oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^1H NMR: δ 3.75 (s, 3H), 3.76 (br s, 2H), 4.13 (d, 1H, $J=1.2$ Hz), 4.73 (dd, 1H, $J=1.2$, 5.1 Hz), 5.4 (d, 1H, $J=5.1$ Hz), 6.80 (d, 2H, $J=9.0$ Hz), 7.0 (m, 2H), 7.18 (d, 2H, $J=9.0$ Hz), 7.35 (m, 7H). ^{13}C NMR: δ 161.9, 156.6, 152.4, 139.4, 130.6, 130.0, 129.8, 128.6, 128.2, 127.3, 124.9, 123.5, 119.2, 118.5, 114.6, 79.5, 59.9, 55.5, 53.6, 51.3. IR ($CHCl_3$, cm^{-1}): ν 3200, 1750, 1590. Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.70; H, 5.70; N, 7.20.

3.5.14. Tricyclic β-lactam (±)-23. From 500 mg (1.40 mmol) of compound **7**, 80 mg (20%) of compound **23** was obtained as a colourless oil after purification by flash chromatography (hexanes/ethyl acetate, 3:1). ^1H NMR: δ 3.75 (d, 1H, $J=15.3$ Hz), 4.58 (d, 1H, $J=15.3$ Hz), 4.91 (d, 1H, $J=4.4$ Hz), 5.12 (s, 1H), 5.53 (d, 1H, $J=4.4$ Hz), 5.60 (s, 1H), 7.15 (m, 8H), 7.63 (d, 1H, $J=7.7$ Hz). ^{13}C NMR: δ 168.5, 157.5, 136.3, 133.3, 129.6, 129.5, 129.2, 128.6, 127.9, 125.4, 122.3, 115.7, 112.2, 82.0, 54.2, 43.7. IR ($CHCl_3$, cm^{-1}): ν 1760. Anal. Calcd for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.84; H, 5.43; N, 5.08.

3.6. Radical reaction of haloaryl β-lactam (+)-3b. Preparation of benzocarbapenem (+)-13b, benzocarbacephem (+)-16, and 1,4-dihydroquinoline 17

According to the general procedure described in Section 3.5. From 150 mg (0.35 mmol) of β-lactam (+)-**3b**, and after column chromatography eluting with hexanes/ethyl acetate (1:1), 40 mg (30%) of the less polar compound (+)-**13b**, 13 mg (10%) of compound (+)-**16**, and 33 mg (25%) of the more polar compound **17** were obtained.

3.6.1. Benzocarbapenem (+)-13b. Colourless oil. $[\alpha]_D = +126.0$ (*c* 0.7, $CHCl_3$). ^1H NMR: δ 0.98 (t, 3H, $J=7.5$ Hz), 1.43 (m, 1H), 1.95 (m, 1H), 3.0 (m, 1H), 4.01 (dd, 1H, $J=5.4$, 8.1 Hz), 4.35 (dd, 1H, $J=6.0$, 9.0 Hz), 4.75 (t, 1H, $J=9.0$ Hz), 4.96 (d, 1H, $J=5.4$ Hz), 5.02 (dd, 1H, $J=6.6$, 9.0 Hz), 7.15 (m, 9H). ^{13}C NMR: δ 170.6, 157.7, 141.6, 141.2, 137.9, 129.4, 129.3, 127.7, 127.7, 125.4, 124.7, 116.8, 71.0, 64.4, 61.9, 59.3, 44.3, 26.5, 11.8. IR ($CHCl_3$, cm^{-1}): ν 1760, 1600. Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.28; H, 5.82; N, 8.07.

3.6.2. Benzocarbacephem (+)-16. Colourless solid. Mp >170 °C (decomp.) (hexanes/ethyl acetate). $[\alpha]_D = +114.6$ (*c* 0.5, $CHCl_3$). ^1H NMR: δ 1.09 (d, 3H, $J=7.2$ Hz), 1.7 (m, 2H), 2.72 (m, 1H), 3.87 (dddd, 1H, $J=1.5$, 4.2, 8.1, 12.0 Hz), 4.34 (dd, 1H, $J=5.4$, 8.7 Hz), 4.70 (t, 1H, $J=8.7$ Hz), 4.81 (d, 1H, $J=4.2$ Hz), 5.01 (dd, 1H, $J=5.4$, 8.7 Hz), 7.0–7.4 (m, 9H). ^{13}C NMR: δ 161.1, 157.9, 138.6, 132.6, 130.0, 129.4, 129.4, 128.0, 127.0, 126.5, 124.2, 118.5, 70.8, 63.2, 59.1, 54.1, 30.0, 29.3, 19.6. IR ($CHCl_3$, cm^{-1}): ν 1760, 1595. Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.54; H, 5.83; N, 8.00.

3.6.3. 1,4-Dihydroquinoline 17. Colourless oil. ^1H NMR: δ 1.19 (d, 3H, $J=7.5$ Hz), 3.35 (m, 1H), 3.55 (d, 1H, $J=17.4$ Hz), 4.18 (t, 1H, $J=7.8$ Hz), 4.64 (d, 1H, $J=17.4$ Hz), 4.75 (t, 1H, $J=9.3$ Hz), 5.19 (t, 1H, $J=8.4$ Hz), 5.51 (dd, 1H, $J=5.1$, 7.2 Hz), 6.56 (d, 1H, $J=7.2$ Hz), 7.25 (m, 8H), 7.95 (d, 1H). IR ($CHCl_3$, cm^{-1}): ν 1750, 1650. Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.52; H, 5.75; N, 8.08.

Acknowledgements

Support for this work by the D.G.I.-M.C.Y.T. (Project BQU2003-07793-C02-01) is gratefully acknowledged.

References and notes

1. (a) *Recent Advances in the Chemistry of Anti-Infective Agents*; Bentley, P. H., Ponsford, R., Eds.; The Royal Society of Chemistry: Cambridge, 1993. (b) Southgate, R.; Branch, C.; Coulton, S.; Hunt, E. In Lukacs, G., Ed.; Recent Progress in the Chemical Synthesis of Antibiotics and Related Microbial Products; Springer: Berlin, 1993; Vol. 2, p 621. (c) *The Chemistry of β-Lactams*; Page, M. I., Ed.; Chapman & Hall: London, 1992. (d) Morin, R. B., Gorman, M., Eds.; Chemistry and Biology of β-Lactam Antibiotics; Academic Press: New York, 1982; Vols. 1–3.
2. See, for example: (a) Ritter, T. K.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2001**, *40*, 3508. (b) Díaz, N.; Suárez, D.; Merz, K. M. M., Jr. *J. Am. Chem. Soc.* **2000**, *122*, 4197. (c) Page, M. I. *Chem. Commun.* **1998**, 1609. (d) Niccolai, D.; Tarsi, L.; Thomas, R. J. *Chem. Commun.* **1997**, 2333. (e) Hook, V. *Chem. Br.* **1997**, *33*, 34. (f) Spratt, B. G. *Science* **1994**, *264*, 388. (g) Davies, J. *Science* **1994**, *264*, 375.
3. (a) Finkelstein, J.; Holden, K. G.; Perchonock, C. D. *Tetrahedron Lett.* **1978**, 1629. (b) Hakimelahi, G. H.; Just, G. *Can. J. Chem.* **1979**, *57*, 1939. (c) Bose, A. K.; Ram, B.; Hoffman, N. A.; Hutchinson, A. J.; Manhas, M. S. *J. Heterocycl. Chem.* **1979**, *16*, 1313. (d) Miyake, H.; Tokutake, N.; Kirisawa, M. *Synthesis* **1983**, 833. (e) Bachi, M. D.; Klein, J. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1925. (f) Hegedus, L. S.; McGuire, M. A.; Schultze, L. M.; Yijun, C.; Anderson, O. P. *J. Am. Chem. Soc.* **1984**, *106*, 2680. (g) Ongania, K. H.; Wallnoefer, M. *Arch. Pharm. (Weinheim, Ger.)* **1985**, *318*, 2. (h) Gilchrist, T. L.; Rahmam, A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1203.
4. (a) Joyeau, R.; Yadav, L. D. S.; Wakselman, M. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1899. (b) Joyeau, R.; Dugenet, Y.; Wakselman, M. *J. Chem. Soc., Chem. Commun.* **1983**, 431.
5. (a) Coulton, S.; Gilchrist, T. L.; Graham, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1193. (b) Gilchrist, T. L.; Graham, K. *Tetrahedron Lett.* **1995**, *36*, 8693.
6. See, for instance: (a) Alcaide, B.; de Murga, R.; Pardo, C.; Rodríguez-Ranera, C. *Tetrahedron Lett.* **2004**, *45*, 7255. (b) Alcaide, B.; Almendros, P.; Alonso, J. M. *J. Org. Chem.* **2004**, *69*, 993. (c) Alcaide, B.; Almendros, P.; Aragoncillo, C.; Rodríguez-Acebes, R. *J. Org. Chem.* **2004**, *69*, 826. (d) Alcaide, B.; Almendros, P.; Alonso, J. M. *Chem. Eur. J.* **2003**, *9*, 5793. (e) Alcaide, B.; Almendros, P.; Aragoncillo, C. *Org. Lett.* **2003**, *5*, 3795. (f) Alcaide, B.; Almendros, P.; Alonso, J. M.; Aly, M. F. *Chem. Eur. J.* **2003**, *9*, 3415.
7. For a preliminary communication of a part of this work, see: (a) Alcaide, B.; Moreno, A. M.; Rodríguez-Vicente, A.; Sierra, M. A. *Tetrahedron: Asymmetry* **1996**, *7*, 2203. (b) After the initiation of our efforts, a report appeared describing the aryl radical cyclization of *N*-allyl-4-(*o*-bromophenyl)-2-azetidinones leading to 1,2-benzocarbacephams. See: Banik, B. K.; Subbaraju, G. V.; Manhas, M. S.; Bose, A. K. *Tetrahedron Lett.* **1996**, *37*, 1363.
8. (a) Staudinger, M. *Liebigs Ann. Chem.* **1907**, *356*, 51. For reviews on the ketene–imine approach to β-lactams, see: (b) Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Curr. Med. Chem.* **2004**, *11*, 1837. (c) Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Eur. J. Org. Chem.* **1999**, 3223. (d) Tidwell, T. T. *Ketenes*; Wiley: New York, 1995; pp 518–527. (e) Georg, G. I.; Ravikumar, V. T. In *The Organic Chemistry of β-Lactams*; Georg, G. I., Ed.; VCH: Weinheim, 1993; Chapter 3, p 295.
9. *N*-(2-Halogenophenyl)imines were prepared by the condensation of an *o*-halogenoaniline with the corresponding α,β-unsaturated aldehyde in the presence of the $ZnCl_2$/α-phenylethylamine complex as catalyst, using benzene as solvent and a Dean–Stark apparatus to remove the water formed during the reaction. Alternatively, *N*-alkylimines required in our work were smoothly prepared by the condensation of the appropriate aldehyde with the corresponding amine in dichloromethane at room temperature in the presence of anhydrous magnesium sulphate. A nearly quantitative yield of the Schiff base was obtained in all cases under these reaction conditions.
10. The assignment of relative stereochemistry was determined by the observed coupling constant for methine protons H3 and H4, which is a very well established criterium in β-lactam chemistry. See, for example: (a) Alcaide, B.; Aly, M. F.; Rodríguez, C.; Rodríguez-Vicente, A. *J. Org. Chem.* **2000**, *65*, 3453. (b) Ren, X. F.; Turos, E.; Lake, C. H.; Churchill, M. R. *J. Org. Chem.* **1995**, *60*, 6468. (c) Alcaide, B.; Domínguez, G.; Escobar, G.; Parreño, U.; Plumet, J. *Heterocycles* **1986**, *24*, 1579. (d) Zamboni, R.; Just, G. *Can. J. Chem.* **1979**, *57*, 1945.
11. Evans, D. A.; Sjogren, E. B. *Tetrahedron Lett.* **1985**, *26*, 3783.
12. Dichlorophenylphosphate was used as the condensating agent in these cases. See: Arrieta, A.; Lecea, B.; Cossío, F. P.; Palomo, C. *J. Org. Chem.* **1988**, *53*, 3784.
13. Alcaide, B.; Martín-Cantalejo, Y.; Pérez-Castells, J.; Rodríguez-López, J.; Sierra, M. A.; Monge, A.; Pérez-García, V. *J. Org. Chem.* **1992**, *57*, 5921.
14. For selected references, see: (a) Zard, S. Z. *Radicals Reactions in Organic Synthesis*; Oxford University Press: New York, 2003. (b) Martínez-Grau, A.; Marco-Contelles, J. *Chem. Soc. Rev.* **1998**, *27*, 155. (c) Rousseau, G.; Homsi, F. *Chem. Soc. Rev.* **1997**, *26*, 453. (d) Curran, D. P.; Porter, N. A.; Giese, B. *Stereochemistry of Radical Reactions*; VCH: New York, 1996. (e) Malacria, M. *Chem. Rev.* **1996**, *96*, 289. (f) Giese, B.; Kopping, B.; Göbel, T.; Dickhaut, J.; Thoma, G.; Kulicke, K. J.; Trach, F. *Org. React.* **1996**, *48*, 301–856. (g) Fossey, J.; Lefort, D.; Sorba, J. *Free Radicals in Organic Chemistry*; Wiley: New York, 1995.
15. Leibner, J. E.; Jacobus, J. *J. Org. Chem.* **1979**, *44*, 449–450.
16. Although construction of five- and six-membered rings has received much attention, problems associated with the formation of medium-sized rings using free radical methodology are widely appreciated. Even the formation of seven-membered rings in the 7-*exo-trig* mode is likely to be unsuccessful in most cases (at 25 °C, $k_{7\text{-exo}} < 7 \times 10^{-1}\ s^{-1}$). However, we and others were able to prepare seven-membered rings by radical cyclization of β-lactam-tethered haloalkenes or alkynes. See: (a) Alcaide, B.; Almendros, P.; Aragoncillo, C. *J. Org. Chem.* **2001**, *66*, 1612. (b) Penfold, D. J.; Pike, K.; Genge, A.; Anson, M.; Kitteringham, J.; Kilburn, J. D. *Tetrahedron Lett.* **2000**, *41*, 10347. (c) Alcaide, B.; Almendros, P.; Aragoncillo, C. *Chem. Commun.* **1999**, 1913. (d) Knight, J.; Parsons, P. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 1237. (e) Knight, J.; Parsons, P. J.; Southgate, R. *J. Chem. Soc.,*

Chem. Commun. **1986**, *1*, 78. (f) Bachi, M. D.; Frolow, F.; Hoornaert, C. *J. Org. Chem.* **1983**, *48*, 1841.
17. For a review on the selective bond cleavage of the β-lactam nucleus, see: Alcaide, B.; Almendros, P. *Synlett* **2002**, 381.
18. See, for example: (a) Crimmings, M. T.; Huang, S.; Guise-Zabacki, L. E. *Tetrahedron Lett.* **1996**, *37*, 6519. (b) Horwell, D. C.; Morrell, A. I.; Roberts, E. *Tetrahedron Lett.* **1995**, *36*, 459.
19. Alcaide, B.; Rodríguez-Campos, I. M.; Rodríguez-López, J.; Rodríguez-Vicente, A. *J. Org. Chem.* **1999**, *64*, 5377.
20. These values are in good agreement with previously reported data for related compounds obtained by different methods. See: Bertha, F.; Fetter, M.; Kajtár-Peredy, M.; Lempert, K.; Czira, G. *Tetrahedron* **1998**, *54*, 15227.
21. (a) Kliegman, J. M.; Barnes, R. K. *J. Org. Chem.* **1970**, *35*, 3140. (b) Kliegman, J. M.; Barnes, R. K. *Tetrahedron* **1970**, *26*, 2555.
22. Schmid, C.; Bryant, J. D.; Dowlatzedah, M.; Phillips, J.; Prather, D. E.; Schantz, R. D.; Sear, N. L.; Vianco, C. S. *J. Org. Chem.* **1991**, *56*, 4056.
23. Ojima, I.; Komata, T.; Qiu, X. *J. Am. Chem. Soc.* **1990**, *112*, 770. See, also Ref. 11.
24. Tako, A.; Ongania, K. H.; Wurst, K. *Monatsh. Chem.* **1997**, *128*, 1156.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2779–2794

Aza-Wittig reaction of fluoroalkylated *N*-vinylic phosphazenes with carbonyl compounds. Usefulness of 2-azadienes for the preparation of fluoroalkyl pyridine derivatives

Francisco Palacios,* Concepción Alonso, Gloria Rubiales and Maite Villegas

Departamento de Química Orgánica I, Facultad de Farmacia, Universidad del País Vasco, Apartado 450, 01080, Spain

Received 25 November 2004; revised 18 January 2005; accepted 20 January 2005

Abstract—A method for the preparation of 3-fluoroalkyl substituted 2-aza-butadienes **6** by aza-Wittig reaction of 3-fluoroalkyl-*N*-vinylic phosphazenes **4** and aldehydes **5** is reported. [4+2] Cycloaddition reaction of these heterodienes **6** with enamines **9** gives fluoroalkyl substituted pyridine **15**, **16**, **24**–**27** and isoquinoline **12**–**14**, **20** derivatives.

1. Introduction

Functionalized 2-azabutadiene systems have proved to be efficient key intermediates in organic synthesis for the preparation of heterocycles[1,2] although the great majority of 2-azadienes studied are substituted with electron-donating groups and are excellent reagents in normal Diels–Alder reactions with electron-poor dienophiles.[1–3] In this context, we have described new methods for the preparation of heterocycles,[4] as well as for the synthesis of neutral azadienes **I** (Fig. 1)[5] and of electron-poor 2-aza-1,3-butadienes derived from α- and β-amino esters **II** (Fig. 1)[6] and we have also reported their use in the preparation of nitrogen heterocyclic compounds.[4–6]

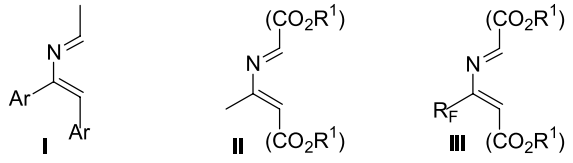


Figure 1.

Moreover, special interest has been focused on developing synthetic methods for the preparation of fluorinated building blocks since they are used for the efficient and/or selective preparation of fluorine-containing molecules with biological activity[7] and commercial applications.[8] Direct fluorination is the simplest way to prepare fluorinated heterocyclic compounds,[9] but usually the use of fluorinated precursors has been of more interest due to the easy formation of the products and to the regioselectivity of the fluorine substituents on the heterocyclic ring.[10] In this context, fluoroalkyl substituted 2-aza-1,3-butadienes **III** ($R_F = CF_3$, C_2F_5, … Fig. 1), despite their potential interest as synthons in organic synthesis for the construction of more complex fluoro-containing acyclic and cyclic compounds, have not received much attention, probably owing to the lack of general methods of synthesis of these compounds. Moreover, the presence of carboxylic groups in position 1 and 4 in compounds **III** (Fig. 1) may open new entries to the formation of heterodienes derived from α- and β-amino-acids. However, as far as we know, there has only been synthesis of 4-alkoxy-1,4-disilyloxy-1-trifluoromethyl-,[11a] 1,1-bis-(trifluoromethyl-),[11b] 4,4-difluoro- and 3-trifluoromethyl-2-aza-1,3-butadienes[11c] and reactions of 4-alkoxy-1,4-disilyloxy-1-trifluoromethyl-2-aza-1,3-butadienes with carbonyl compounds[12a] and 1,1-bis-(trifluoromethyl)-2-aza-1,3-butadiene with bromide, amines, mercaptans,[12b] diazomethane[12c] and phosphines[12d] have been described. As a continuation of our work on the design of new building blocks, we report herein an easy and versatile method for the synthesis of fluoroalkyl substituted 2-azadienes **III** involving aza-Wittig reaction[13] of *N*-vinylic phosphazenes **IV** with aldehydes and the use of these substrates as starting materials for the construction of fluoroalkyl functionalized heterocycles (Fig. 2).[14]

Keywords: *N*-vinylic phosphazenes; Aza-Wittig reaction; 2-Aza-1; 3-butadienes; Fluoroalkyl derivatives; Heterocycles.
* Corresponding author. Tel.: +34 945013103; fax: +34 945013049; e-mail: qoppagaf@vc.ehu.es

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.080

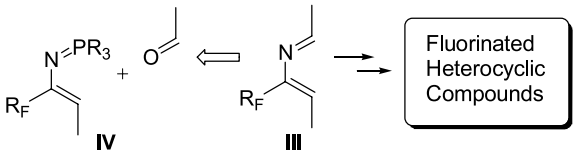
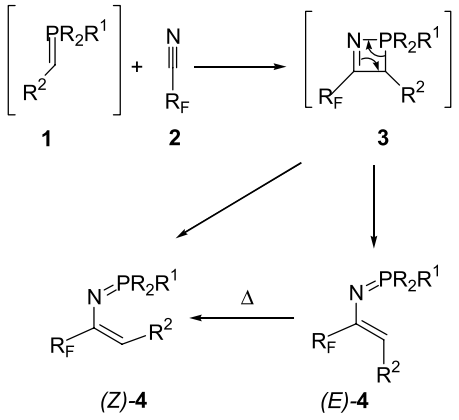


Figure 2.



Scheme 1.

2. Results and discussion

2.1. Synthesis of *N*-vinylic phosphazenes

Fluoroalkyl substituted *N*-vinylic phosphazenes **4** were prepared by reaction of phosphorus ylides and perfluoroalkyl nitriles.[15] Gas nitriles **2a** (R_F=CF_3) and **2b** (R_F=C_2F_5) were freshly generated[16] and bubbled through a solution of phosphorus ylides **1** (R^2=Ph, CO_2Me, CN),[17] affording the corresponding *N*-vinylic phosphazenes **4a**, **4b**, **4d**, **4e**, **4g**, **4i**, **4l** and **4m** (Scheme 1) in good yields (Table 1, entries 1, 2, 4, 5, 7, 9, 12 and 13). Commercially available nitrile **2c** (R_F=C_7F_{15}) was also used for the synthesis of phosphazenes **4c**, **4f**, **4h**, **4j**, **4k**, **4n** (Table 1, entries 3, 6, 8, 10, 11 and 14). Inorganic salts were eliminated by filtration and some phosphazenes were crystallized from ethyl acetate and isolated. However, other phosphazenes were less stable and, for this reason, were used without isolation (Table 1, entries 6–11) from the crude mixtures.

Crystalline compounds **4** were characterized on the basis of their spectroscopic data as *E* or *Z* isomers. Phosphazenes **4a–c** with a phenyl group (R^2=Ph) were exclusively obtained as *E* isomers (Table 1, entries 1–3), while phosphazenes **4d–h** derived from methoxycarbonyl ylides (R^2=CO_2Me) afforded phosphazenes whose HOESY ^{19}F–^{1}H experiments showed cross signals between fluorinated groups and vinylic protons, suggesting the formation of *Z* isomers (Table 1, entries 4–8).

However, when ylides containing a cyan substituent were employed (R^2=CN) either only *E* isomers (Table 1, entries 10, 11 and 14) or mixtures of *E* and *Z* isomers (Table 1, entries 9, 12 and 13) with a higher proportion of *E* isomers were obtained. Formation of conjugated phosphazenes **4** can be explained through [2+2] cycloaddition of phosphorus ylides **1** and nitriles **2** followed by ring opening of the unstable four-membered cyclic compounds **3**[5b,18] (Scheme 1). In this context, it is noteworthy that, isomerization of *E* isomer towards *Z* isomer was observed during purification (recrystallization or column chromatography) or thermal treatment.

For instance, spectroscopic analysis confirmed the total thermal isomerization to the new *Z* isomers when only the *E* isomers (phosphazenes **4a–c** and **4n**) as well as mixtures of *E*- and *Z*-isomers (phosphazenes **4i**, **4l** and **4m**) were heated at 110 °C in toluene. For example, ^{1}H NMR monitoring of *E*-phosphazene **4a** upon heating showed a singlet at δ_H 6.23 ppm for the vinylic proton of *Z*-phosphazene **4a**, instead of the doublet corresponding to the *E* precursor (δ_H=5.70 ppm, $^{4}J_{PH}$=3.2 Hz). Configuration of vinylic double bonds was also determined on the basis of heteronuclear HOESY ^{19}F–^{1}H experiments. Similar isomerizations have been observed previously by us[19] and by others.[20]

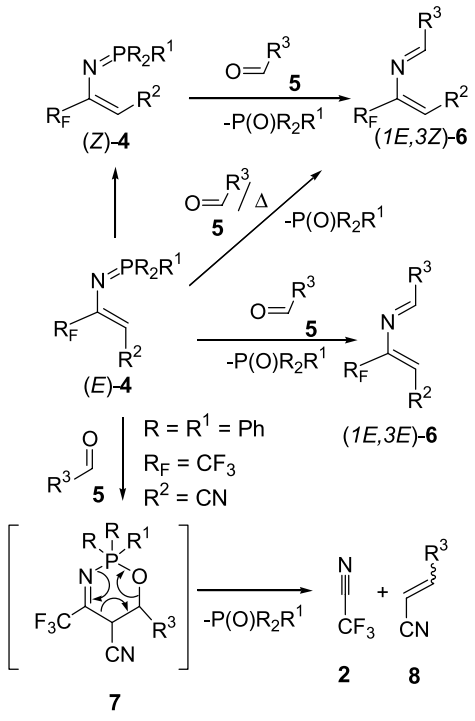
2.2. Aza-Wittig reaction of fluoroalkyl substituted *N*-vinylic phosphazenes 4 with carbonyl compounds 5

We then turned our attention to the preparation of

Table 1. *N*-Vinylic phosphazenes **4** obtained

Entry	Compound	R	R^1	R^2	R_F	Time (h)	Solvent	Yield (%)	Mp (°C)	*E*/*Z* (%)
1	**4a**	Ph	Ph	Ph	CF_3	12	Et_2O	90[a]	134–135	100:0[b,c]
2	**4b**	Ph	Ph	Ph	C_2F_5	24	Et_2O	81[a]	105–106	100:0[b,c]
3	**4c**	Ph	Ph	Ph	C_7F_{15}	12	Toluene	83[a]	120–121	100:0[c]
4	**4d**	Ph	Ph	CO_2Me	CF_3	12	Et_2O	65[a]	101–102	0:100
5	**4e**	Ph	Ph	CO_2Me	C_2F_5	24	Et_2O	99[a]	93–94	0:100
6	**4f**	Ph	Me	CO_2Me	C_7F_{15}	48	Toluene	—[d]	—	0:100
7	**4g**	Me	Me	CO_2Me	CF_3	12	Et_2O	—[d]	—	0:100
8	**4h**	Me	Me	CO_2Me	C_7F_{15}	12	Et_2O	—[d]	—	0:100
9	**4i**	Ph	Ph	CN	CF_3	24	Et_2O	—[d]	—	60:40[c]
10	**4j**	Ph	Ph	CN	C_7F_{15}	48	Toluene	—[d]	—	100:0
11	**4k**	Ph	Me	CN	C_7F_{15}	48	Toluene	—[d]	—	100:0
12	**4l**	Me	Me	CN	CF_3	12	THF	86[a]	—	80:20[c]
13	**4m**	Me	Me	CN	C_2F_5	12	THF	66[a]	—	75:25[c]
14	**4n**	Me	Me	CN	C_7F_{15}	12	THF	40[a]	98–99	100:0[c]

[a] Yield of isolated compounds.
[b] Isomerization towards *Z* isomer was observed when purification by column chromatography was performed (see Section 4).
[c] Isomerization of *E* isomer towards *Z* isomer was observed when a solution of *E* isomer or the *E*/*Z* mixture was heated at 110 °C in toluene (see Section 4).
[d] Not isolated, used in situ.



Scheme 2.

2-azadienes **III** (Fig. 2) containing a carboxylate group or a synthetic equivalent either in position 1 or in position 4, because these substrates are heterodienes derived from α- and β-amino acids respectively, containing fluoroalkyl substituents on 3 position. The aza-Wittig reaction[13] of fluoroalkyl *E*-*N*-vinylic phosphazenes **4a–c** derived from triphenylphosphine (R=R^1=Ph) with ethyl glyoxalate **5** (R^3=CO$_2$Et) at room temperature (Scheme 2) gave fluoroalkyl 2-azadienes **6a–c** (Table 2, entries 1–3) keeping the *E* configuration of the vinylic double bond (1,2-addition). These heterodienes **6a–c** were unstable to distillation or chromatography and therefore were not isolated and used in situ for the following reactions, but the presence of the non isolable compounds was established on the basis of the spectroscopic data of their crude mixtures. In the ^{1}H NMR spectrum of crude reaction of compound **6b** the olefinic hydrogen appeared as a singlet at δ 6.85 ppm and the iminic hydrogen as a singlet at δ 7.90 ppm. A significant signal enhancement (1.6% NOESY) was observed between the vinylic proton at δ 6.85 ppm of 2-azadiene and iminic proton at δ 7.90 ppm for compound **6b**. These data are consistent with the 1*E*,3*E*-configuration of heterodienes **6a–c**.

However, no aza-Wittig reaction (1,2-addition) was observed between ethyl glyoxalate or aromatic aldehydes **5** with fluoroalkyl *N*-vinylic phosphazenes derived from triphenylphosphine **4d**,**4e** (R=R^1=Ph, R^2=CO$_2$Me) or **4i**,**4j** (R=R^1=Ph, R^2=CN) or derived from diphenylmethylphosphine **4f** (R=Ph, R^1=Me, R^2=CO$_2$Me) and **4k** (R=Ph, R^1=Me, R^2=CN). The electron withdrawing effect of these groups (CN, CO$_2$Me) on 4 position seems to decrease the reactivity of conjugated phosphazenes **4** through the P=N linkage in the aza-Wittig reaction. Conversely, when phosphazene **4i** (R=R^1=Ph, R^2=CN, R$_F$=CF$_3$) was treated with *p*-nitrobenzaldehyde **5** (R^3=4-NO$_2$–C$_6$H$_4$), olefinic derivative **8a** (R^3=4-NO$_2$–C$_6$H$_4$)[21] and triphenylphosphine oxide were isolated from the crude mixture (Scheme 2). This result suggests an enaminic behaviour (1,4-addition) of the conjugated phosphazene **4i**. Formation of compound **8a** could be explained by an initial addition of aldehyde **5** to the β-carbon atom of phosphazene **4i** (1,4-addition) to give unstable 1,3,2-oxaazaphosphorane **7**, whose opening afforded the corresponding triphenylphosphine oxide (R=R^1=Ph), the very volatile nitrile **2** (R$_F$=CF$_3$) and alkene derivative **8a**.

Substitution in the phosphorus atom of phosphazenes of phenyl by methyl groups increases the reactivity of the phosphazene[6b] (1,2-addition) in a similar way to that observed for isosteric phosphorus ylides,[22] therefore more reactive phosphazenes derived from trimethylphosphine were used. Initially, we explored the reaction of *Z*-conjugated phosphazene with an ester group in 4 position **4g** (R=R^1=Me, R^2=CO$_2$Me, R$_F$=CF$_3$) with aromatic aldehydes **5** (R^3=4-NO$_2$–C$_6$H$_4$, 2,4-(NO$_2$)$_2$–C$_6$H$_3$). After heating the respective reaction mixtures in refluxing toluene the expected (1*E*,3*Z*)-2-azadienes **6d** (R^3=4-NO$_2$–C$_6$H$_4$) or **6e** (R^3=2,4-(NO$_2$)$_2$–C$_6$H$_3$) (Table 2, entries 4 and 5) were obtained, keeping the *Z*-configuration of the starting vinyl group from conjugated phosphazene **4g**. Similar results were observed when aza-Wittig reaction was attempted with *Z*-conjugated phosphazenes containing a cyano group **4l**

Table 2. 3-Fluoroalkyl-2-azadienes **6** obtained

	Compound	Starting material	R^2	R$_F$	R^3	*T* (°C)	Time (h)	Solvent	Yield (%)
1	(1*E*,3*E*)-**6a**	(*E*)-**4a**	Ph	CF$_3$	CO$_2$Et	20	1	CHCl$_3$	—[a]
2	(1*E*,3*E*)-**6b**	(*E*)-**4b**	Ph	C$_2$F$_5$	CO$_2$Et	20	1	CHCl$_3$	—[a]
3	(1*E*,3*E*)-**6c**	(*E*)-**4c**	Ph	C$_7$F$_{15}$	CO$_2$Et	20	0.5	CHCl$_3$	—[a]
4	(1*E*,3*Z*)-**6d**	(*Z*)-**4g**	CO$_2$Me	CF$_3$	4-NO$_2$–C$_6$H$_4$	110	3	Toluene	—[a]
5	(1*E*,3*Z*)-**6e**	(*Z*)-**4g**	CO$_2$Me	CF$_3$	2,4-(NO$_2$)$_2$–C$_6$H$_3$	110	3	Toluene	—[a]
6	(1*E*,3*Z*)-**6f**	(*Z*)-**4l**	CN	CF$_3$	4-NO$_2$–C$_6$H$_4$	110	2	Toluene	45[b]
7	(1*E*,3*Z*)-**6f**	(*E*)-**4l**	CN	CF$_3$	4-NO$_2$–C$_6$H$_4$	110	3	Toluene	35[b]
8	(1*E*,3*Z*)-**6g**	(*E*)-**4l**	CN	CF$_3$	2,4-(NO$_2$)$_2$–C$_6$H$_3$	110	6	Toluene	30[b]
9	(1*E*,3*Z*)-**6g**	(*E*+*Z*)-**4l**	CN	CF$_3$	2,4-(NO$_2$)$_2$–C$_6$H$_3$	61	12	CHCl$_3$	45[b]
10	(1*E*,3*Z*)-**6h**	(*E*+*Z*)-**4m**	CN	C$_2$F$_5$	4-NO$_2$–C$_6$H$_4$	110	120	Toluene	—[a]
11	(1*E*,3*Z*)-**6i**	(*Z*)-**4n**	CN	C$_7$F$_{15}$	4-NO$_2$–C$_6$H$_4$	110	120	Toluene	—[a]
12	(1*E*,3*Z*)-**6i**	(*E*)-**4n**	CN	C$_7$F$_{15}$	4-NO$_2$–C$_6$H$_4$	110	138	Toluene	—[a]
13	(1*E*,3*Z*)-**6j**	(*E*)-**4n**	CN	C$_7$F$_{15}$	2,4-(NO$_2$)$_2$–C$_6$H$_3$	110	192	Toluene	—[a]

[a] Not isolated, used in situ for the next reactions.
[b] Yield of isolated compounds by column chromatography.

(R=R^1=Me, R^2=CN, R$_F$=CF$_3$) and **4n** (R=R^1=Me, R^2=CN, R$_F$=C$_7$F$_{15}$) to give (1*E*,3*Z*)-heterodienes **6f** and **6i** (Table 2, entries 6 and 11).

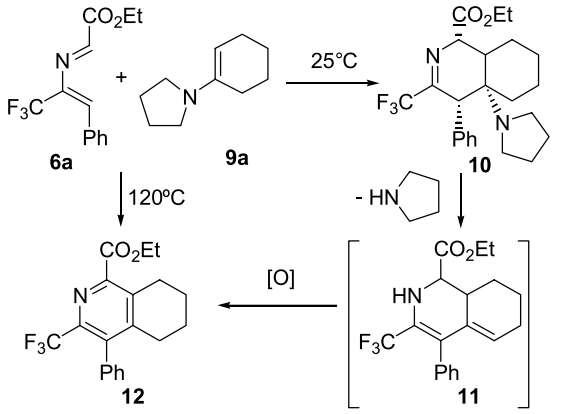
However, these azadienes **6f** and **6i** with the same configuration (1*E*,3*Z*) were also obtained when *E* isomers **4l** and **4n** were used, although longer reaction times were necessary than for 3-*Z* isomers (Table 2, entries 7 and 12). ^{1}H NMR monitoring of reaction of *E* isomer of phosphazene **4l** with *p*-nitrobenzaldehyde (R^3=4-NO$_2$–C$_6$H$_4$) was performed. Initially, signals corresponding to conversion of *E*-phosphazene towards *Z* isomer were observed, the latter being the precursor of corresponding (1*E*,3*Z*)-2-azadiene **6f**, whose signals began to be visible after isomerization. In a similar way, reaction *E* isomer of phosphazenes **4l** and **4n** with aromatic aldehyde **5** (R^3=2,4-(NO$_2$)$_2$–C$_6$H$_3$) (Table 2, entries 8 and 13) or reaction of *E*,*Z* mixtures of phosphazene **4l** (3*E*/3*Z*=80/20) with aromatic aldehyde **5** (R^3=2,4-(NO$_2$)$_2$–C$_6$H$_3$) or phosphazene **4m** (3*E*/3*Z*=75/25) with 4-nitrobenzaldehyde **5** (R^3=4-NO$_2$–C$_6$H$_4$) led to the formation of (1*E*,3*Z*)-azadienes **6g**,**h**,**j**, respectively (Table 2, entries 9, 10 and 13). HOESY ^{19}F–^{1}H experiment for compound **6j** showed cross signal between fluorinated group and vinylic proton, confirming the formation of (1*E*,3*Z*) isomer. As far as we know, this strategy describes the first synthesis[14] of 3-perfluoroalkyl-2-azabutadienes derived from α-amino esters **6a–c** (R$_F$=CF$_3$, C$_2$F$_5$, C$_7$F$_{15}$) and 3-perfluoroalkyl-2-azabutadienes derived from β-amino esters **6d**,**e** (R$_F$=CF$_3$) and from β-amino nitriles **6f–j** (R$_F$=CF$_3$, C$_2$F$_5$, C$_7$F$_{15}$).

2.3. Reaction of 3-fluoromethyl-2-azadienes 6 with enamines 9. [4+2] versus [2+2] cycloaddition processes

Pyridine nuclei are widespread in the alkaloid family and constitute an important class of compounds in pharmaceuticals, agrochemicals and dyestuffs.[23,24] For this reason, in order to test the synthetic usefulness of the new fluoroalkyl substituted azadienes **6** as key intermediates in organic synthesis and specially in the preparation of new nitrogen-containing heterocycles, the cycloaddition reaction of fluoroalkyl substituted 2-azadienes **6** was explored. Cycloaddition reactions with a range of dienophiles (diethyl ketomalonate, *trans*-cyclooctene, …) were inefficient. Indeed, even on prolonged heating and at higher temperature no significant cycloaddition was observed, and so the reaction of heterodienes **6** with electron-rich olefins such as enamines was studied.

Initially, the reaction of 2-azadiene derived from α-amino esters **6a** was explored, because this substrate would be an interesting starting material for the preparation of pipecolic acid derivatives.[25] When the reaction of 2-azadiene **6a** with *N*-cyclohex-1-enyl pyrrolidine **9a** was performed in refluxing toluene, only the aromatic bicyclic compound **12** (Scheme 3, 40%) was obtained.

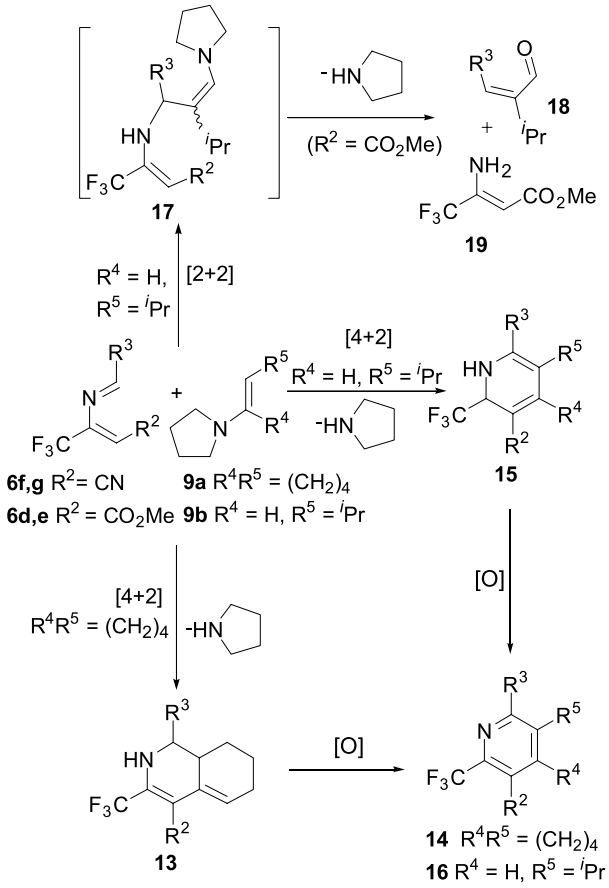
The formation of this tetrahydroisoquinoline can be explained through a [4+2] cycloaddition reaction of heterodiene **6a** with enamine **9a** and formation of cycloadduct **10** followed by the loss of pyrrolidine and oxidation. Then, we tried to stop the process in proposed intermediates when the reaction was achieved in very mild



Scheme 3.

reaction conditions. At room temperature in CHCl$_3$, alkylfluorinated 2-azadiene **6a**, underwent efficient regio- and stereoselective cycloaddition with enamine **9a** affording the *endo*-cycloadduct **10** (Scheme 3) in good yield (80%) in a regio- and stereoselective fashion. The structure of compound **10** was assigned on the basis of the 1D and 2D spectroscopy, including COSY, NOE, HMQC and HMBC experiments and mass spectral data. As far as we know, this strategy describes the first synthesis[14] of 3-trifluoromethyl-isoquinolines derived from α-amino esters **10** and **12**.

Then, we tried to extend the process to 3-trifluoromethyl-2-azadienes derived from β-amino nitrile. Thus, 2-azadiene **6f**



Scheme 4.

Table 3. Pyridine derivatives **13–16** obtained from azadienes **6** and enamines **9**

Entry	Compound	R^2	R^3	R^4	R^5	Time (h)	Yield (%)
1	**13a**	CN	4-NO_2–C_6H_4	–$(CH_2)_4$–		2	29[a]
2	**13b**	CN	2,4-$(NO_2)_2$–C_6H_3	–$(CH_2)_4$–		3	—[b,c]
3	**14a**	CN	4-NO_2–C_6H_4	–$(CH_2)_4$–		2/72[d]	29[a]/93[e]
4	**14b**	CN	2,4-$(NO_2)_2$–C_6H_3	–$(CH_2)_4$–		3/144[f]	—[b,c]/70[d]
5	**15a**	CO_2Me	2,4-$(NO_2)_2$–C_6H_3	H	^{i}Pr	3	35[a]
6	**15b**	CO_2Me	4-NO_2–C_6H_4	H	^{i}Pr	24	60[a]
7	**16a**	CO_2Me	2,4-$(NO_2)_2$–C_6H_3	H	^{i}Pr	3/24[i]	43[a]/98[f]
8	**16b**	CO_2Me	4-NO_2–C_6H_4	H	^{i}Pr	48[i]	72[f]
9	**16c**	CN	4-NO_2–C_6H_4	H	^{i}Pr	3	52[a]
10	**16d**	CN	2,4-$(NO_2)_2$–C_6H_3	H	^{i}Pr	3	64[a]

[a] Isolated by column chromatography.
[b] Not isolated.
[c] Proportion **13b**:**14b**, 6:1.
[d] Obtained by oxidation of the mixture of **13** and **14** with *p*-benzoquinone in dioxane at 80 °C.
[e] Obtained by oxidation of **13a** with *p*-benzoquinone in dioxane at 80 °C.
[f] Obtained by oxidation of **15** with *p*-benzoquinone in dioxane at 80 °C.

(R^2=CN, R^3=4-NO_2–C_6H_4) reacted with *N*-cyclohex-1-enylpyrrolidine **9a** at room temperature until disappearance of starting material, affording a mixture (1:1) of bicyclic cycloadduct **13a** (29%) and the corresponding aromatic heterocycle **14a** (29%) (Scheme 4, Table 3, entries 1 and 3). Oxidation of **13a** with *p*-benzoquinone in dioxane at 80 °C (72 h) gave only 3-trifluoromethyl-5,6,7,8-tetrahydro-isoquinoline **14a** in very high yield (93%). Similarly, azadiene **6g** (R^2=CN, R^3=2,4-$(NO_2)_2$–C_6H_3) was treated with enamine **9a** at room temperature to give an inseparable mixture of heterocyclic derivatives **13b** and **14b** (6:1) (Scheme 4, Table 3, entries 2 and 4). Oxidation of the mixture with *p*-benzoquinone in dioxane at 80 °C gave only the aromatic pyridine **14b** (Scheme 4, Table 3, entry 4).

Formation of compounds **13** and **14** (R^4R^5=$(CH_2)_4$) could be explained, as before, by formation of a [4+2]-cycloadduct followed by the loss of pyrrolidine and oxidation in a similar way to that described in Scheme 3.

The reaction was extended to *N*-(3-methyl)but-1-enyl-pyrrolidine **9b** (R^4=H, R^5=^{i}Pr). In this process, the electron-withdrawing effect of substituents at position 4 (CO_2Me, CN) seems to play an important role. 2-Azadiene **6e** derived from β-aminoester (R^2=CO_2Me, R^3=2,4-$(NO_2)_2$–C_6H_3) reacted with enamine **9b** in $CHCl_3$ at room temperature to give heterocycles **15a** (R^2=CO_2Me, R^3=2,4-$(NO_2)_2$–C_6H_3, R^4=H, R^5=^{i}Pr) and **16a** (R^2=CO_2Me, R^3=2,4-$(NO_2)_2$–C_6H_3, (Scheme 4, Table 3, entries 5 and 7) along with a small amount (<10%) of aldehyde **18a** (R^3=2,4-$(NO_2)_2$–C_6H_3) and enamine **19**.[26] Formation of heterocycles **15** and **16** (R^4=H, R^5=^{i}Pr) could be explained, as before, by formation of a [4+2]-cycloadduct followed by the loss of pyrrolidine and oxidation in a similar way to that described in Scheme 3. However, the formation of aldehyde **18a** (R^3=2,4-$(NO_2)_2$–C_6H_3) and primary enamine **19** could be explained by a competitive [2+2] cycloaddition of the enamine with the iminic double bond of heterodiene[27] followed by ring opening of the four-membered ring and formation of the intermediate **17** (Scheme 4), whose subsequent C–N bond cleavage gave pyrrolidine, carbonyl compound **18** and primary enamine **19**.

On the other hand, when 2-azadiene **6d** (R^2=CO_2Me, R^3=4-NO_2–C_6H_4) reacted in $CHCl_3$ at room temperature with the same enamine **9b** a complex mixture of several compounds including decomposition products of starting azadiene was obtained after very long period of time (10 days). Nevertheless, if the reaction was performed by heating in toluene, only the 1,2-dihydropyridine **15b** (R^2=CO_2Me, R^3=4-NO_2–C_6H_4, R^4=H, R^5=^{i}Pr) was obtained (Scheme 4, Table 3, entry 6). Oxidation of 1,2-dihydropyridines **15a**,**b** with *p*-benzoquinone in dioxane at 80 °C (for 24 and 48 h respectively) gave pyridines **16a**,**b** (98 and 72% see Table 3, entries 7 and 8).

Aromatic pyridines **16** can also be directly prepared in the case of 2-azadienes derived from β-amino nitriles **6f**,**g** (R^2=CN). Reactions of 2-azadienes **6f** (R^3=4-NO_2–C_6H_4) and **6g** (R^3=2,4-$(NO_2)_2$–C_6H_3) with enamine **9b** was performed in $CHCl_3$ at room temperature to give only

Table 4. Pyridine derivatives **20**, **24–27** obtained from azadienes **6** and enamines **9**

Entry	Compound	R^3	R^6	R^4	R^5	Yield
1	**20**	—	CF_3	–$(CH_2)_4$–	60[a]	
2	**24a**	—	CF_3	–$(CH_2)_3$–	42[a]	
3	**24b**	—	C_6F_{13}	–$(CH_2)_3$–	48[a]	
4	**25a**	4-NO_2–C_6H_4	CF_3	H	^{i}Pr	40[a]
5	**25b**	4-NO_2–C_6H_4	C_6F_{13}	H	^{i}Pr	35[a]
6	**25c**	2,4-$(NO_2)_2$–C_6H_3	C_6F_{13}	H	^{i}Pr	—[b]
7	**26a**	4-NO_2–C_6H_4	CF_3	H	^{i}Pr	18[a]
8	**26b**	4-NO_2–C_6H_4	C_6F_{13}	H	^{i}Pr	18[a]
9	**26c**	2,4-$(NO_2)_2$–C_6H_3	C_6F_{13}	H	^{i}Pr	—[b]
10	**27b**	4-NO_2–C_6H_4	C_6F_{13}	H	^{i}Pr	22[a]

[a] % Isolated by column chromatography.
[b] Obtained as an inseparable mixture of **25c** and **26c** (1:2).

pyridines **16c** and **16d**, respectively (Scheme 4, Table 3, entries 9 and 10).

2.4. Reaction of 3-perfluoroethyl-2-azadienes and 3-perfluorohepthyl-2-azadienes with enamines

In order to test the influence of perfluoroalkyl substituents at 3 position on the reactivity of heterodienes **6** derived from α-amino esters ($R^3=CO_2Et$), we tried to extend the study to the reaction of 3-perfluoroethyl-2-azadiene **6b** ($R^6=CF_3$) and 3-perfluorohepthyl-2-azadiene **6c** ($R^6=C_6F_{13}$) with enamines **9** (Scheme 5). However, an unexpected behaviour was observed when 2-azadiene derived from ethyl glyoxalate **6b** ($R^6=CF_3$) was treated with enamine **9a** ($R^4R^5=(CH_2)_4$). Spectroscopic data for the compounds obtained showed that pyridine **20** ($R^6=CF_3$, $R^4R^5=(CH_2)_4$) was obtained (Scheme 5, Table 4, entry 1) instead of the expected compound **21** ($R^4R^5=(CH_2)_4$).

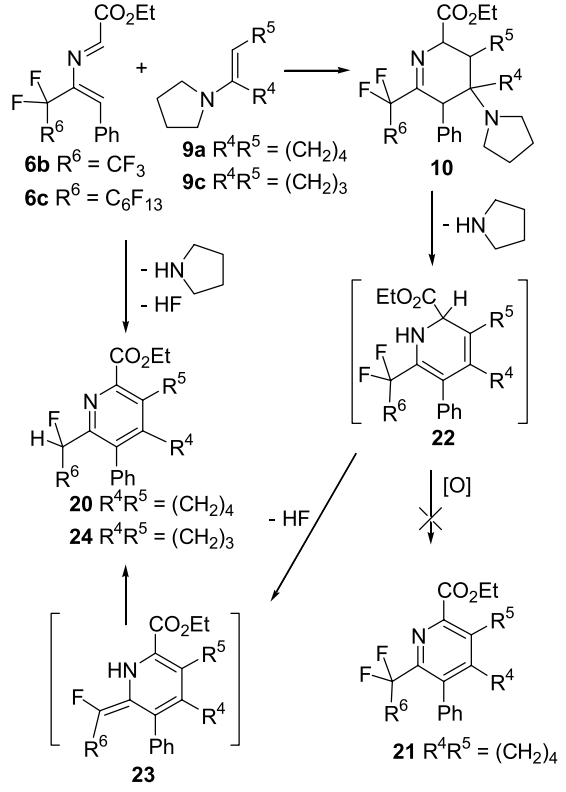
Mass spectrometry of compound **20** showed the molecular ion (*m*/*z* 381, 73%), which corresponds to the loss of HF from expected cycloadduct **22**, confirmed also by ^{1}H NMR and ^{13}C NMR spectra. Thus, ^{1}H NMR of compound **20** ($R^6=CF_3$, $R^4R^5=(CH_2)_4$) showed a double quadruplet at δ 5.45 ppm with coupling constants of $^2J_{HF}=46$ Hz and $^3J_{HF}=6$ Hz and ^{13}C NMR spectrum shows a double quadruplet at δ 122.0 ppm with coupling constants of $^1J_{CF}=281.0$ Hz and $^2J_{CF}=29.0$ Hz.

Formation of this fluoroalkyl heterocycle **20** could be explained through a [4+2]-cycloadduct **10** followed by the loss of pyrrolidine, dehydrofluorination and formation of **23**. Enamine-imine tautomerization of **23** may give aromatic 1-ethoxycarbonyl-4-phenyl-3-(1,2,2,2-tetrafluoroethyl)-5,6,7,8-tetrahydroisoquinoline **20** (Scheme 5).[28]

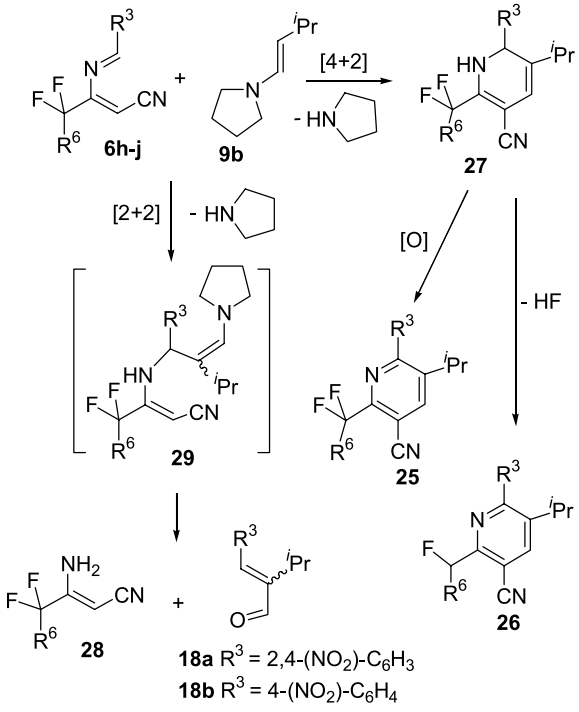
Likewise, heterodienes derived from glyoxalate **6b** ($R^6=CF_3$) and **6c** ($R^6=C_6F_{13}$) with *N*-cyclopent-1-enyl pyrrolidine **9c** ($R^4R^5=(CH_2)_3$) led also to the formation of bicyclic heterocycles **24a** ($R^6=CF_3$, $R^4R^5=(CH_2)_3$) and **24b** ($R^6=C_6F_{13}$, $R^4R^5=(CH_2)_3$) containing fluoroalkyl substituents at 3 position (Scheme 5, Table 4, entries 2 and 3). In these processes the presence of an electron-withdrawing group (CO_2Et) at 1 position seems to favour the [4+2] cycloaddition reaction with exclusive formation of aromatic pyridine derivatives **20** and **24**.

However, when heterodienes with an aryl group (1 position) and derived from β-amino nitriles ($R^2=CN$) such as 2-azadiene **6h** ($R^6=CF_3$, $R^3=4\text{-}NO_2\text{–}C_6H_4$) were treated with enamine **9b**, not only aromatic pyridines **25a** and **26a** (Scheme 6, Table 4, entries 4 and 7) but also unsaturated aldehydes **18b** ($R^3=4\text{-}NO_2\text{–}C_6H_5$, *E* and *Z*) and enamine **28a** ($R^6=CF_3$) were obtained (<10%, Scheme 6).

Similar behaviour was observed in the reaction of 2-azadienes **6i** ($R^6=C_6F_{13}$, $R^3=4\text{-}NO_2\text{–}C_6H_4$) and **6j** ($R^6=C_6F_{13}$, $R^3=2,4\text{-}(NO_2)_2\text{–}C_6H_3$) with this enamine **9b** to give mixtures of aromatic perfluoro substituted (position 2) pyridines **25b**,**c** (Scheme 6) and pyridines with only a fluorine atom in Cα of C-2 **26b**,**c** (Table 4, entries 5, 6, 8 and 9). Formation of compounds **25**, **26** could be explained, as before, through a normal [4+2] cycloaddition reaction of heterodienes and enamines with formation of cycloadducts **27**. These intermediates could give either pyridines **25**, by oxidation under reaction conditions, or pyridines **26** by dehydrofluorination, in a similar way to that observed before in Scheme 5. Moreover, in the case of the reaction of heterodiene **6i**, the dihydropyridine derivative **27b**



Scheme 5.



Scheme 6.

($R^6=C_6F_{13}$, $R^3=4$-(NO_2)–C_6H_4) can also be isolated (Table 4, entry 10). Spectroscopic data 1D and 2D, including HMQC and HMBC experiments were consistent with the structure of compound **27**. In these processes a small amount (<10%) of the corresponding unsaturated aldehydes **18b** ($R^3=4$-(NO_2)–C_6H_4) and **18a** ($R^3=2,4$-$(NO_2)_2$–C_6H_3) and enamine **28b** ($R^6=C_6F_{13}$, 6% and 8% respectively) were obtained. The formation of compounds **28** and **18** could also be explained by a competitive [2+2] cycloaddition of the enamine with the iminic double bond of heterodiene and formation of intermediate **29** in a similar manner to that reported in Scheme 4.

3. Conclusion

In summary, fluoroalkyl *N*-vinylic phosphazenes **4** can be prepared readily from fluoroalkyl nitriles **2** and phosphorus ylides **1**. These conjugated phosphazenes react cleanly and in good yields with aldehydes, by means of an aza-Wittig reaction, to afford fluoroalkyl functionalized 2-azadienes **6**, which are excellent building blocks for the preparation of fluorinated heterocycles. For instance, fluoroalkylated heterocycles esters such as 5,6,7,8-tetrahydroisoquinolines **12**, **14** and **20**, substituted pyridines derived from pipecolic esters **24**, as well as pyridine compounds derived from β-amino nitriles and β-amino esters such as 1,2,6,7,8,8a-hexahydroisoquinolines **13**, dihydropyridines **15** and **27** and substituted pyridines **16**, **25** and **26** can be prepared through a [4+2] cycloaddition strategy involving heterodienes **6** with electron-rich dienophiles such as enamines. Most of them are described for the first time. These fluoroalkylated 2-aza-1,3-butadienes may be important synthons in organic synthesis and in the preparation of fluoroalkyl substituted acyclic and heterocycles.[1,8]

4. Experimental

4.1. General

Chemicals were purchased from Aldrich, Lancaster, Fluorochem and Acros Chemical Companies. Solvents for extraction and chromatography were technical grade. All solvents used in reactions were freshly distilled from appropriate drying agents before use. All other reagents were recrystallized or distilled as necessary. All reactions were performed under an atmosphere of dry nitrogen. Analytical TLC was performed with Merck silica gel 60 F_{254} and aluminium oxide N/UV_{254} plates. Visualization was accomplished by UV light. Flash chromatography was carried out using Merck silica gel 60 (230–400 mesh ASTM) and aluminium oxide 90 active neutre (70–230 mesh ASTM). Melting points were determined with an Electrothermal IA9100 Digital Melting Point Apparatus and are uncorrected. ^{1}H (400, 300 and 250 MHz), ^{13}C (100 and 75 MHz), ^{19}F NMR (376 and 282 MHz) and ^{31}P NMR (120 MHz) spectra were recorded on a Bruker Avance 400 MHZ and a Varian VXR 300 MHz spectrometer using $CDCl_3$ or CD_3OD solutions with TMS as an internal reference ($\delta=0.00$ ppm) for ^{1}H and ^{13}C NMR spectra, $FCCl_3$ as an internal reference ($\delta=0.00$ ppm) for ^{19}F NMR spectra, and phosphoric acid (85%) ($\delta=0.0$ ppm) for ^{31}P NMR spectra. Chemical shifts (δ) are reported in ppm. Coupling constants (J) are reported in Hertz. Low-resolution mass spectra (MS) were obtained at 50–70 eV by electron impact (EIMS) on a Hewlett–Packard 5971 or 5973 spectrometer. Data are reported in the form *m/z* (intensity relative to base=100). Infrared spectra (IR) were taken on a Nicolet IRFT Magna 550 spectrometer, and were obtained as solids in KBr or as neat oils. Peaks are reported in cm^{-1}. Elemental analyses were performed in a LECO CHNS-932 apparatus.

4.2. General procedure A for the preparation of phosphazenes 4

A 1.6 M solution of methyllithium (3.125 mL, 5 mmol) in ether was added dropwise to a solution of 5 mmol of phosphonium salt in ether or toluene (20 mL) cooled to 0 °C under N_2. The clear red solution was heated at reflux for 1 h. Fluoroalkylated nitrile was added dropwise or bubbled to the ylide solution at 0 or −35 °C and the mixture was stirred at room temperature. Inorganic salts were filtered under N_2 and filtrate was concentrated to afford an oil.

4.3. General procedure B for the preparation of phosphazenes 4

A 0.5 M solution of KHMDS in toluene (10 mL, 5 mmol) was added dropwise to a solution of 5 mmol of phosphonium salt in THF (20 mL) cooled to 0 °C under N_2 and was stirred for 4.5 h at room temperature. Inorganic salts were filtered under N_2 and fluoroalkylated nitrile was added dropwise or bubbled to the ylide solution at −35 °C. The mixture was stirred at room temperature overnight. Evaporation of solvent afforded an oil.

4.3.1. (3*E*)-1,1,1,4-Tetraphenyl-3-trifluoromethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4a). The general procedure A was followed using benzyltriphenylphosphonium iodide (2.40 g) in ether and bubbling trifluoroacetonitrile (CF_3CN) in excess at 0 °C for 12 h. Crystallization from ethyl acetate gave the (*E*) isomer of **4a** as a yellow solid (2.01 g, 90%) mp 134–135 °C (ethyl acetate). IR (KBr) ν 1600, 1341 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.70 (d, $^4J_{PH}=3.2$ Hz, 1H), 6.98–7.85 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 113.8 (dd, $^3J_{CP}=11.3$ Hz, $^3J_{CF}=1.7$ Hz), 122.8 (dq, $^1J_{CF}=278.0$ Hz, $^3J_{CP}=24.5$ Hz), 125.5–133.7 (m), 137.0 (q, $^2J_{CF}=21.3$ Hz). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 7.94. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −64.1. MS (EI) *m/z* 447 (M^+, 100). Anal. Calcd for $C_{27}H_{21}F_3NP$ (447): C, 72.48; H, 4.73; N, 3.13. Found: C, 72.02; H, 4.68; N, 3.10.

4.3.2. (3*E*)-3-Perfluoroethyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4b). The general procedure A was followed using benzyltriphenylphosphonium iodide (2.40 g) in ether and bubbling perfluoropropanenitrile (C_2F_5CN) in excess at 0 °C for 24 h. Crystallization from ethyl acetate gave the (*E*) isomer of **4b** as a yellow solid (2.01 g, 81%) mp 105–106 °C (ethyl acetate). IR (KBr) ν 1608, 1203 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.73 (s, 1H), 6.91–7.8 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 112.4 (tq, $^1J_{CP}=259.9$ Hz, $^2J_{CF}=40.8$ Hz), 119.3 (tq, $^1J_{CF}=286.6$ Hz, $^2J_{CF}=40.8$ Hz), 125.9–136.6 (m). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 7.46. ^{19}F NMR (282 MHz,

$CDCl_3$) δ: −64.3, −94.5; MS (EI) *m/z* 497 (M^+, 100). Anal. Calcd for $C_{28}H_{21}F_5NP$ (497): C, 67.61; H, 4.25; N, 2.82. Found: C, 67.72; H, 4.21; N, 2.81.

4.3.3. (3*E*)-3-Perfluorohepthyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4c). The general procedure A was followed using benzyltriphenylphosphonium iodide (2.40 g) at 0 °C in toluene and adding dropwise 1.97 g (5 mmol) of perfluorooctanenitrile ($C_7F_{15}CN$). The mixture was stirred at room temperature for 12 h. Crystallization from ethyl acetate gave the (*E*) isomer of **4c** as a yellow solid (3.10 g, 83%) mp 120–121 °C (ethyl acetate) IR (KBr) ν 1611, 1206 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 5.74 (s, 1H), 6.89–7.81 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 109.3–115.8 (m), 125.9–136.6 (m). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 7.03. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.2 (t, $^3J_{FF}$=9.2 Hz), −107.1–126.5 (m); MS (EI) *m/z* 747 (M^+, 40). Anal. Calcd for $C_{33}H_{21}F_{15}NP$ (747): C, 53.03; H, 2.83; N, 1.87. Found: C, 53.20; H, 2.80; N, 1.82.

4.3.4. (3*Z*)-4-Methoxycarbonyl-3-trifluoromethyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4d). Trifluoroacetonitrile in excess (CF_3CN) was bubbled to a suspension of commercial ylide (methoxycarbonyl-methylen)-triphenylphosphoran ($Ph_3P{=}CHCO_2CH_3$) in Et_2O at 0 °C and the mixture was stirred at room temperature for 12 h. Filtration and evaporation of solvent afforded the (*Z*) isomer of **4d** as an orange solid (1.39 g, 65%) mp 101–102 °C ($CHCl_3$). IR (KBr) ν 1702, 1208 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.17, (s, 3H), 5.41 (s, 1H), 7.31–7.58 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 50.2, 96.7, 120.9 (q, $^1J_{CF}$=280 Hz), 128.2–132.5 (m), 167.1. ^{31}P NMR (120 MHz, $CDCl_3$) δ: 10.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −71.3. MS (EI) *m/z* 429 (M^+, 18). Anal. Calcd for $C_{23}H_{19}F_3NO_2P$ (429): C, 64.34; H, 4.46; N, 3.26. Found: C, 64.58; H, 4.51; N, 3.13.

4.3.5. (3*Z*)-4-Methoxycarbonyl-3-perfluoroethyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4e). A solution of commercial (methoxycarbonylmethylen)-triphenylphosphorane (1.672 g, 5 mmol) in CH_2Cl_2 was cooled to 0 °C under N_2. Perfluoropropanenitrile in excess (C_2F_5CN) was bubbled and the mixture was stirred for 24 h. Crystallization from ethyl acetate gave the (*Z*) isomer of **4e** as a white solid (2.37 g, 99%) mp 93–94 °C (ethyl acetate). IR (KBr) ν 1709, 1178 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.13, (d, $^4J_{PH}$=0.97 Hz, 3H), 5.43 (s, 1H), 7.40–7.71 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 50.0, 95.8 (d, $^3J_{PC}$=4.5 Hz), 111.1 (tq, $^1J_{CF}$=258 Hz, $^2J_{CF}$=31 Hz) 119.2 (tq, $^1J_{CF}$=287 Hz, $^2J_{CF}$=37.8 Hz), 127.7–132.4 (m), 149.1 (t, $^2J_{CF}$=27.7 Hz), 167.0. ^{31}P NMR (120 MHz, $CDCl_3$) δ: 9.56. ^{19}F NMR (282 MHz, $CDCl_3$) δ −59.6, −95.0. MS (EI) *m/z* 479 (M^+, 100). Anal. Calcd for $C_{24}H_{19}F_5NO_2P$ (479): C, 60.13; H, 3.99; N, 2.92. Found: C, 60.21; H, 3.88; N, 2.86.

4.3.6. (3*Z*)-1,1-Diphenyl-4-methoxycarbonyl-3-perfluorohepthyl-1-methyl-2-aza-1λ^5-phosphabuta-1,3-diene (4f). The general procedure A was followed using methoxycarbonylmethyldiphenylphosphonium bromide (1.76 g) at 0 °C in toluene and adding dropwise 1.97 g (5 mmol) of perfluorooctanenitrile ($C_7F_{15}CN$). The mixture was stirred at room temperature for 48 h. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture [(3*Z*)-**4f**]: ^1H NMR (300 MHz, $CDCl_3$) δ: 2.36 (d, $^2J_{PH}$= 13.4 Hz, 3H), 3.34 (s, 3H), 5.37 (s, 1H), 7.26–7.76 (m, 10H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 11.2.

4.3.7. (3*Z*)-4-Methoxycarbonyl-3-trifluoromehyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4g). The general procedure A was followed using (methoxycarbonyl)-tetramethylphosphonium bromide (1.15 g) at −35 °C in ether and bubbling trifluoroacetonitrile in excess (CF_3CN) for 12 h. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture [(3*Z*)-**4g**]: ^1H NMR (400 MHz, $CDCl_3$) δ: 1.81 (d, $^2J_{PH}$=12.8 Hz, 9H), 3.67 (s, 3H), 5.44 (d, $^4J_{PH}$=1.4 Hz, 1H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 22.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −71.4.

4.3.8. (3*Z*)-4-Methoxycarbonyl-3-perfluorohepthyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4h). The general procedure A was followed using (methoxycarbonyl)-tetramethylphosphonium bromide (1.15 g) in ether at 0 °C and adding dropwise 1.97 g (5 mmol) of perfluorooctanenitrile ($C_7F_{15}CN$). The mixture was stirred at room temperature for 12 h. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture [(3*Z*)-**4h**]: ^1H NMR (300 MHz, $CDCl_3$) δ: 1.70 (d, $^2J_{PH}$=12.8 Hz, 9H), 3.63 (s, 3H), 5.40 (s, 1H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 19.8. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.0, −114.4 to −126.4 (m).

4.3.9. (3*E*/3*Z*)-4-Cyano-3-trifluoromethyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4i). The general procedure A was followed using cyanomethyltriphenylphosphonium chloride obtained in situ [from triphenylposphine 1.31 g (5 mmol) and 1-chloroacetonitrile 0.80 mL (12.5 mmol) the mixture was stirred at 70 °C for 12 h] in ether and bubbling trifluoroacetonitrile in excess (CF_3CN) for 24 h to give **4i** as a mixture of isomers 3*E*/3*Z* (60/40). The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture [(3*E* and 3*Z*)-**4i**]: ^1H NMR (300 MHz, $CDCl_3$) δ: 3.87 (s, 1H), 4.86 (s, 1H), 7.50–7.78 (m, 30H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 9.1, 12.8. ^{19}F NMR (282 MHz, $CDCl_3$) δ −67.2, −71.2.

4.3.10. (3*E*)-4-Cyano-3-perfluorohepthyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4j). The general procedure A was followed using cyanomethyltriphenylphosphonium chloride (1.69 g) in toluene at 0 °C and adding dropwise 1.97 g (5 mmol) of perfluorooctanenitrile ($C_7F_{15}CN$). The mixture was stirred at room temperature for 48 h. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture [(3*E*)-**4j**]: ^1H NMR (300 MHz, $CDCl_3$) δ: 4.77 (d, $^4J_{PH}$=1.07 Hz, 1H),

7.14–7.79 (m, 15H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 11.1. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1, −113.8 to −126.5 (m).

4.3.11. (3*E*)-4-Cyano-1,1-diphenyl-1-methyl-3-perfluorohepthyl-2-aza-1λ^5-phosphabuta-1,3-diene (4k). The general procedure B was followed using cyanomethylmethyldiphenyl phosphonium chloride (1.20 g) at 0 °C in toluene and adding dropwise 1.97 g (5 mmol) of perfluorooctanenitrile ($C_7F_{15}CN$). The mixture was stirred at room temperature for 48 h. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture [(3*E*)-**4k**]: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.14 (d, $^2J_{PH}$=13.3 Hz, 3H), 4.74 (s, 1H), 7.18–7.79 (m, 10H). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 14.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1 (t, $^3J_{FF}$=10.7 Hz) −111.9 to −126.5 (m).

4.3.12. 4-Cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4l). The general procedure B was followed using cyanotetramethylphosphonium chloride (0.76 g) in THF and bubbling trifluoroacetonitrile in excess (CF_3CN) for 12 h to give **4l** (0.90 g, 86%) as a mixture of isomers 3*E*/3*Z* (80/20). ^{1}H NMR (300 MHz, $CDCl_3$) of crude reaction mixture [(3*E* and 3*Z*)-**4l**] δ: 1.72 (d, $^2J_{PH}$=12.8 Hz, 9H), 1.80 (d, $^2J_{PH}$=12.9 Hz, 9H), 4.02 (d, $^4J_{PH}$=0.6 Hz, 1H), 4.71 (s, 1H). Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.54 g (52%) of *E* isomer **4l** as a white solid; mp 133–134 °C ($CHCl_3$/hexane). IR (KBr) ν 2195, 1360 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.72 (d, $^2J_{PH}$=12.8 Hz, 9H), 4.02 (d, $^4J_{PH}$=0.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.5 (d, $^1J_{CP}$=68.0 Hz), 70.5, 116.5 (q, $^1J_{CF}$=279 Hz), 117.7, 155.4 (q, $^2J_{CF}$=32 Hz). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 21.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −68.5. MS (EI) *m*/*z* 210 (M^+, 100). Anal. Calcd for $C_7H_{10}F_3N_2P$ (210): C, 40.01; H, 4.80; N, 13.33. Found C, 39.96; H, 4.56; N, 13.12.

4.3.13. 4-Cyano-3-perfluoroethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4m). The general procedure B was followed using cyanotetramethylphosphonium chloride (0.76 g) in THF and bubbling perfluoropropanenitrile in excess (C_2F_5CN) for 12 h to give **4m** (0.86 g, 66%) as a mixture of isomers 3*E*/3*Z* (75/25). ^{1}H NMR (300 MHz, $CDCl_3$) of crude reaction mixture [(3*E* and 3*Z*)-**4m**] δ: 1.71 (d, $^2J_{PH}$=12.8 Hz, 9H), 1.80 (d, $^2J_{PH}$=13.0 Hz, 9H), 4.09 (d, $^4J_{PH}$=0.6 Hz, 1H), 4.73 (s, 1H). Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.56 g (43%) of *E* isomer **4m** as a brown solid; mp 143–144 °C ($CHCl_3$/hexane). IR (KBr) 2199, 1371 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.71 (d, $^2J_{PH}$=12.8 Hz, 9H), 4.09 (d, $^4J_{PH}$=0.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8 (d, $^1J_{CP}$=68 Hz), 72.0 (d, $^3J_{CF}$=16 Hz), 111.7 (tq, $^1J_{CF}$=143 Hz, $^3J_{CF}$=37 Hz), 117.7, 118.8 (tq, $^1J_{CF}$=288 Hz, $^3J_{CF}$=37 Hz), 156.3. ^{31}P NMR (120 MHz, $CDCl_3$) δ: 21.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −82.4, −115.6 (d, $^2J_{FF}$=7.6 Hz); MS (CI) *m*/*z* 261 (M^++1, 100). Anal. Calcd for $C_8H_{10}F_5N_2P$ (260): C, 36.94; H, 3.87; N, 10.77. Found: C, 36.65; H, 3.58; N, 10.63.

4.3.14. (3*E*)-4-Cyano-3-perfluoroheptyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4n). The general procedure B was followed using cyanotetramethylphosphonium chloride (0.76 g) in THF at 0 °C and adding dropwise 1.97 g (5 mmol) of perfluorooctanenitrile ($C_7F_{15}CN$). The mixture was stirred at room temperature for 12 h to give the (*E*) isomer of **4n** as a brown solid (1.02 g, 40%) mp 98–99 °C ($CHCl_3$). IR (KBr) ν 2196, 1379 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (d, $^2J_{PH}$=12.8 Hz, 9H), 4.09 (d, $^4J_{PH}$=0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2 (d, $^1J_{CP}$=68 Hz), 72.7 (d, $^3J_{CF}$=16 Hz), 110.5–115.7 (m), 117.5, 155.0–157.2 (m). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 21.1. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1, −112.1 to −126.4 (m). MS (CI) *m*/*z* 511 (M^++1, 100). Anal. Calcd for $C_{13}H_{10}F_{15}N_2P$ (510): C, 30.60; H, 1.98; N, 5.49. Found: C, 30.49; H, 1.86; N, 5.47.

4.4. General procedure A for isomerization of (*E*) isomer towards (*Z*) isomer of phosphazenes 4

Purification by colum chromatography of (*E*)-phosphazene **4** (2 mmol) on neutral alumina using ethyl acetate as eluent.

4.5. General procedure B for isomerization of (*E*) isomer towards (*Z*) isomer of phosphazenes 4

A solution of (*E*)-phosphazene **4** or a mixture of *E*/*Z* isomers of phosphazene (2 mmol) in toluene under N_2, was stirred at reflux (110 °C), until ^{1}H NMR of crude reaction mixture indicated the disappearance of (*E*)-isomer of phosphazene.

4.5.1. (3*Z*)-3-Trifluoromethyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4a). The general procedure A was followed using (3*E*)-1,1,1,4-tetraphenyl-3-trifluoromethyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4a**) and 0.54 g (60%) of (3*Z*)-**4a** were obtained. When the general procedure B was followed for 12 h, 0.88 g (98%) of (3*Z*)-**4a** were obtained. In both cases evaporation of solvent and crystallization from ethyl acetate give a yellow solid mp 121–122 °C. IR (KBr) ν 1613, 1467 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.23 (s, 1H), 7.05–7.81 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 112.6–112.8 (m), 122.5 (dq, $^1J_{CF}$=279.0 Hz, $^3J_{CP}$=3.7 Hz), 125.9–133.7 (m), 133.7 (q, $^2J_{CF}$=29.5 Hz). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 2.13; ^{19}F NMR (282 MHz, $CDCl_3$) δ −68.2. MS (EI) *m*/*z* 447 (M^+, 100). Anal. Calcd for $C_{27}H_{21}F_3NP$ (447): C, 72.48; H, 4.73; N, 3.13. Found: C, 72.22; H, 4.70; N, 3.10.

4.5.2. (3*Z*)-3-Perfluoroethyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4b). The general procedure A was followed using (3*E*)-3-perfluoroethyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4b**) and 0.61 g (62%) of (3*Z*)-**4b** were obtained. When the general procedure B was followed for 12 h, 0.97 g, (98%)of (3*Z*)-**4b** were obtained. In both cases evaporation of solvent and crystallization from ethyl acetate give a yellow solid mp 97–98 °C (ethyl acetate). IR (KBr): ν 1626, 1328, 1150 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.17 (s, 1H), 7.08–7.67 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 112.4 (tq, $^1J_{CP}$=259.8 Hz, $^2J_{CF}$=36.7 Hz), 115.3 (t, $^3J_{CF}$=7.3 Hz), 119.4 (tq, $^1J_{CF}$=287.0 Hz, $^2J_{CF}$=39.8 Hz), 125.8–132.9 (m), 133.5 (t, $^2J_{CF}$=21.9 Hz). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 2.35. ^{19}F NMR (282 MHz, $CDCl_3$) δ : −66.3, −96.5. MS (EI) *m*/*z* 497 (M^+, 76). Anal. Calcd for $C_{28}H_{21}F_5NP$ (497):

C, 67.61; H, 4.25; N, 2.82. Found: C, 67.70; H, 4.29; N, 2.79.

4.5.3. (3*Z*)-3-Perfluorohepthyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4c). The general procedure B was followed using (3*E*)-3-perfluorohepthyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4c**) for 12 h, and 1.46 g (98%) of (3*Z*)-**4c** were obtained. Evaporation of solvent give a yellow oil; R_f=0.13 (1/10, ethyl acetate/hexane). IR (KBr) ν 1612, 1438, 1206 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.07 (s, 1H), 6.93–7.82 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 107.2–119.1 (m), 125.8–137.3 (m). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 1.44. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.15 to −82.3 (m), −109.8 (t, $^3J_{FF}$=15.2 Hz), −117.0 to −127.2 (m). MS (EI) *m*/*z* 747 (M^+, 69). Anal. Calcd for $C_{33}H_{21}F_{15}NP$ (747): C, 53.03; H, 2.83; N, 1.87. Found: C, 52.90; H, 2.86; N, 1.86.

4.5.4. (3*Z*)-4-Cyano-3-trifluoromethyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (4i). The general procedure B was followed for 24 h using a mixture of 3*E*/3*Z* (60/40) of 4-cyano-3-trifluoromethyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4i**) prepared in situ. Evaporation of solvent and crystallization gave the (*Z*) isomer of **4i** (0.68 g, 86%) as a white solid mp 122–123 °C (hexane/ethyl acetate). IR (KBr) ν 2207, 1432 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.86 (s, 1H), 7.50–7.78 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 75.8, 119.1, 120.5 (q, $^1J_{CF}$=280 Hz), 128.6–132.7 (m), 152.6 (q, $^2J_{CF}$=32 Hz). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 9.03. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −71.2. MS (EI) *m*/*z* 396 (M^+, 100). Anal. Calcd for $C_{22}H_{16}F_3N_2P$ (396): C, 66.67; H, 4.07; N, 7.07. Found: C, 66.76; H, 3.86; N, 7.17.

4.5.5. (3*Z*)-4-Cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4l). The general procedure B was followed for 4 h using (3*E*)-4-cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4l**). Evaporation of solvent gave the (*Z*) isomer of **4l** (0.38 g, 91%) as a yelow oil; R_f=0.17 (ethyl acetate). IR (KBr) ν 2175, 1295 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.80 (d, $^2J_{PH}$=12.9 Hz, 9H), 4.71 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.8 (d, $^1J_{CP}$=69 Hz), 69.8 (dd, $^3J_{CP}$=11 Hz, $^3J_{CF}$=4 Hz), 121.8 (dq, $^1J_{CF}$=253 Hz, $^3J_{CP}$=27 Hz), 121.6, 154.7 (q, $^2J_{CF}$=31 Hz). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 19.7. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −72.5. M/S (EI) *m*/*z* 210 (M^+, 84). Anal. Calcd for $C_7H_{10}F_3N_2P$ (210): C, 40.01; H, 4.80; N, 13.33. Found C, 40.09; H, 4.65; N, 13.27.

4.5.6. (3*Z*)-4-Cyano-3-perfluoroethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4m). The general procedure B was followed for 120 h using (3*E*)-4-cyano-3-perfluoroethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4m**). Evaporation of solvent and crystallization gave the (*Z*) isomer of **4m** (0.49 g, 95%) as a brown solid; mp 43–44 °C (ethyl acetate). IR (KBr) ν 2189, 1326 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.80 (d, $^2J_{PH}$=13 Hz, 9H), 4.73 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ =17.9 (d, $^1J_{CP}$=69 Hz), 70.7, 110.2–111.5 (m), 118.8 (tq, $^1J_{CF}$=287 Hz, $^2J_{CF}$=38 Hz), 121.9, 154.4–155.1 (m). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 18.8. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.9, −118.2. MS (EI) *m*/*z* 260 (M^+, 85). Anal. Calcd for $C_8H_{10}F_5N_2P$ (260): C, 36.94; H, 3.87; N, 10.77. Found C, 36.89; H, 3.75; N, 10.72.

4.5.7. (3*Z*)-4-Cyano-3-perfluorohepthyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (4n). The general procedure B was followed for 120 h using (3*E*)-4-cyano-3-perfluorohepthyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene (**4n**). Evaporation of solvent and crystallization gave the (*Z*) isomer of **4n** (0.87 g, 85%) as a white solid; mp 90–91 °C ($CHCl_3$). IR (KBr) ν 2211, 1248 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.80 (d, $^2J_{PH}$=12.9 Hz, 9H), 4.71 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.0 (d, $^1J_{CP}$=69 Hz), 70.8, 110.5–115.7 (m), 122, 157.0–157.5 (m). ^{31}P NMR (120 MHz, $CDCl_3$) δ: 18.9. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1 (t, $^3J_{FF}$=9 Hz), −118.1 to −126.4 (m). MS (EI) *m*/*z* 510 (M^+, 95). Anal. Calcd for $C_{13}H_{10}F_{15}N_2P$ (510): C, 30.60; H, 1.98; N, 5.49. Found: C, 30.69; H, 2.02; N, 5.51.

4.6. General procedure for preparation of azadienes 6

Aldehyde (2 mmol) was added to a 0–10 °C solution of phosphazene **4** (2 mmol) in $CHCl_3$, toluene or xylenes under N_2, and the mixture was stirred at room temperature or reflux, until TLC indicated the disappearance of phosphazene.

4.6.1. (1*E*,3*E*)-1-Ethoxycarbonyl-4-phenyl-3-trifluoromethyl-2-azabuta-1,3-diene (6a). The general procedure was followed using (3*E*)-3-trifluoromethyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (0.89 g) **4a** and ethyl glyoxalate (0.20 g) for 1 h at room temperature in $CHCl_3$. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture (**6a**+Ph_3PO): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.26 (t, *J*=7.1 Hz, 3H), 4.26 (q, *J*=7.1 Hz, 2H), 6.89 (s, 1H), 7.09–7.56 (m, 20H), 7.82 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −71.2.

4.6.2. (1*E*,3*E*)-1-Ethoxycarbonyl-4-phenyl-3-perfluoroethyl-2-azabuta-1,3-diene (6b). The general procedure was followed using (3*E*)-3-perfluoroethyl-1,1,1,4-tetraphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (0.99 g) **4b** and ethyl glyoxalate (0.20 g) for 2 h at room temperature in $CHCl_3$. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture (**6b**+Ph_3PO): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (t, *J*=7.2 Hz, 3H), 4.45 (q, *J*=7.2 Hz, 2H), 6.85 (s, 1H), 7.40–7.80 (m, 20H), 7.90 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −62.0, −93.6.

4.6.3. (1*E*,3*E*)-1-Ethoxycarbonyl-4-phenyl-3-perfluorohepthyl-2-azabuta-1,3-diene (6c). The general procedure was followed using (3*E*)-4-phenyl-3-perfluorohepthyl-1,1,1-triphenyl-2-aza-1λ^5-phosphabuta-1,3-diene (1.50 g) **4c** and ethyl glyoxalate (0.20 g) for 0.5 h at room temperature in $CHCl_3$. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used in situ for the following reactions. Spectroscopic data of crude reaction mixture (**6c**+Ph_3PO): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.22 (t, *J*=7.2 Hz, 3H), 4.21 (q, *J*=7.2 Hz, 2H), 6.63 (s, 1H),

7.16–7.59 (m, 20H), 7.70 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1 (t, $^3J_{FF}$=9.2 Hz), −111.3 to −126.5 (m).

4.6.4. (1*E*,3*Z*)-4-Methoxycarbonyl-1-(4-nitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene (6d). The general procedure was followed using (3*Z*)-4-methoxycarbonyl-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4g** obtained in situ and 4-nitrobenzaldehyde (0.31 g) for 3 h at 110 °C in toluene. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used for the following reactions. Spectroscopic data of crude reaction mixture (**6d**+Me_3PO). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.53 (d, $^2J_{HP}$=12.9 Hz, 9H), 3.71 (s 3H), 5.96 (s, 1H), 8.10 (d, J=8.8 Hz, 2H), 8.36 (d, J=8.8 Hz, 2H), 8.50 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −70.8.

4.6.5. (1*E*,3*Z*)-1-(2,4-Dinitrophenyl)-4-methoxycarbonyl-3-trifluoromethyl-2-azabuta-1,3-diene (6e). The general procedure was followed using (3*Z*)-4-methoxycarbonyl-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4g** obtained in situ and 2,4-dinitrobenzaldehyde (0.39 g) for 3 h at 110 °C in toluene. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used for the following reactions. Spectroscopic data of crude reaction mixture (**6e**+Me_3PO). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.53 (d, $^2J_{HP}$=12.9 Hz, 9H), 3.73 (s, 3H), 5.98 (s, 1H), 8.48 (d, J=8.5 Hz, 1H), 8.61 (dd, J=8.5, 2.1 Hz, 1H), 8.93 (s, 1H), 8.99 (d, J=2.1 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −70.6.

4.6.6. (1*E*,3*Z*)-4-Cyano-1-(4-nitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene (6f). The general procedure was followed using (3*Z*)-4-cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4l** (0.42 g) and 4-nitrobenzaldehyde (0.31 g) for 2 h at 110 °C in toluene. Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.24 g (45%) of **6f** as a yellow oil; R_f=0.50 (1/5, ethyl acetate/hexane). When (3*E*)-4-cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4l** (0.42 g) was used the mixture was stirred for 3 h at 110 °C in toluene and chromatographic separation (10/1, hexane/ethyl acetate) gave 0.19 g (35%) of **6f**. IR (KBr) ν 2204, 1708 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.56 (s, 1H), 8.14 (d, J=8.8 Hz, 2H), 8.37 (d, J=8.8 Hz, 2H), 8.68 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 87.7, 113.5, 119.5 (q, $^1J_{CF}$=276 Hz), 124.2, 130.9, 138.5, 150.7, 155.7 (q, $^2J_{CF}$=33 Hz), 165.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −69.8. MS (EI) m/z 269 (M^+, 100).

4.6.7. (1*E*,3*Z*)-4-Cyano-1-(2,4-dinitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene (6g). The general procedure was followed using a mixture 80/20 of (3*E*/3*Z*)-4-cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4l** (0.42 g) and 2,4-dinitrobenzaldehyde (0.39 g) for 12 h at 61 °C in $CHCl_3$. Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.29 g (45%) of **6g** as an orange oil; R_f=0.48 (1/5, ethyl acetate/hexane). When (3*E*)-4-cyano-3-trifluoromethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4l** (0.42 g) was used the mixture was stirred for 6 h at 110 °C in toluene and chromatographic separation (10/1, hexane/ethyl acetate) gave 0.19 g (30%) of **6g**. IR (KBr) ν 2210, 1708 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.67 (s, 1H), 8.47 (d, J=8.5 Hz, 1H), 8.61 (dd, J=8.5, 2.1 Hz, 1H), 9.01 (d, J=2.1 Hz, 1H), 9.19 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 90.0, 112.9, 119.3 (q, $^1J_{CF}$=277 Hz), 120.6, 128.2, 132.1, 133.4, 149.3, 149.9, 154.6 (q, $^2J_{CF}$=34 Hz), 162.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −69.5. M/S (EI) m/z 314 (M^+, 4).

4.6.8. (1*E*,3*Z*)-4-Cyano-1-(4-nitrophenyl)-3-perfluoroethyl-2-azabuta-1,3-diene (6h). The general procedure was followed using a mixture 75/25 of (3*E*/3*Z*)-4-cyano-3-perfluoroethyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4m** (0.52 g) and 4-nitrobenzaldehyde (0.31 g) for 120 h at 120 °C in toluene. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used for the following reactions. Spectroscopic data of crude reaction mixture (**6h**+Me_3PO): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.53 (d, $^2J_{HP}$=12.9 Hz, 9H), 5.50 (s, 1H), 8.10 (d, $^3J_{HH}$=8.6 Hz, 2H), 8.37 (d, $^3J_{HH}$=8.8 Hz, 2H), 8.65 (s, 1H).^{19}F NMR (282 MHz, $CDCl_3$) δ: −82.4, −114.3.

4.6.9. (1*E*,3*Z*)-4-Cyano-1-(4-nitrophenyl)-3-perfluoroheptyl-2-azabuta-1,3-diene (6i). The general procedure was followed using (3*Z*)-4-cyano-3-perfluoroepthyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4n** (1.02 g) and 4-nitrobenzaldehyde (0.31 g) for 120 h at 110 °C in toluene. When (3*E*)-4-cyano-3-perfluoroepthyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4n** (1.02 g) was used the mixture was stirred for 138 h at 110 °C in toluene. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used for the following reactions. Spectroscopic data of crude reaction mixture (**6i**+Me_3PO): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.53 (d, $^2J_{HP}$=12.9 Hz, 9H), 5.47 (s, 1H), 8.10 (d, J=8.7 Hz, 2H), 8.36 (d, J=8.7 Hz, 2H), 8.66 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1, −115.4 to −126.4 (m).

4.6.10. (1*E*,3*Z*)-4-Cyano-1-(2,4-dinitrophenyl)-3-perfluorohepthyl-2-azabuta-1,3-diene (6j). The general procedure was followed using (3*E*)-4-cyano-3-perfluoroheptyl-1,1,1-trimethyl-2-aza-1λ^5-phosphabuta-1,3-diene **4n** (1.02 g) and 2,4-dinitrobenzaldehyde (0.39 g) for 192 h at 120 °C in toluene. The reaction product is unstable to distillation or chromatography and therefore was not isolated and used for the following reactions. Spectroscopic data of crude reaction mixture (**6j**+Me_3PO): ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.53 (d, $^2J_{HP}$=12.9 Hz, 9H), 5.56 (s, 1H), 8.39 (d, J=8.4 Hz, 1H), 8.63 (dd, J=8.3, 1.4 Hz, 1H), 9.04 (d, J=1.5 Hz, 1H), 9.17 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.0, −118.1 to −126.3 (m).

4.7. General procedure for reactions of 2-azadienes (6) with enamines (9)

Enamine (5 mmol) was added to a 0–10 °C solution of azadiene **6** (5 mmol) in $CHCl_3$ or toluene (15 mL) under N_2, and the mixture was stirred to adequate temperature, until TLC indicated the disappearance of azadiene.

4.7.1. Reaction of 2-azadiene (6a) with enamine (9a).

4.7.1.1. 1-Ethoxycarbonyl-3-trifluoromethyl-4-phenyl-4a-pyrrolidyl-1,4,5,6,7,8-hexahydroisoquinoline (10). The general procedure was followed using (1*E*,3*E*)-1-

ethoxycarbonyl-4-phenyl-3-trifluoromethyl-2-azabuta-1,3-diene **6a** obtained in situ and 1-cyclohex-1-enylpyrrolidine **9a** (0.23 g) at room temperature in $CHCl_3$ for 15 h. Chromatographic separation (15/1, hexane/ethyl acetate) gave 0.51 g (80%) of **10** as a yellow oil; R_f=0.42 (1/5, ethyl acetate/hexane). IR (KBr) ν 1733, 1193 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.09–1.72 (m, 16H), 2.41 (s, 1H), 2.53–2.56 (m, 1H), 2.86–2.88 (m, 1H), 3.06–3.09 (m, 1H), 3.84 (s, 1H), 4.30–4.36 (m, 2H), 4.79 (d, J=9.8 Hz, 1H), 7.23–7.37 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.1, 19.3, 20.6, 22.5, 24.8, 25.2, 32.3, 32.5, 43.2, 43.7, 49.2, 56.6, 61.7, 65.2, 119.3 (q, $^1J_{CF}$=280.8 Hz), 127.7–135.7 (m), 161.4 (q, $^2J_{CF}$=32.0 Hz), 171.6. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −71.3. MS (EI) m/z 422 (M^+, 2). Anal. Calcd for $C_{23}H_{29}F_3N_2O_2$ (422): C, 65.39; H, 6.92; N, 6.63. Found C, 65.41; H, 6.90; N, 6.68.

4.7.1.2. 1-Ethoxycarbonyl-3-trifluoromethyl-4-phenyl-5,6,7,8-tetrahydroisoquinoline (12). The general procedure was followed using (1*E*,3*E*)-1-ethoxycarbonyl-4-phenyl-3-trifluoromethyl-2-azabuta-1,3-diene **6a** obtained in situ and 1-cyclohex-1-enylpyrrolidine **9a** (0.23 g) in refluxing toluene (110 °C) for 48 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.70 g (40%) of **12** as a yellow oil; R_f=0.25 (1/10, ethyl acetate/hexane). IR (KBr) ν 1738, 1210 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, J=7.1 Hz, 3H), 1.88–2.00 (m, 4H), 2.82 (t, J=6.2 Hz, 2H), 3.08 (t, J=6.4 Hz 2H), 4.00 (q, J=7.1 Hz, 2H) 7.26–7.40 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.6, 22.0, 22.3, 26.1, 32.7, 61.6, 122.0 (q, $^1J_{FC}$=33.0 Hz), 123.2–157.3 (m), 166.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −61.3. MS (70 eV) m/z 349 (M^+, 5). Anal. Calcd for $C_{19}H_{18}F_3NO_2$ (349): C, 65.32; H, 5.19; N, 4.01. Found C, 65.29; H, 5.23; N, 3.98.

4.7.2. Reaction of 2-azadiene (6f) with enamine (9a).

4.7.2.1. 4-Cyano-1-(4-nitrophenyl)-3-trifluoromethyl-1,2,6,7,8,8a-hexahydroisoquinoline (13a) and 4-cyano-1-(4-nitrophenyl)-3-trifluoromethyl-5,6,7,8-tetrahydroisoquinoline (14a). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(4-nitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene **6f** obtained in situ and 1-cyclohex-1-enylpyrrolidine **9a** (0.23 g) at room temperature in $CHCl_3$ for 2 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.20 g (29%) of **13a** as a brown solid mp 156–157 °C (CH_2Cl_2/hexane) and 0.20 g (29%) of **14a** as a brown oil R_f=0.42 (1/5, ethyl acetate/hexane). *Data for* **13a**: IR (KBr) ν 3296, 2211, 1522 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.06–2.44 (m, 7H), 4.00 (d, J=10.7 Hz, 1H), 4.76 (s, 1H), 6.21 (s, 1H), 7.52 (d, J=8.7 Hz, 2H), 8.30 (d, J=8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.5, 25.4, 29.6, 39.6, 61.1, 85.3, 114.7, 119.9 (q, $^1J_{CF}$=276 Hz), 124.3, 126.2, 127.3, 128.7, 140.0 (q, $^2J_{CF}$=33 Hz), 145.5, 148.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −66.6. MS (EI) m/z 349 (M^+, 5). Anal. Calcd for $C_{17}H_{14}F_3N_3O_2$ (349): C, 58.45; H, 4.04; N, 12.03. Found C, 58.91; H, 3.93; N, 12.13. *Data for* **14a**: IR (KBr) ν 2234, 1522 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.84 (m, 2H), 1.97 (m, 2H), 2.79 (t, J=6.4 Hz, 2H), 3.16 (t, J=6.1 Hz, 2H), 7.74 (d, J=8.8 Hz, 2H), 8.36 (d, J=8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.0, 21.7, 107.4, 112.6, 120.3 (q, $^1J_{CF}$=276 Hz), 123.6, 130.1, 135.2, 143.9, 146.8 (q, $^2J_{CF}$=35 Hz), 148.2, 153.9, 158.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −65.9. MS (EI) m/z 347 (M^+, 100). Anal. Calcd for $C_{17}H_{12}F_3N_3O_2$ (347): C, 58.79; H, 3.48; N, 12.10. Found C, 58.81; H, 3.53; N, 12.07.

4.7.3. Reaction of 2-azadiene (6g) with enamine (9a).

4.7.3.1. 4-Cyano-1-(2,4-dinitrophenyl)-3-trifluoromethyl-1,2,6,7,8,8a-hexahydroisoquinoline (13b) and 4-cyano-1-(2,4-dinitrophenyl)-3-trifluoromethyl-5,6,7,8-tetrahydroisoquinoline (14b). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(2,4-dinitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene (**6g**, 1.57 g) and 1-cyclohex-1-enylpyrrolidine **9a** (0.23 g) at room temperature in $CHCl_3$ for 3 h. After column chromatography (hexane/ethyl acetate 10/1) a mixture (1.348 g) of compounds **13b** and **14b** (6/1) was obtained. Spectroscopic data of mixture **13b** and **14b**: ^1H NMR (300 MHz, $CDCl_3$) δ: 1.11–2.35 (m, 11H), 2.48 (m, 2H), 3.16 (t, J=6.1 Hz, 2H), 4.66 (d, J=10.4 Hz, 1H), 5.38 (s, 1H), 6.21 (s, 1H), 7.64 (d, J=8.4 Hz, 1H), 7.90 (d, J=8.7 Hz, 1H), 8.54 (dd, J=8.7, 2.2 Hz, 1H), 8.62 (dd, J=8.4, 2.3 Hz, 1H), 8.70 (d, J=2.1 Hz, 1H) 9.09 (d, J=2.3 Hz, 1H).^{19}F NMR (282 MHz, $CDCl_3$) δ: −65.8, −71.5. Compound **13b** is unstable and the separation of both products was not possible. Therefore *p*-benzoquinone (0.15 g) was added to a solution of (0.39 g) of **13b** and **14b** mixture in dioxane and was heated at 80 °C for 144 h. Evaporation of solvent and chromatographic separation (10/1, hexane/ethyl acetate) gave 0.27 g (70%) of **14b** as a brown oil; R_f=0.40 (1/5, ethyl acetate/hexane). *Data for* **14b**: IR (KBr) ν 2210. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.85 (m, 2H), 1.96 (m, 2H), 2.48 (m, 2H), 3.16 (t, J=6.1 Hz, 2H), 7.64 (d, J=8.4 Hz, 1H), 8.62 (dd, J=8.4, 2.3 Hz, 1H), 9.09 (d, J=2.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.9, 21.3, 26.7, 28.6, 108.2, 112.3, 118.3 (q, $^1J_{CF}$=269 Hz) 120.7, 128.1, 132.4, 135.2, 139.0, 147.5.(q, $^2J_{CF}$=42 Hz), 148.3, 153.7, 156.7. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −65.8. MS (EI) m/z 392 (M^+, 11).

4.7.4. Reaction of 2-azadiene (6e) with enamine (9b).

4.7.4.1. 5-Isopropyl-6-(2,4-dinitrophenyl)-2-trifluoromethyl-1,2-dihydropiridine-3-carboxylic acid methyl ester (15a) and 5-isopropyl-6-(2,4-dinitrophenyl)-2-trifluoromethylnicotinic acid methyl ester (16a). The general procedure was followed using (1*E*,3*Z*)-1-(2,4-dinitrophenyl)-4-methoxycarbonyl-3-trifluoromethyl-2-azabuta-1,3-diene **6e** obtained in situ and *trans* 3-methyl-1-pyrrolidyl-but-1-ene **9b** (0.67 g) at room temperature in $CHCl_3$ for 3 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.72 g (35%) of **15a** as a brown oil; R_f=0.26 (1/5, ethyl acetate/hexane) and 0.88 g (43%) of **16a** as a brown oil; R_f=0.39 (1/5, ethyl acetate/hexane), 0.09 g of **18a** (7%) and 0.06 g of **19** (9%).[26] *Data for* **15a**: IR (KBr) ν 3362, 2867, 1692 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.88 (d, J=6.8 Hz, 3H), 0.93 (d, J=6.8 Hz, 3H), 1.90 (dq, J=6.8 Hz, 1H), 3.82 (s, 3H), 4.30 (d, J=5.6 Hz, 1H), 5.21 (dq, $^3J_{HF}$=7.3 Hz, J=6.7 Hz, 1H), 7.45 (s, 1H), 7.72 (d, J=8.3 Hz, 1H), 8.53 (dd, J=8.3, 2.1 Hz, 1H), 8.81 (d, J=2.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.1, 23.3, 28.1, 51.9, 52.1 (q, $^2J_{CF}$=32 Hz), 117.1, 118.3 (q, $^1J_{CF}$=284 Hz), 119.7, 127.5, 134.4, 135.9, 136.2, 136.6, 142.5, 148.1, 148.4, 165.6. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.3. MS (EI) m/z 415 (M^+, 3). *Data for* **16a**: IR (KBr) ν 2873, 1744, 1601 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.23 (d, J=6.7 Hz, 6H), 2.76 (m, J=6.8 Hz, 1H), 4.02 (s, 3H), 7.65

(d, J=8.2 Hz, 1H), 8.17 (s, 1H), 8.59 (dd, J=8.3, 2.2 Hz, 1H), 9.05 (d, J=2.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.2, 30.1, 53.4, 120.1, 120.5, 120.6 (q, $^1J_{CF}$=275 Hz), 127.6, 132.8, 136.9, 139.5, 145.2, 148.1, 153.7–154.1 (m), 154.3, 165.7. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −64.6. MS (EI) m/z 413 (M^+, 5). ^{1}H NMR (300 MHz, $CDCl_3$) for **18a** δ: 1.22 (d, J=6.7 Hz, 6H), 3.02–3.11 (m, 1H), 7.58 (d, J=8.4 Hz, 1H), 7.70 (s, 1H), 8.49 (dq, J=8.4, 2.4 Hz, 1H), 9.06 (d, J=2.4 Hz, 1H), 9.63 (s, 1H).

4.7.5. Reaction of 2-azadiene (6d) with enamine (9b).

4.7.5.1. 5-Isopropyl-6-(4-nitrophenyl)-2-trifluoromethyl-1,2-dihydropiridine-3-carboxylic acid methyl ester (15b). The general procedure was followed using (1*E*,3*Z*)-4-methoxycarbonyl-1-(4-nitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene **6d** obtained in situ and *trans* 3-methyl-1-pyrrolidylbut-1-ene **9b** (0.67 g) at 120 °C in toluene for 24 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 1.11 g (60%) of **15b** as an orange solid; mp 155–157 °C (hexane/CH_2Cl_2). IR (KBr) ν 3426, 2852, 1679 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.96 (d, J=6.8 Hz, 3H), 1.19 (d, J=6.9 Hz, 3H), 2.48 (dq, J=6.9, 6.8 Hz, 1H), 3.85 (s, 3H), 4.15 (d, J=5.2 Hz, 1H) 5.20 (q, $^3J_{HF}$=7.5 Hz, $^3J_{HH}$=5.6 Hz, 1H), 7.57 (d, J=8.8 Hz, 2H), 7.58 (s, 1H), 8.30 (d, J=8.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 23.7, 29.7, 52.6–53.2 (m), 53.3, 117.0, 119.4 (q, $^1J_{CF}$=285 Hz), 123.7, 130.2, 137.2, 141.6, 144.5, 145.1, 148.1, 165.9. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −64.8. MS (EI) m/z 370 (M^+, 9). Anal. Calcd for $C_{17}H_{17}F_3N_2O_4$ (370): C, 55.14; H, 4.63; N, 7.56. Found: C, 55.22; H, 4.72; N, 7.61.

4.7.5.2. 5-Isopropyl-6-(4-nitrophenyl)-2-trifluoromethylnicotinic acid methyl ester (16b). *p*-Benzoquinone (0.32 g, 3 mmol) was added to a solution of **15b** (0.74 g, 2 mmol) in dioxane and the mixture was heated at 80 °C for 48 h. Evaporation of solvent and chromatographic separation (10/1, hexane/ethyl acetate) gave 0.53 g (72%) of **16b** as a green solid; mp 145–146 °C (hexane/ethyl acetate). IR (KBr) ν 1727, 1606, 1522 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.26 (d, J=6.9 Hz, 6H), 3.17 (q, J=6.9 Hz, 1H), 4.00 (s, 3H), 7.68 (d, J=8.8 Hz, 2H), 8.15 (s, 1H), 8.35 (d, J=8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.7, 29.2, 53.3, 120.9 (q, $^1J_{CF}$=275 Hz), 123.7, 130.1, 137.1, 142.8 (q, $^2J_{CF}$=37 Hz), 144.5, 145.1, 148.1, 156.9, 165.9. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −64.7. MS (EI) m/z 368 (M^+, 47). Anal. Calcd for $C_{17}H_{15}F_3N_2O_4$ (368): C, 55.44; H, 4.11; N, 7.61. Found: C, 55.99; H, 4.23; N, 7.58.

4.7.6. Reaction of 2-azadiene (6f) with enamine (9b).

4.7.6.1. 5-Isopropyl-6-(4-nitrophenyl)-2-trifluoromethyl-3-cyanopiridine (16c). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(4-nitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene **6f** (1.35 g) and *trans* 3-methyl-1-pyrrolidylbut-1-ene **9b** (0.67 g) at room temperature in $CHCl_3$ for 3 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 0.87 g (52%) of **16c** as a white solid; mp 143–144 °C (hexane/ethyl acetate). IR (KBr) ν 2234, 1600, 1526 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28 (d, J=6.8 Hz, 6H), 3.22 (dq, J=6.8 Hz, 1H), 7.69 (d, J=8.9 Hz, 2H), 8.22 (s, 1H), 8.39 (d, J=8.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.6, 29.3, 107.3, 113.7, 120.1 (q, $^1J_{CF}$=276 Hz), 123.8, 130.1, 141.3, 143.5, 145.7, 146.4 (q, $^2J_{CF}$=36 Hz), 148.4, 158.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −65.9. MS (EI) m/z 334 (M^+−1, 35). Anal. Calcd for $C_{16}H_{12}F_3N_3O_2$ (335): C, 57.32; H, 3.61; N, 12.53. Found: C, 57.33; H, 3.65; N, 12.50.

4.7.7. Reaction of 2-azadiene (6g) with enamine (9b).

4.7.7.1. 5-Isopropyl-6-(2,4-dinitrophenyl)-2-trifluoromethyl-3-cyanopiridine (16d). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(2,4-dinitrophenyl)-3-trifluoromethyl-2-azabuta-1,3-diene **6g** (1.57 g) and *trans* 3-methyl-1-pyrrolidylbut-1-ene **9b** (0.67 g) at room temperature in $CHCl_3$ for 3 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 1.22 g (64%) of **16d** as an orange solid; mp 96–97 °C (hexane/ethyl acetate). IR (KBr) ν 2240, 1731, 1602 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.25 (d, J=6.9 Hz, 6H), 2.76 (dq, J=6.9 Hz, 1H), 7.65 (d, J=8.4 Hz, 1H), 8.24 (s, 1H), 8.64 (dd, J=8.3, 2.2 Hz, 1H), 9.10 (d, J=2.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 23.1, 30.2, 108.1, 113.5, 119.9 (q, $^1J_{CF}$=276 Hz), 120.7, 127.9, 132.4, 138.6, 140.9, 145.8, 146.5 (q, $^2J_{CF}$=36 Hz), 147.9, 148.5, 156.2; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −65.9. MS (CI) m/z 381 (M^++1, 100). Anal. Calcd for $C_{16}H_{11}F_3N_3O_4$ (380): C, 50.53; H, 2.92; N, 14.99. Found: C, 50.33; H, 2.94; N, 15.01.

4.7.8. Reaction of 2-azadiene (6b) with enamine (9a).

4.7.8.1. 1-Ethoxycarbonyl-3-(1,2,2,2-tetrafluoroethyl)-4-phenyl-5,6,7,8-tetrahydroisoquinoline (20). The general procedure was followed using (1*E*,3*E*)-1-ethoxycarbonyl-4-phenyl-3-pentafluoroethyl-2-azabuta-1,3-diene **6b** in situ and 1-cyclohex-1-enylpyrrolidine **9a** (0.68 g) in refluxing $CHCl_3$ for 60 h. Chromatographic separation (15/1, hexane/ethyl acetate) gave 1.33 g (70%) of **20** as a yellow solid, mp 84–85 °C (ethyl acetate/hexane). IR (KBr) ν 1747, 1173 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 (t, J=7.2 Hz, 3H), 1.64–1.85 (m, 4H), 2.35–2.41 (m, 2H), 3.00–3.02 (m, 2H), 4.48 (q, J=7.2 Hz, 2H), 5.45 (dq, $^2J_{HF}$=46 Hz, $^3J_{HF}$=6.0 Hz, 1H), 7.13–7.55 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 21.7, 21.8, 26.1, 28.5, 61.7, 85.0 (dq, $^1J_{CF}$=187.8 Hz, $^2J_{CF}$=34.7 Hz), 122.0 (dq, $^1J_{CF}$=281.0 Hz, $^2J_{CF}$=29.0 Hz), 128.3–160.2 (m), 166.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −75.6 (dd, $^3J_{FF}$=14 Hz, $^3J_{HF}$=6 Hz), −190.1 (dq, $^2J_{HF}$=46 Hz, $^3J_{FF}$=14 Hz). MS (EI) m/z 381 (M^+, 73). Anal. Calcd for $C_{20}H_{19}F_4NO_2$ (381): C, 62.99; H, 5.02; N, 3.67. Found C, 63.01; H, 5.00; N, 3.66.

4.7.9. Reaction of 2-azadiene (6b) with enamine (9c).

4.7.9.1. 2-Ethoxycarbonyl-6-(1,2,2,2-tetrafluoroethyl)-3,4-trimethylen-5-phenylpyridine (24a). The general procedure was followed using (1*E*,3*E*)-1-ethoxycarbonyl-4-phenyl-3-pentafluoroethyl-2-azabuta-1,3-diene **6b** obtained in situ and 1-cyclopent-1-enylpyrrolidine **9c** (0.67 g) in $CHCl_3$ at room temperature for 2 h. Chromatographic separation (15/1, hexane/ethyl acetate) gave 0.77 g (42%) of **24a** as a colorless oil; R_f=0.23 (1/10, ethyl acetate/hexane). IR (KBr) ν 1724, 1294 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (t, J=7.1 Hz, 3H), 2.10–2.17 (m, 2H), 2.70–2.76 (m, 2H), 3.41–3.45 (m, 2H), 4.47 (m, 2H), 5.67 (dq, $^2J_{HF}$=45 Hz, $^3J_{HF}$=6.0 Hz, 1H), 7.23–7.51 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 24.4, 32.4, 33.1, 61.6, 85.9 (dq, $^1J_{CF}$=188.6 Hz, $^2J_{CF}$=34.6 Hz), 122.0 (dq, $^1J_{CF}$=282.7 Hz, $^2J_{CF}$=29.1 Hz), 126.4–156.7

(m), 165.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −75.2 (dd, $^3J_{FF}$=15 Hz, $^3J_{HF}$=6 Hz), −190.0 (dq, $^2J_{HF}$=45 Hz, $^3J_{FF}$=15 Hz). MS (EI) *m/z* 367 (M^+, 3). Anal. Calcd for $C_{25}H_{19}F_4NO_2$ (367): C, 62.12; H, 4.66; N, 3.81. Found: C, 61.99; H, 4.70; N, 3.83.

4.7.10. Reaction of 2-azadiene (6c) with enamine (9c).

4.7.10.1. 2-Ethoxycarbonyl-6-(1,2,2,3,3,4,4,5,5,6,6,7,7,7-tetradecafluoro-heptyl)-3,4-trimethylen-5-phenyl-pyridine (24b). The general procedure was followed using (1*E*,3*E*)-1-ethoxycarbonyl-4-phenyl-3-perfluorohepthyl-2-azabuta-1,3-diene **6c** obtained in situ and 1-cyclopent-1-enylpyrrolidine **9c** (0.67 g) in $CHCl_3$ at room temperature for 15 h. Chromatographic separation (15/1, hexane/ethyl acetate) gave 1.76 g (56%) of **24b** as a colorless oil; R_f=0.43 (1/5, ethyl acetate/hexane). IR (KBr) ν 1724, 1224 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (t, *J*=7.1 Hz, 3H), 2.08–2.18 (m, 2H), 2.71–2.76 (m, 2H), 3.41–3.48 (m, 2H), 4.44–4.55 (m, 2H), 5.83–5.99 (m, 1H), 7.18–7.51 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.2, 24.3, 32.4, 33.1, 61.6, 82.5–85.0 (m), 107.0–120.2 (m), 128.1–156.6 (m), 165.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.2, −117.1 to −126.6 (m). MS (EI) *m/z* 617 (M^+, 2). Anal. Calcd for $C_{24}H_{17}F_{14}NO_2$ (617): C, 46.69; H, 2.78; N, 2.27. Found: C, 46.73; H, 2.81; N, 2.25.

4.7.11. Reaction of 2-azadiene (6h) with enamine (9b).

4.7.11.1. 5-Isopropyl-6-(4-nitrophenyl)-2-perfluoro-ethyl-3-cyanopiridine (25a) and 5-isopropyl-6-(4-nitro-phenyl)-2-(1,2,2,2-tetrafluoroethyl)-3-cyanopiridine (26a). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(4-nitrophenyl)-3-perfluoroethyl-2-azabuta-1,3-diene (**6h**) obtained in situ and *trans* 3-methyl-1-pyrro-lidylbut-1-ene **9b** (0.67 g) in $CHCl_3$ at room temperature for 3 h. Chromatographic separation (15/1, hexane/ethyl acetate) gave 0.78 g (40%) of **25a** as a white solid; mp 120–121 °C (ethyl acetate/hexane); 0.33 g (18%) of **26a** as an orange oil, R_f=0.50 (1/5, ethyl acetate/hexane); 0.04 g (4%) of *E*-**18b** as yellow oil, R_f=0.27 (1/5, ethyl acetate/hexane); 0.03 g (3%) of *Z*-**18b** and as a orange oil, R_f=0.45 (1/5, ethyl acetate/hexane) and 0.06 g (6%) of **28a** as an orange solid; mp 69–70 °C (ethyl acetate/hexane). *Data for* **25a**: IR (KBr) ν 2237, 1520 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (d, *J*=6.8 Hz, 6H), 3.26 (dq, *J*=6.8 Hz, 1H), 7.68 (d, *J*=8.7 Hz, 2H), 8.24 (s, 1H), 8.38 (d, *J*=8.7 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 23.6, 29.2, 108.6, 110.9 (tq, $^1J_{CF}$=258 Hz, $^2J_{CF}$=39 Hz), 113.8, 118.5 (tq, $^1J_{CF}$=287 Hz, $^2J_{CF}$=36 Hz), 123.8, 130.1, 141.7, 143.4, 145.6, 145.8–146.4 (m), 148.5, 158.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −82.4, −113.3. MS (EI) *m/z* 385 (M^+, 23). Anal. Calcd for $C_{17}H_{12}F_5N_3O_2$ (385): C, 52.99; H, 3.14; N, 10.91. Found: C, 52.48; H, 3.31; N, 10.81. *Data for* **26a**: IR (KBr) ν 2236, 1522 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.26 (d, *J*=7.1 Hz, 3H), 1.28 (d, *J*=7.0 Hz, 3H), 3.20 (dq, *J*=7.0, 7.1 Hz, 1H), 5.97 (dq, $^2J_{HF}$=44.3 Hz, $^3J_{HF}$=6.0 Hz, 1H), 7.66 (d, *J*=8.8 Hz, 2H), 8.16 (s, 1H), 8.37 (d, *J*=8.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 23.6, 23.7, 29.2, 87.8 (dq, $^1J_{CF}$=194 Hz, $^2J_{CF}$=35 Hz), 109.2, 114.5, 121.5 (dq, $^1J_{CF}$=283 Hz, $^2J_{CF}$=28 Hz), 123.7, 130.0, 140.6, 143.9, 144.2, 148.3, 158.6. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −76.6 (dd, $^3J_{FF}$=12.5 Hz, $^3J_{HF}$=6.0 Hz), −197.9 (dq, $^2J_{HF}$=44.3 Hz, $^3J_{FF}$=12.5 Hz). MS (CI, 80V) *m/z* 368 (M^++1, 100). Anal. Calcd for $C_{17}H_{13}F_4N_3O_2$ (367): C, 55.59; H, 3.57; N, 11.44. Found: C, 55.48; H, 3.38; N, 11.31. ^{1}H NMR (400 MHz, $CDCl_3$) for *E*-isomer of **18b**, δ: 1.28 (d, *J*=7.0 Hz, 6H), 3.02 (dq, *J*=7.0 Hz, 1H), 7.50 (s, 1H), 7.51 (d, *J*=8.8 Hz, 2H), 8.30 (d, *J*=8.8 Hz, 2H), 9.59 (s, 1H). ^{1}H NMR (400 MHz, $CDCl_3$) for *Z*-isomer of **18b**, δ: 1.18 (d, *J*=6.8 Hz, 6H), 3.05 (dq, *J*=6.8 Hz, 1H), 7.46 (d, *J*=8.7 Hz, 2H), 7.50 (s, 1H), 8.26 (d, *J*=8.7 Hz, 2H), 9.82 (s, 1H). ^{1}H NMR (400 MHz, $CDCl_3$) for **28a**, δ: 4.51 (s, 1H), 5.19 (bs, 2H).

4.7.12. Reaction of 2-azadiene (6i) with enamine (9b).

4.7.12.1. 5-Isopropyl-6-(4-nitrophenyl)-2-perfluoro-hepthyl-3-cyanopiridine (25b), 5-isopropyl-6-(4-nitro-phenyl)-2-(1,2,2,3,3,4,4,5,5,6,6,7,7,7-tetradecafluoro-hepthyl)-3-cyanopiridine (26b) and 3-isopropyl-2-(4-nitrophenyl)-6-perfluorohepthyl-1,2-dihydro-5-cyano-piridine (27b). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(4-nitrophenyl)-3-perfluorohepthyl-2-azabuta-1,3-diene (**6i**) obtained in situ and and *trans* 3-methyl-1-pyrrolidylbut-1-ene **9b** (0.67 g) in $CHCl_3$ at room temperature for 5 h. Chromatographic separation (10/1, hexane/ethyl acetate) gave 1.11 g (35%) of **25b** as an orange solid; mp 80–81 °C (CH_2Cl_2/hexane); 0.56 g (18%) of **26b** as a yellow solid; mp 93–94 °C (CH_2Cl_2/hexane); 0.68 g (22%) of **27b** as a yellow solid; mp 90–91 °C (ethyl acetate/hexane) 0.03 g (3%) of **18b** (see reaction of 2-azadiene **6h** with enamine **9b**) and 0.15 g (7%) of **28b** as a white solid mp 127–128 °C (hexane/ethyl acetate). *Data for* **25b**: IR (KBr) ν 2233, 1523 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (d, *J*=6.9 Hz, 6H), 3.25 (dq, *J*=6.9 Hz, 1H), 7.68 (d, *J*=8.8 Hz, 2H), 8.23 (s, 1H), 8.38 (d, *J*=8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.6, 29.7, 109.2, 110.2–111.1 (m), 113.9, 123.8, 130.1, 141.6, 143.4, 145.6, 145.7–146.4 (m), 148.5, 158.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.0, −110.8 to −126.3 (m). MS (EI) *m/z* 635 (M^+, 20). Anal. Calcd for $C_{22}H_{12}F_{15}N_3O_2$ (635): C, 41.59; H, 1.90; N, 6.61. Found: C, 41.31; H, 1.91; N, 6.79. *Data for* **26b**: IR (KBr) ν 2235, 1524 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (d, *J*=6.7 Hz, 3H), 1.30 (d, *J*=6.7 Hz, 3H), 3.21 (dq, *J*=6.7 Hz, 1H), 6.20 (dd, $^1J_{HF}$=24.9 Hz, $^3J_{HF}$=19.1 Hz, 1H), 7.66 (d, *J*=8.8 Hz, 2H), 8.18 (s, 1H), 8.37 (d, *J*=8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.6, 29.2, 87.3 (dt, $^1J_{CF}$=195 Hz, $^2J_{CF}$=23 Hz), 109.8, 110.2–111.2 (m), 114.5, 123.7, 123.7, 130.0, 140.7, 143.1–143.6 (m), 144.0, 144.2, 148.3, 158.6. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1, −110.8 (t, $^3J_{FF}$=13.7 Hz), −120.8 to −126.4 (m), −198.6 (d, $^3J_{FF}$=13.7 Hz). MS (CI) *m/z* 618 (M^++1, 100). Anal. Calcd for $C_{22}H_{13}F_{14}N_3O_2$ (617): C, 42.80; H, 2.12; N, 6.81. Found: C, 42.77; H, 2.09; N, 6.85. *Data for* **27b**: IR (KBr) ν 3284, 2214, 1531 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.00 (d, *J*=6.8 Hz, 3H), 1.05 (d, *J*=6.7 Hz, 3H), 2.07 (dq, *J*=6.8, 6.7 Hz, 1H), 5.25 (s, 1H), 5.33 (d, *J*=3.3 Hz, 1H), 6.08 (s, 1H), 7.50 (d, *J*=8.7 Hz, 2H), 8.26 (d, *J*=8.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.7, 21.2, 31.6, 57.1, 83.8, 109.8–110.9 (m), 116.0, 116.6, 124.4, 128.0, 137.6, 143.5–144.2 (m), 147.4, 148.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1, −112.7 to −126.4 (m). MS (CI) *m/z* 638 (M^++1, 100). Anal. Calcd for $C_{22}H_{14}F_{15}N_3O_2$ (637): C, 41.46; H, 2.21; N, 6.59. Found: C, 41.41; H, 2.26; N, 6.57. ^{1}H NMR (400 MHz, $CDCl_3$) for **28b**, δ: 4.56 (s, 1H), 5.30 (bs, 2H).

4.7.13. Reaction of 2-azadiene (6j) with enamine (9b).

4.7.13.1. 5-Isopropyl-6-(2,4-dinitrophenyl)-2-perfluorohepthyl-3-cyanopiridine (25c) and 5-isopropyl-6-(2,4-dinitrophenyl)-2-(1,2,2,3,3,4,4,5,5,6,6,7,7,7-tetrafluorohepthyl-3-cyanopiridine (26c). The general procedure was followed using (1*E*,3*Z*)-4-cyano-1-(2,4-dinitrophenyl)-3-perfluorohepthyl-2-azabuta-1,3-diene (**6j**) obtained in situ and **9b** (0.23 g) at room temperature in $CHCl_3$ for 5 h. After column chromatography (hexane/ethyl acetate 10/1) 2.0 g of an inseparable mixture of compounds **25c** and **26c** (1/2), 0.05 g (4%) of **18a** (see reaction of 2-azadiene **6e** with enamine **9b**) and 0.18 g (8%) of **28b** (see reaction of 2-azadiene **6g** with enamine **9b**) were obtained. Spectroscopic data of mixture **25c** and **26c**: IR (KBr) ν 2239, 1640, 1547 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.19–1.28 (m, 18H), 2.77–2.86 (m, 2H) for **25c** and **26c**, 6.12 (dq $^2J_{FH}=25.1$ Hz, $^3J_{FH}=2.8$ Hz, 1H) for **26c**, 7.65 (d, $J=8.3$ Hz, 4H) for **25c** and **26c**, 8.21 (s, 1H) for **26c**, 8.27 (s, 1H) for **25c**, 8.64 (d, $J=8.3$ Hz, 2H) for **26c**, 8.66 (d, $J=8.3$ Hz, 2H) for **25c**, 9.07–9.13 (m, 2H) for **25c** and **26c**. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 23.1, 30.1, 87.3 (dt, $^1J_{CF}=167$ Hz, $^2J_{CF}=23$ Hz), 107.0–119.1 (m), 120.8, 127.8, 132.2, 138.5–148.5 (m), 156.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −81.1, −111.3 to −126.5 (m), −198.6 to −199.6 (m).

Acknowledgements

The authors thank the Ministerio de Ciencia y Tecnología (MCYT, Madrid DGI, PPQ2003-0910) and the Universidad del País Vasco (UPV-GC/2002) for supporting this work. C. A. thanks the Departamento de Educación, Universidades e Investigación of Gobierno Vasco for a Postdoctoral Fellowship and M. V. thanks the Universidad del País Vasco for a Predoctoral Fellowship.

References and notes

1. For reviews see: (a) Nicolaou, K. C.; Snyder, S. A.; Montagnon, T.; Vassilikogiannakis, G. *Angew. Chem. Int. Ed.* **2002**, *41*, 1668–1698. (b) Jayakumar, S.; Ishar, M. P. S.; Mahajan, M. P. *Tetrahedron* **2002**, *58*, 379–471. (c) Buonora, P.; Olsen, J.-C.; Oh, T. *Tetrahedron* **2001**, *57*, 6099–6138.
2. For recent contributions, see: (a) Bongini, A.; Panunzio, M.; Tamanini, E.; Martelli, G.; Vicennati, P.; Moneri, M. *Tetrahedron: Asymmetry* **2003**, *14*, 993–998. (b) Alves, M. J.; Duraes, M. M.; Gil, A. *Tetrahedron Lett.* **2003**, *44*, 5079–5082. (c) Nicolaou, K. C.; Nevalainen, M.; Safina, B. S.; Zak, M.; Bulat, S. *Angew. Chem. Int. Ed.* **2002**, *41*, 1941–1945. (d) Moody, C. J.; Hughes, R. A.; Thompson, S. P.; Alcaraz, L. *Chem. Commun.* **2002**, 1760–1761. (e) Ntirampebura, D.; Ghosez, L. *Synthesis* **2002**, 2043–2052.
3. (a) Jnoff, E.; Ghosez, L. *J. Am. Chem. Soc.* **1999**, *121*, 2617–2618. (b) Bandini, E.; Martelli, G.; Spunta, G.; Bongini, A.; Panunzio, M. *Synlett* **1999**, 1735–1738. (c) Ntirampebura, D.; Ghosez, L. *Tetrahedron Lett.* **1999**, *40*, 7079–7082. (d) Ghosez, L. *Pure Appl. Chem.* **1996**, *68*, 15–22.
4. (a) Palacios, F.; Aparicio, D.; Ochoa de Retana, A. M.; de los Santos, J. M.; Gil, J. I.; Alonso, J. M. *J. Org. Chem.* **2002**, *67*, 7283–7288. (b) Palacios, F.; Ochoa de Retana, A. M.; Gil, J. I.; Alonso, J. M. *Tetrahedron: Asymmetry* **2002**, *13*, 2541–2552. (c) Palacios, F.; Ochoa de Retana, A. M.; Gil, J. I.; López de Munain, R. *Org. Lett.* **2002**, *4*, 2405–2408.
5. (a) Palacios, F.; Alonso, C.; Amezua, P.; Rubiales, G. *J. Org. Chem.* **2002**, *67*, 1941–1946. (b) Palacios, F.; Alonso, C.; Rubiales, G. *J. Org. Chem.* **1997**, *62*, 1146–1154.
6. (a) Palacios, F.; Herrán, E.; Rubiales, G.; Ezpeleta, J. M. *J. Org. Chem.* **2002**, *67*, 2131–2135. (b) Palacios, F.; Herrán, E.; Rubiales, G. *J. Org. Chem.* **1999**, *64*, 6239–6246.
7. (a) Chambers, R. 2nd ed.; Fluorine in Organic Chemistry; Blackwell: London, 2004. (b) *Organofluorine Compounds: Chemistry and Applications*; Hiyama, T., Ed.; Springer: Berlin, 2000. (c) *Asymmetric Fluoroorganic Chemistry: Synthesis, Applications, and Future Directions*; Ramachandran, P. V., Ed.; American Chemical Society: Washington DC, 1999.
8. (a) Kirsch, P.; Modern Fluoroorganic Chemistry: Synthesis, Reactivity, Applications; Wiley: New York, 2004. (b) *Enantiocontrolled Synthesis of Fluoro-Organic Compounds: Stereochemical Challenges and Biomedical Targets*; Soloshonok, V. A., Ed.; Wiley: New York, 1999. (c) *Fluorine-Containing Amino Acids: Synthesis and Applications*; Kukhar, V. P., Soloshonok, V. A., Eds.; Wiley: New York, 1995.
9. (a) Hou, Y.; Higashiya, S.; Fuchigami, T. *J. Org. Chem.* **1997**, *62*, 8773–8776. (b) Hou, Y.; Higashiya, S.; Fuchigami, T. *Synlett* **1997**, *655*, 656.
10. (a) Ichikawa, J.; Wada, Y.; Miyazaki, H.; Mori, T.; Kuroki, H. *Org. Lett.* **2003**, *5*, 1455–1458. (b) Shen, Y.; Zhang, Y.; Sun, J. *J. Fluorine Chem.* **2002**, *116*, 157–161. (c) Peng, W.; Zhu, S. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3204–3218.
11. (a) Oesterle, T.; Simchen, G. *Synthesis* **1985**, 403–406. (b) Burger, K.; Dirsteiner, G.; Fehn, J. *Liebigs Ann. Chem.* **1971**, *747*, 45–50. (c) Ono, T.; Kukhar, V. P.; Soloshonok, V. A. *J. Org. Chem.* **1996**, *61*, 6563–6569.
12. (a) Simchen, G.; Pürkner, E. *Synthesis* **1990**, 525–527. (b) Burger, K.; George, G.; Fehn, J. *Liebigs Ann. Chem.* **1972**, *757*, 1–8. (c) Burger, K.; Fehn, J. *Liebigs Ann. Chem.* **1972**, *757*, 14–19. (d) Burger, K.; Meffert, A. *Liebigs Ann. Chem.* **1975**, 316–322.
13. Aza-Wittig reaction affords an excellent strategy for the construction of the C=N double bonds. For reviews see: (a) Wamhoff, H.; Richard, G.; Stölben, S. *Adv. Heterocycl. Chem.* **1995**, *64*, 159–249. (b) Barluenga, J.; Palacios, F. *Org. Prep. Proced. Int.* **1991**, *23*, 1–65.
14. For a preliminary communication see: Palacios, F.; Alonso, C.; Rubiales, G.; Villegas, M. *Tetrahedron Lett.* **2004**, *45*, 4031–4034.
15. $C_7F_{15}CN$ was purchased from Lancaster, however CF_3CN and C_2F_5CN were freshly prepared by dehydration of the corresponding amides (CF_3CONH_2 and $C_2F_5CONH_2$, respectively).[16]
16. Reisner, D. B.; Horning, E. C. In *Organic Syntheses*, *Collect. Vol. IV*; Wiley: New York, 1963; pp 144–145.
17. Phosphorus ylides **1** were generated in situ from theirs corresponding phosphonium salts and methyl lithium in diethyl ether.
18. (a) Barluenga, J.; Ferrero, M.; Lopez, F.; Palacios, F. *J. Chem. Soc., Perkin Trans. 1* **1989**, 615–618. (b) Ciganek, E. *J. Org. Chem.* **1970**, *35*, 3631–3636.
19. Palacios, F.; Legido, M.; Pérez de Heredia, I.; Rubiales, G. *Heterocycles* **2000**, *52*, 1057–1064.

20. Oikawa, T.; Kanomata, N.; Tada, M. *J. Org. Chem.* **1993**, *58*, 2046–2051.
21. Huang, X.; Xie, L.; Wu, H. *J. Org. Chem.* **1988**, *53*, 4862–4864.
22. *Ylides and Imines of Phosphorus*; Johnson, A. W., Kahsa, W. S., Starzewski, K. A. D., Dixon, D. A., Eds.; Wiley: New York, 1993.
23. (a) Coffey, D. S.; Kolis, S. P.; May, S. A. In Gribble, G. W., Gilchrist, T. L., Eds.; Progress in Heterocyclic Chemistry; Pergamon: Amsterdam, 2002; Vol. 14; Chapter 6.1. (b) Bentley, K. W.; The Isoquinoline Alkaloids; Harwood Academic: Amsterdam, 1998. (c) Yates, F. S. In Katritzky, A. R., Rees, C. W., Eds.; Comprehensive Heterocyclic Chemistry; Pergamon: New York, 1984; Vol. 2; Chapter 2.09.
24. (a) Dai, G.; Larock, R. C. *J. Org. Chem.* **2002**, *67*, 7042. (b) Huang, Q.; Hunter, J. A.; Larock, R. C. *J. Org. Chem.* **2002**, *67*, 3437–3444.
25. (a) Hofle, G.; Glaser, N.; Leibold, T.; Karama, U.; Sasse, F.; Steinmetz, H. *Pure Appl. Chem.* **2003**, *75*, 167–178. (b) Cativiela, C.; Diaz de Villegas, M. D. *Tetrahedron: Asymmetry* **2000**, *11*, 645–742. (c) Couty, F. *Amino Acids* **1999**, *16*, 297–320.
26. Goure, W. F. *J. Heterocycl. Chem.* **1993**, *30*, 71–80.
27. [2+2] Cycloaddition reaction of imines with electron-rich alkenes (enamines and enol ethers) has been reported. See: Aben, R. W. M.; Smit, R.; Scheeren, J. W. *J. Org. Chem.* **1987**, *52*, 365–370.
28. Mori, T.; Ichikawa, J. *Chem. Lett.* **2004**, *33*, 1206.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2795–2802

Hairpin conformation of amphidinols possibly accounting for potent membrane permeabilizing activities

Toshihiro Houdai,[a] Shigeru Matsuoka,[a] Nagy Morsy,[a] Nobuaki Matsumori,[a] Masayuki Satake[b] and Michio Murata[a,*]

[a]*Department of Chemistry, Graduate School of Science, Osaka University, 1-16 Machikaneyama, Toyonaka, Osaka 560-0043, Japan*
[b]*Graduate School of Agricultural Science, Tohoku University, Tsutsumidori-Amemiya, Aoba-ku, Sendai 981-8555, Japan*

Received 24 November 2004; revised 19 January 2005; accepted 20 January 2005

Abstract—Amphidinols are a unique dinoflagellate metabolite with potent antifungal activity. We examined membrane permeabilizing action by amphidinol analogues with structural variations in polyhydroxy and polyene moieties. Consequently, the polyene and polyhydroxy moieties turned out to play important roles in binding to lipid bilayer membrane and in forming ion-permeable pore/lesion across membrane, respectively. NMR-constrained modeling experiments have revealed for the fist time that amphidinols in membrane generally take a hairpin configuration, which plausibly accounts for their potent antifungal and other membrane permeabilizing activities.

1. Introduction

Dinoflagellates are known to be a rich source of bioactive secondary metabolites.[1,2] Polyketide metabolites with unique structures and powerful bioactivities have been frequently discovered from the species implicated in seafood poisoning; e.g. brevetoxins, ciguatoxins, maitotoxin, okadaic acid, and palytoxin. Amphidinols (AMs) have been isolated as a potent antifungal agent from the dinoflagellate *Amphidinium klebsii*. Eight congeners including those bearing closely related structures with different names have been so far reported,[3–6] among which the absolute configuration of AM3 has been recently determined by our group (Fig. 1)[7]. Their structures are best characterized by a long carbon chain encompassing multiple hydroxyl groups and polyolefins, which endow amphiphilic nature to the molecule. AMs enhance the permeability of the biological membrane by direct interaction with the membrane lipids, which is thought to be responsible for their potent antifungal activity.[4–6]

A number of membrane-active peptides such as melittin, alamethicin and magainin are known to spontaneously induce transmembrane pores in biomembrane. These bilayer pores deprive the affected organisms of their electrochemical gradients across membrane, which elicits the influx of water and leads to cell swelling, osmolysis and death. Most of them are short, helical and amphiphilic peptides; one side along the helix largely consists of hydrophobic side chains, and the other side comprises hydrophilic residues.[8–12] Some non-peptide antibiotics such as amphotericin B possess a similar amphiphilic structure. The barrel-stave model was proposed for the ion channel formed by amphotericin B.[13,14] In our previous study on the membrane permeabilizing actions of AM3 in comparison with those of polyene antibiotics, amphotericin B and filipin,[15] membrane pores or lesion formed by AM3 have shown the similar features to those of amphotericin B, implying that the membrane activity is caused by the molecular assemblage formed in biomembrane. On the other hand, the size of pore/lesion in erythrocyte membrane was estimated to be 2.0–2.9 nm in diameter, which is much larger than that of amphotericin B, 0.8 nm.

To gain a better understanding of the mechanism of membrane permeabilizing activity, we examined the structure–activity relationship using AM congeners, and the conformation of AM3 in sodium dodecyl sulfate (SDS) micelles on the basis of high-resolution NMR and simulations. In this report we present these results.

2. Results and discussion

2.1. Structure and membrane permeability activity

We measured the antifungal and hemolytic activities of AM

Keywords: Amphidinol membrane-permeabilization.
* Corresponding author. Tel./fax: +81 66850 5774;
e-mail: murata@ch.wani.osaka-u.ac.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.069

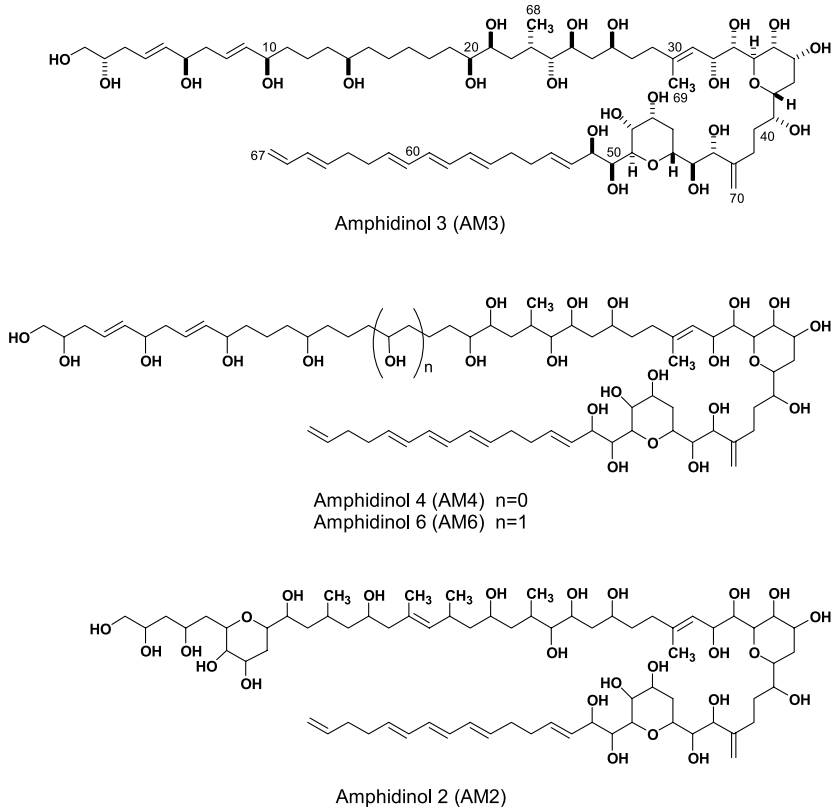


Figure 1. Absolute configuration of AM3 and structures of amphidinol congeners, AM4, AM6 and AM2.

congeners; antifungal on *Aspergillus niger* in minimal effective concentration, AM3: 10–20 μg/disk, AM4: 20–40 μg/disk, AM2: 20–40 μg/disk and hemolysis on human erythrocytes in EC_{50}, AM3: 0.15 μM, AM4: 0.6 μM, AM6: 1.0 μM and AM2: 1.5 μM. AM3 revealed very potent hemolytic activity, which was somewhat less potent than the reported value,[4,6] but still significantly exceeded those of AM4 and AM2. The interaction of AMs with phospholipid vesicles was further investigated by measuring ion efflux through the liposomal membrane. For evaluating their membrane permeability activities, we employed NMR-based Na^+ efflux assays using liposomes. From the concentration-dependence shown in Figure 2, AM3 revealed the most potent ion efflux activity, followed by AM4, and AM2, the latter of which elicited 30% permeability even at 60 μM. The activities of AM4, which possesses a vinyl group in lieu of butadiene in AM3 at the polyene terminus were less potent than that of AM3. AM2 structurally differing in the polyhydroxy part showed the weakest activities among them.

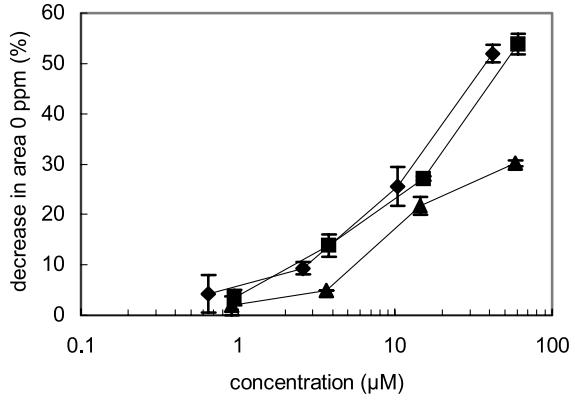


Figure 2. Concentration dependence of Na^+ efflux from liposomes for AM3 (diamonds), AM4 (squares) and AM2 (triangles). Na^+ efflux was measured with pure eggPC liposomes at the lipid concentration of 25 mM. The *Y*-axis represents percentage of decrease in peak areas at 0 ppm in ^{23}Na NMR spectra as described in Section 4.1.

The middle core part (C30–C50) of the molecule is structurally common among amphidinol family including luteophanol A[16] and lingshuiols.[17] Interestingly, luteophanol A is reported to exhibit no antifungal activity.[16] AMs comprise a hydrophobic polyene region in one end of the molecule whereas the corresponding portion of luteophanol A is substituted with three hydroxyl groups, which makes this side of molecule less hydrophobic, implying the importance of the hydrophobicity of the polyene chain in the membrane permeabilizing activities.

2.2. Partition coefficients to lipid bilayer

It is known that retention time on reversed-phase HPLC is generally in parallel with the membrane affinity of amphipathic peptides.[18,19] We measured retention times of AMs with an octadecylsilyl (ODS) column and aqueous acetonitrile as a mobile phase; AM3 at 30.1 min, AM4 at 14.6 min and AM2 at 33.5 min. The retention times of melittin analogues on reversed-phase HPLC are known to reflect their affinity to biomembranes, and is roughly correlated with their hemolytic activities.[20] However, no such correlation could be obtained for AM congeners.

We then determined the apparent partition coefficients to multi lamellar vesicle (MLV) membrane using a method reported by Betageri et al.[21] Figure 3 depicts the plot of mass ratio of eggPC/water [W_2/W_1] versus mole ratio of AM in lipid/AM in water [$(C_t-C_w)/C_w$] for the AM congeners (see Section 4). The slope corresponds to apparent molar partition coefficient, K'_m, from equation 1, AM3: 22.2×10^3, AM4, 2.24×10^3 and AM2, 0.77×10^3. The difference in the values suggest that AM3 with a diene terminus binds most efficiently to eggPC membrane and their order in K'_m values is same as that in the membrane permeabilizing activities (Figs. 2 and 3). The greater retention of AM2 compared with that of AM4 on the HPLC, despite their polyolefin region bearing the same structure, can be explained by the interaction between the octadecyl group of the ODS surface and the methyl groups of polyhydroxy region; five methyl groups in AM2 versus two in AM4. Thus, the length of the polyolefin region of AM is predominantly responsible for binding to lipid bilayer membrane.

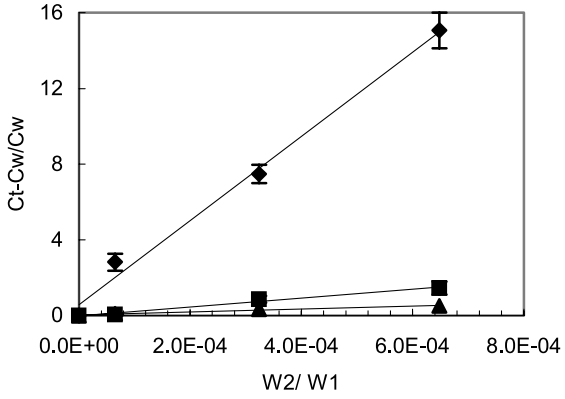


Figure 3. Partition coefficients derived from dependence of mass ratio of eggPC/water [W_2/W_1] versus mole ratio of AM in lipid/AM in water [$(C_t-C_w)/C_w$] for AM3 (diamonds), AM4 (squares) and AM2 (triangles). AMs incubated with the various concentrations of eggPC MLV in phosphate buffer. After incubation, MLVs solutions were centrifuged. The concentrations of AMs in supernatant were determined by peak area on HPLC. The slope express apparent molal partition coefficient, K'_m.

2.3. Estimation of pore size

To estimate the size of pores formed by AMs in biomembrane, we carried out colloid osmotic protection assays. This method with use of erythrocytes is widely applied for measuring the size of channels.[22,23] When a osmotic-protecting agent added to the medium is too large in size to pass through a channel formed on erythrocytes, no hemolysis takes place; the osmotic pressure of the intracellular hemoglobin is balanced by a protecting agent added outside. Thus, size of the pore in the membrane can be estimated by the molecular size of the efficacious protecting agent.

In our previous study[15], AM3 caused the rapid permeabilization of erythrocyte membrane, which led to the simultaneous leakage of macromolecules including hemoglobin. These observations indicate that the pore size of AM3 increases with increased concentrations of AM3, as reported for melittin.[22] The concentrations used in these experiments, therefore, were taken as least doses necessary for hemolysis. According to the results in Figure 4, the size

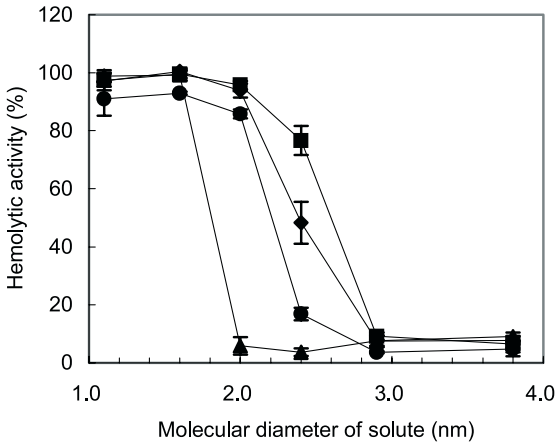


Figure 4. Osmotic protection experiments for AM3 (diamonds), AM4 (squares), AM6 (circles) and AM2 (triangles) using erythrocytes. The size of a pore or lesion formed by these agents was estimated by hemolytic tests in the presence of various osmotic protectants. A blood cell suspension at 1% hematocrit was suspended in 90% PBS buffer and added with 30 mM of the following solutes as an osmotic protectant; raffinose, polyethylene glycols 600, 1000, 1540, 2000, and 4000, whose diameters were estimated as 1.1, 1.6, 2.0, 2.4, 2.9, and 3.8 nm, respectively. The concentrations of AM3, AM4, AM6 and AM2 were 0.28, 1.1, 2.0, and 3.1 μM, respectively, where 100% hemolysis occurs in PBS in the absence of a protectant.

of a pore formed by AM3 and AM4 was deduced to 2.0–2.9 nm since the hemolysis occurred in the presence of polyethylene glycol 1000 (PEG 1000) and was suppressed by PEG 2000, while that of AM2 pore was estimated significantly smaller around 1.6–2.0 nm.

The antibacterial activity of alamethicin analogues is known

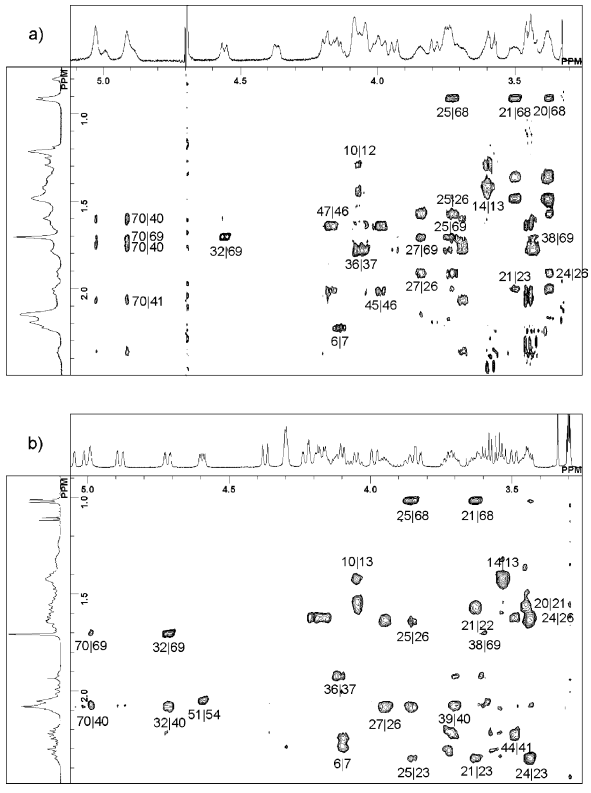


Figure 5. Partial NOESY spectrum of AM3 with SDS-d_{25} in D_2O (a) and partial ROESY spectrum in CD_3OD/pyridine-d_5 (2:1) (b).

to be correlated with the mean number of monomers per conducting aggregate or channel, which roughly corresponds to their pore size.[24] When their size becomes smaller, the single-channel conductance decreased as well.[25] In our study the diameter of AM2 pore was smaller than that of AM4 (Fig. 4), implying that their difference in the membrane permeability activities can be partly attributable to the pore size formed in bilayer membrane. In addition, the pore side of AM6, which possesses an additional hydroxyl group in the polyhydroxy region compared with AM4[4], was also deduced to be 2.0–2.9 nm from the same experiment. The tetrahydropyrane (THP) ring in the end of polyhydroxy region of AM2 presumably narrows the pore size.

2.4. Conformation analysis in SDS micelles

To further investigate AM's mode of action, the conformation of AM3 in SDS micelle and that in organic solvent were compared by two-dimensional NMR experiments. Micelle dispersions of small membrane peptides have been often used to reproduce the membrane environments, where orientational averaging due to smaller and spherical micelles facilitates high-resolution NMR measurements. Because of their high curvature, use of micelles as a bilayer membrane model is sometimes controversial. SDS micelles are, however, reported to mimic the amphipathic nature of a bilayer membrane even for a conformationally sensitive peptide such as gramicidin A; the conformation of the peptide was shown to form a stable right-handed $\beta^{6.3}$-helix in SDS micelles,[26] which was also observed in 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayer membrane[27,28] while different conformations were shown in organic solvents such as MeOH/$CHCl_3$,[29] trifluoroethanol (TFE)[30] and dimethyl sulfoxide (DMSO).[31]

Signal assignment was effected by DQF-COSY, TOCSY, and NOESY (ROESY) (Fig. 5) experiments. The chemical shifts and NOE interactions of AM3 in SDS-d_{25} micelles are listed in Table 1. As shown in the table, most of NOEs including conformationally relevant ones (e.g., H25-H69, H69-H70, and H43-H51) were observed in the middle region (the C20–C51 moiety) including the core part. From the 83 structures calculated for AM3 with NOE constrains by Molecular Dynamics, five of them were retained for AM3 on the basis of their low potential energy. Figure 6a shows superimpose of conformation ensemble in SDS micelles.

Molecular Dynamics simulations reveal partly converged

Table 1. ^{1}H NMR data of AM3 with SDS-d_{25} micelle (D_2O at 4.7 ppm)

Position	δ_H	NOEs[a]	Position	δ_H	NOEs[a]
1	3.44, 3.59	2[b]	35	4.09	37[b], 38[b]
2	3.75		36	4.06	37[b]
3	2.14, 2.17		37	1.79	38[b], 39[c]
4	5.68	6[c]	38	3.44	39[c], 69[c], 70*l*[c]
5	5.59	6[c]	39	3.70	41*h*[c]
6	4.15	7[b]	40	1.61, 1.75	70*h*[c], 70*l*[c]
7	2.23		41	2.07, 2.37	44[c], 70*h*[c], 70*l*[c]
8	5.64	10[d]	42	—	
9	5.56	10[c]	43	4.20	44[d], 51[b], 53[c], 70*h*[b], 70*l*[d]
10	4.08	11[c], 12*h*[c]	44	3.46	45[d], 46*h*[b]
11	1.46, 1.53		45	3.99	46*h*[c], 46*l*[c], 47[d], 49[b]
12	1.29, 1.63	13[c]	46	1.65, 2.02	47[c]
13	1.43	14[b]	47	4.17	48[d]
14	3.60		48	4.05	49[d]
15	n.d.		49	3.80	51[c]
16	n.d.		50	4.01	51[d]
17	n.d.		51	4.37	53[b]
18	n.d.		52	5.62	53[b]
19	1.37, 1.49	20[d], 21[c]	53	5.81	54[b]
20	3.39	21[b], 22[d], 68[d]	54	2.20	
21	3.50	22[b], 23[c], 68[d]	55	2.20	57[d]
22*h*	1.36	68[c]	56	5.68	
22*l*	1.50	68[b]	57	6.05	
23	2.00	24[b], 68[d]	58	n.d.	
24	3.38	25[b], 26*h*[c], 26*l*[c]	59	n.d.	
25	3.73	26*h*[c], 26*l*[c], 27[c], 68[d], 69[b]	60	6.04	62[d]
26*h*	1.58	27[c], 29[c], 69[d]	61	5.61	
26*l*	1.92	27[c]	62	2.15	
27	3.84	69[b]	63	2.15	
28	n.d.		64	5.64	
29	2.15	31[d], 69[d]	65	6.00	
30	—		66	6.22	
31	5.51	32[b], 33[c], 69[b]	67	4.90, 5.01	
32	4.56	33[d], 34[c], 35[c], 38[d], 69[d]	68	0.92	
33	3.75	34[b], 35[d], 36[b], 38[d]	69	1.71	70*h*[b], 70*l*[c]
34	3.94	35[d]	70	4.91, 5.03	

h: high field resonance, *l*: low field resonance, n.d. not assigned by the overlapped crosspeaks.
[a] The NOEs connectivities are listed once according to the proton having the lower number.
[b] Intensity of the NOEs are medium.
[c] Intensity of the NOEs are weak.
[d] Intensity of the NOEs are strong.

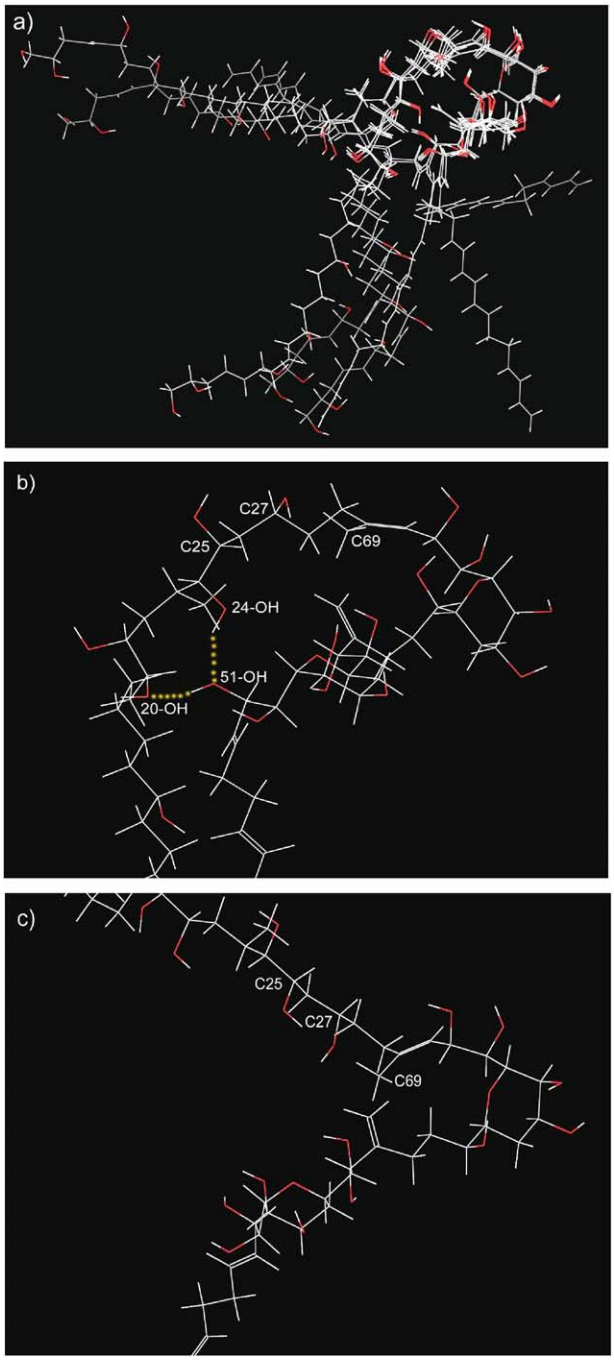


Figure 6. Superimpose of five low potential energy structures (a) and the lowest energy structure calculated for AM3 in SDS micelles derived from NOE data (b). The dotted lines between hydroxyl groups expressed the hydrogen bonds network involving $O^{H}20$–$H^{O}51$, $H^{O}24$–$O^{H}51$. One of the lowest energy structures calculated in MeOH/pyridine (2:1) (c).

conformation, which takes a hairpin-like shape for the vicinity of two THP rings, and is rather dispersed for polyhydroxy and polyolefinic side chains. In the case of a MeOH/pyridine solution, the restraints were also abundant for the core region, by which the simulation reveals the similar hairpin-like arrangement as one of major conformers (Fig. 6c). In particular, the conformation of the C28–C51 moiety in MeOH/pyridine, which was previously obtained from the *J*-based configuration analysis,[7,32] resembles that in micelles (Fig. 6a), supporting the hairpin shape for AM3. However, there are small but significant differences between them. In the micelles, hydrogen-bonding network stabilizing the conformation is formed with $O^{H}20$–$H^{O}51$, $H^{O}24$–$O^{H}51$ and $O^{H}24$–$H^{O}51$ (Fig. 6b) while hydrogen bonds were not apparent in MeOH/pyridine (Fig. 6c); highly polar solvents such as MeOH and pyridine generally weaken hydrogen bond.[33,34] In addition to long-range NOEs such as H43/H51 and H69/H70, middle-range NOEs for H69/H25, H69/H26, H69/H27, and H69/H29 were observed for the polyhydroxy side chan in SDS micelles (Fig. 5a) while these middle-range interactions were not seen on ROESY in MeOH/pyridine (Fig. 5b). The results suggest that the conformation of the core part including the hairpin shape is stabilized by rotational restriction with respect to each acyclic C–C bond and by intramolecular hydrogen bonds. In the case of AM2, the observed NOEs of the middle part in SDS micelle were similar to those of AM3, indicating that the conformation of the core structure is common among AM congeners.

Protegrins are short and amphiphilic peptides bearing potent antimicrobial activity. Their conformations are known to take a rigid antiparallel two-stranded β-sheet that is stabilized by two disulfide bonds.[35–37] Such a disulfide linked β-sheet motif is common in other antimicrobial peptides such as human defensins and tachyplesin.[34] Breaking these disulfide bonds has greatly reduced the activity.[38–40] The presence of the disulfide bonds is key to the stability of the β-sheet structure and to the membrane disturbing activity. A hairpin-like structure of the central region of AM molecule somewhat mimics these peptidic antimicrobes, implying its important role in the membrane activity.

To determine the position of AM3 molecule relative to the surface and interior of the SDS micelles, spin-lattice relaxation time T_1 was determined from ^{1}H NMR spectra in the presence and absence of paramagnetic manganese ion, Mn^{2+} (Fig. 7). Although a large portion of proton

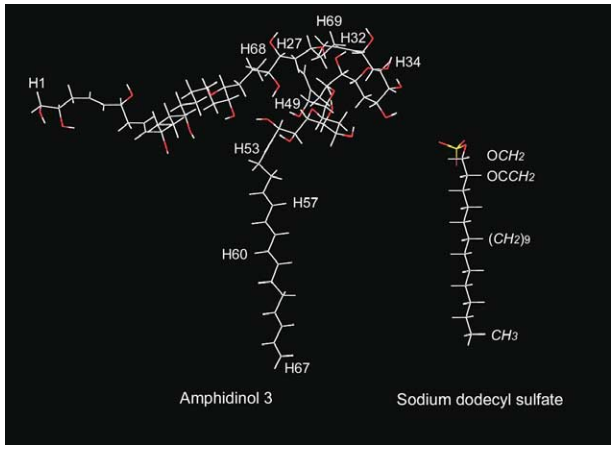


Figure 7. Hypothetical illustration of the relative positions of AM3 and SDS in micelle with respect to depth from the surface. The detectable paramagnetic contribution to the spin-lattice relaxation time T_{1M} (in seconds) are following data; H1, 0.47; H68, 2.06; H27, 1.75; H69, 1.36; H32, 2.20; H34, 1.17; H49, 0.92; H53, 1.82; H57,60, 6.58; H67, 11.35 for AM3 in SDS-d_{25} micelles and OCH_2, 1.25; OCCH_2, 2.14; $(CH_2)_9$: 9.11; CH_3: 12.00 for pure SDS micelles. The chemical shifts of pure SDS in D_2O are OCH_2: 4.0 ppm, OCCH_2: 1.6 ppm, $(CH_2)_9$: 1.2 and 0.8 ppm. The concentration of Mn^{2+} used in this experiment was 10 μM.

signals heavily overlap and prevent T_1 measurements, the relaxation data for methylene protons (H1), methine protons (H27, H34 and H49), methyl groups (H68, H69), terminal olefin (H67) and olefinic protons (H53 and H57,60) in SDS-d_{25} micelles were obtained. The relaxation times of signals from SDS micelles without AM3 were also measured for comparison. The T_{1M} values calculated from Eq. 2 are related to the distance between the proton of interest and Mn^{2+}. Figure 7 shows the relative distance of AM3 protons from the surface of micelles, which indicates that H1, H27, H32, H34,, H49, H68, H69 and H53 reside near the surface while the other olefin protons (H57,60 and H67) inside the micelle. In other words, the hydrophilic region of the molecule is predominantly present in the surface while the hydrophobic polyolefin region penetrating in the interior of micelle.

3. Conclusion

All these findings allow us to hypothesize the molecular mode of action for AMs as follows: (a) binding to bilayer membrane chiefly with the polyolefin region (the C52-moiety) and the membrane affinity is markedly dependent on the length of the polyolefinic chain; (b), the size of the pore formed in the membrane is influenced by the polyhydroxy region (the C1–C20 moiety); (c) the central region (the C20–C52 moiety) takes hairpin-shaped conformation, which is stabilized by hydrogen bonds in amphipathic environments. The conformation of AMs in membrane environment is reminiscent of amphotericin B; the hairpin-like conformation for the vicinity of two THP rings and closing two strands (one is a hydrophilic and the other is a hydrophobic). Thus, the activity may be accounted for by the facial amphiphilic interaction with membrane lipids although further experiments are necessary to clarify the mechanism of membrane action by AM.

4. Experimental

4.1. Materials

Dysprosium chloride hexahydrate ($DyCl_3 \cdot 6H_2O$), sodium tripolyphosphate (Na_5PPP), Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$), raffinose and polyethylene glycols (PEG 600, 1000, 1540, 2000, and 4000) were purchased from Wako Pure Chemical Industries (Osaka, Japan), egg york phosphatidylcholine (eggPC) from Nacalai Tesque (Osaka, Japan). Polycarbonate filters were obtained from Nuclepore (Pleasanton, CA). Sodium dodecyl sulfate (SDS-d_{25}) was from Cambridge Isotope Laboratories, Inc. (Andover, MA). Other chemicals were from standard commercial sources and used without further purification otherwise noted.

4.2. Culture and isolation

The marine dinoflagellate *Amphidinium klebsii* was separated from Aburatsubo-Bay, Kanagawa, Japan, and deposited in National Institute of Environmental Studies (NIES 613). The culture medium was artificial seawater (Marin Art Hi, Tomita Pharmaceutical, 3% w/v) enriched with ES-1 supplement. *Amphidinium carterae* was separated from Kauaroa, South Island, New Zealand. This was used for production of AM4. Briefly, the unialgal culture was grown in a three-liter glass flask containing 2 L of 80% seawater enriched with GSe supplements.

Extract and purification of amphidinols were carried out as previously reported.[42] Briefly, the cultured cells were extracted with methanol and acetone, and the combine extract, after the solvents were removed, was subjected to ethyl acetate–H_2O partition and the resultant aqueous layer was extracted with 1-butanol. The butanol layer was further purified by chromatography over HW-40F (Toyopearl, methanol–H_2O, 1:1) and then HPLC (YMC-Pack ODS-AM, MeCN–H_2O, 1:2) to furnish 1.0 mg of AM3 and 0.2 mg of AM2 from 10 L of the culture media. Similar extraction and purification from the cells of *A. carterae* in 10 L of the media provided 1.0 mg of AM4.

4.3. Na^+ efflux assay using ^{23}Na NMR

For liposome preparation, eggPC was dissolved in chloroform to prepare stock solutions. The solvent was then evaporated to form a thin film on the bottom of the flask. To ensure complete removal of solvent, the flask was placed under vacuum for over night. The filmy residue was hydrated with a 100 mM NaCl solution containing 10 mM Tris–HCl at pH 7.2 or 10 mM phosphate buffer at pH 7.4, and incubated for 2 h to form multi-lamellar vesicle (MLV). The MLVs solution was frozen and thawed three times. The sized liposomes thus obtained were extruded through polycarbonate membrane with the pore size of 800 nm (AVESTIN Liposofast®). Na^+ efflux assays were carried out basically following a method by Kimura et al.[43] Liposomes (800 nm in diameter) in 100 mM NaCl/10 mM Tris–HCl (pH 7.2) buffer was dialyzed against 2 L of 120 mM KCl/10 mM Tris–HCl buffer for 12 h to replace external Na^+ with K^+. An NMR sample was made by mixing 100 μL of D_2O, 2.0 μL of 89.3 mM sodium bis(tripolyphosphate) dysprosium, $Na_7Dy(PPP)_2$, and 500 μL of liposome solution. The shift reagent was prepared by mixing 50 μL of 250 mM $DyCl_3 \cdot 6H_2O$ and 90 μL of 500 mM $Na_5(PPP)$ at pH 7.0[43]. Na^+ efflux was measured by ^{23}Na NMR at 30 °C with the lipid concentration of 25 mM. The ^{23}Na NMR spectra were determined with an liposome suspension containing 100 mM NaCl inside and 100 mM KCl outside the vesicles. The residual Na^+ outside liposomes was shifted to up-field around −1.0 ppm by shift reagent $Na_7Dy(PPP)_2$,[44] whereas leaving a signal due to the inside ions at 0 ppm unaffected. An aliquot of a sample solution in methanol (10 μL) was added to liposome suspensions (600 μL) and incubated for 2 h at room temperature.

When an agent permeabilizes the membrane, the peak around −1.0 ppm increases while a peak at 0 ppm decreases, which clearly shows that inside Na^+ and outside K^+ was exchange through liposomal membrane. Percentage of a reduction of peak area at 0 ppm was expressed as $(Na^+{}_0 – Na^+{}_t)/Na^+{}_0 \times 100$, where $Na^+{}_t$ is the molar quantity of Na^+ inside the vesicles in the presence of a sample, and $Na^+{}_0$ is that in the absence of a sample.

4.4. Determination of partition coefficients

Apparent molar partition coefficients of AM congeners into bilayer membrane were determined by following a method by Betageri et al.[21] AMs incubated with the various concentration (0, 0.10, 0.50 and 1.0 mM) of eggPC MLV in 10 mM phosphate buffer (100 μL) for 3 h at room temperature After incubation, MLV solutions were centrifuged at 18,800*g* for 30 min. The concentrations of AMs in supernatant (50 μL) were determined by peak area on HPLC analysis (Cosmosil 5C18-AR Waters 4.6×150 mm, MeCN–H_2O, 13:7). The retention time of AM3, AM4 and AM2 is 30.1, 14.6 and 33.5 min, respectively. The apparent molar partition coefficients, K'_m, were calculated from

$$K'_m = \frac{(C_t - C_w)W_1}{C_w W_2} \quad (1)$$

where C_t: peak area of AM without eggPC MLV, C_w: peak area of AM of aqueous phase with eggPC MLV, W_1: weight of aqueous phase (g), W_2: weight of phospholipid (g).

4.5. NMR measurements

Samples for NMR experiments were prepared by dissolving a mixture of 2.8 mg of AM3 and 6.6 mg of perdeuterated SDS (SDS-d_{25}) in 0.55 mL of D_2O or by dissolving 4.4 mg of AM3 in 500 μL of CD_3OD/pyridine-d_5 (2:1). The $MnCl_2$ was dissolved in D_2O before added to a micelle suspension. The spin-lattice relaxation time, T_1, was measured with the absence and presence of 10 μM of $MnCl_2$.

The NMR spectra were recorded on a LA500 instrument. The 2D NMR spectra such as DQF-COSY, TOCSY and NOESY were measured with a sweep width of 4000 Hz in both dimensions, 512 real data points in F1 and 1000 real data points in F2, 16 scans per increment. Mixing times of 150 and 300 ms were used for NOESY experiments. The data were apodized with shifted sine-bell window functions in both F1 and F2 dimensions after zerofilling in the F1 dimension to obtain a final matrix of 1024 (F1)×1024 (F2) real data points. The spin-lattice relaxation time T_1 were determined using a standard 180-τ-90 inversion recovery pulse sequence with $>5T_1$ delay between pulse sequence allowing the spin system to relax to equilibrium. Twenty τ values between 0.01 and 10 s were applied and evaluated by the linear curve fitting software of the spectrometer. All the spectra were recorded at 303 K.

The paramagnetic contribution to the relaxation time, T_{1M}, were calculated from

$$\frac{1}{T_{1M}} = \frac{1}{T_1} - \frac{1}{T_1^0} \quad (2)$$

$$\frac{1}{T_{1M}} \propto r^{-6}$$

where T_1 and T_1^0: spin-lattice relaxation time in the presence and absence of paramagnetic ion, Mn^{2+}, r: time average distance between the proton of interest and Mn^{2+} ion.[41] The concentration of Mn^{2+} ion used in this experiments was 10 μM.

4.6. Molecular dynamics calculations

The force field used in this calculation was MMFFs under vacuum since NOEs data were supposed to incorporate solvent effects. All interproton-distance restraints were derived from two-dimensional NOESY experiments. The restraints were classified into three categories. Upper bounds were fixed at 2.6, 3.4 and 5.0 Å for strong, medium and weak correlations, respectively. The THP rings and all double bonds were fixed as a chair conformation and *E* configurations, respectively, judging from ^3J-coupling constants on DQF-COSY. Simulated annealing[45] performed three different tasks with the NMR-based restraints; (1) 10 ps equilibrium run at 1000 K, (2) 20 ps equilibrium run at 300 K, (3) 20 ps equilibrium run at 100 K. After simulated annealing, Conformational search in MacroModel® was carried out. This process typically cycles through the sequence of generating a new structure, minimizing it, and then determining if the structure should be retained. The same force field and restraints were used in simulated annealing. The MCMM method was used during a calculation and a 50 kJ/mol cutoff was applied, eventually leaving several final conformations.

4.7. Antifungal assay, hemolytic test, and osmotic protection experiments

The fungus *Aspergillus niger* was cultured in a GP liquid medium (2% glucose, 0.2% yeast extract, 0.5% polypeptone, 0.05% $MgSO_4$, and 0.1% KH_2PO_4) at 25 °C for 2 days. An aliquot of the broth was then spread onto a GP agar plate (1.5% agar). Samples dissolved in methanol were spotted on paper disks (8 mm in diameter). They were then placed on an agar plate spread with *A. niger* mycelia. After incubation at 25 °C for 2 days, the diameter of the inhibitory zone on each paper disk was measured. For each hemolytic test, human blood cells were collected in 3.13% sodium citrate and immediately separated from the plasma by centrifugation at 1000*g* for 5 min. Sedimented cells were washed three times with phosphate buffer saline (PBS), containing 137 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4, and 1.47 mM KH_2PO_4 at pH 7.4. A sample dissolved in methanol (10 μL) was added to 190 μL of the blood cell suspension in 1% hematocrit PBS and incubated for 12 h at 30 °C. After incubation, the resultant supernatant was subjected to colorimetric measurements at 450 nm on micro-plate reader (Molecular Devices) to determine the absorbance (A_{s450}). Total hemoglobin in the suspension was obtained from the value upon complete hemolysis (A_{h450}). The percentage amount of hemoglobin released from erythrocytes was calculated as $A_{s450}/A_{h450}\times 100$. From dose–response curves, the concentration that caused 50% hemolysis (EC_{50}) was determined. In osmotic protection experiments, a blood cell suspension with 1% hematocrit was mixed with 90% PBS, and added with 30 mM of the following solutes as an osmotic protectant; raffinose, and polyethylene glycols (PEG 600, 1000, 1540, 2000, and 4000), whose molecular diameters are estimated to be 1.1, 1.6, 2.0, 2.4, 2.9 and 3.8 nm, respectively.[22,46]

Acknowledgements

We are grateful to Seiji Adachi in Department of Chemistry, Osaka University for measuring NMR spectra; to Megumi Mori and Saori Seki in our laboratory for their help in culturing dinoflagellates; and to Prof. Tohru Oishi in our laboratory for discussion and suggestions. This study was supported by Yamada Science Foundation and by a Grant-In-Aide for Scientific Research on Priority Area (A) (No. 12045243) from MEXT, Japan.

References and notes

1. Shimizu, Y. *Chem. Rev.* **1993**, 1685–1698.
2. Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, 1897–1909.
3. Satake, M.; Murata, M.; Yasumoto, M.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc.* **1991**, *113*, 9859–9861.
4. Paul, G. K.; Matsumori, N.; Konoki, K.; Murata, M.; Tachibana, K. *J. Mar. Biotechnol.* **1997**, *5*, 124–128.
5. Paul, G. K.; Matsumori, N.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 6279–6282.
6. Paul, G. K.; Matsumori, N.; Konoki, K.; Sasaki, M.; Murata, M.; Tachibana, K. *Harmful and Toxic Algal Blooms, Intergovernmental Oceanographic Commission of UNESCO, Sendai*; Yasumoto, T., Oshima, Y., Fukuyo, Y., Eds.; 1996, 503–506.
7. Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc.* **1999**, *121*, 870–871.
8. Terwilliger, T. C.; Weissman, L.; Eisenberg, D. *Biophys. J.* **1982**, *37*, 353–361.
9. Inagaki, F.; Shimada, I.; Kawaguchi, K.; Hirano, M.; Terasawa, I.; Ikura, T.; Go, N. *Biochemistry* **1989**, *28*, 5985–5991.
10. Dempsey, C. E.; Butler, G. S. *Biochemistry* **1992**, *31*, 11973–11977.
11. Bechinger, B.; Zasloff, M.; Opella, S. J. *Protein Sci.* **1993**, *2*, 2077–2084.
12. Fox, R. O., Jr; Richards, F. M. *Nature* **1982**, *300*, 325–330.
13. De Kruijff, B.; Demel, R. A. *Biochim. Biophys. Acta* **1974**, *339*, 57–70.
14. Andreoli, T. E. *Ann. N. Y. Acad. Sci.* **1974**, *235*, 448–468.
15. Houdai, T.; Matsuoka, S.; Matsumori, N.; Murata, M. *Biochim. Biophys. Acta* **2004**, *1667*, 91–100.
16. Doi, Y.; Ishibashi, M.; Nakamichi, H.; Kosaka, T.; Ishikawa, T.; Kobayashi, J. *J. Org. Chem.* **1997**, *62*, 3820–3823.
17. Huang, X. C.; Zhao, D.; Guo, Y. W.; Wu, H. M.; Trivellone, E. *Tetrahedron Lett.* **2004**, *45*, 5501–5504.
18. Parker, J. M. R.; Guo, D.; Hodges, R. S. *Biochemistry* **1986**, *25*, 5425–5432.
19. Guo, D.; Mant, C. T.; Taneja, A. K.; Parker, J. M. R.; Rodges, R. S. *J. Chromatogr.* **1986**, *359*, 499–517.
20. Blondelle, S. E.; Houghten, R. A. *Biochemistry* **1991**, *30*, 4671–4678.
21. Betageri, G. V.; Rogers, J. A. *Int. J. Pharm.* **1988**, *46*, 95–102.
22. Katsu, T.; Ninomiya, C.; Kuroko, M.; Kobayashi, H.; Hirota, T.; Fujita, Y. *Biochim. Biophys. Acta* **1988**, *939*, 57–63.
23. Katsu, T. *Biol. Pharm. Bull.* **1999**, *22*, 978–980.
24. Beven, L.; Helluin, O.; Molle, G.; Duclohier, H.; Wroblewski, H. *Biochim. Biophys. Acta* **1999**, *1421*, 53–63.
25. Duclohier, H.; Molle, G.; Dugast, J.; Spach, G. *Biophys. J.* **1992**, *63*, 868–873.
26. Arseniev, A. S.; Barsukov, I. L.; Bystrov, V. F.; Lomize, A. L. *FEBS Lett.* **1985**, *186*, 168–174.
27. Fares, C.; Sharom, F. J.; Davis, J. H. *J. Am. Chem. Soc.* **2002**, *124*, 11232–11233.
28. Ketchem, R. R.; Lee, K. C.; Huo, S. *J. Biomol. NMR* **1996**, *8*, 1–14.
29. Arseniev, A. S.; Barsukov, I. L.; Bystrov, V. F. *FEBS Lett.* **1985**, *180*, 33–39.
30. Abdul-Manan, N.; Hinton, J. F. *Biochemistry* **1994**, *33*, 6773–6783.
31. Hawkes, G. E.; Lian, L. Y.; Randall, E. W.; Sales, K. D.; Curzon, E. H. *Eur. J. Biochem.* **1987**, *166*, 437–445.
32. Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866–876.
33. Hong, D. P.; Hoshino, M.; Kuboi, R.; Goto, Y. *J. Am. Chem. Soc.* **1999**, *121*, 8427–8433.
34. Uversky, V. N.; Narizhneva, N. V.; Kirschstein, S. O.; Winter, S.; Lober, G. *Fold. Des.* **1997**, *2*, 163–172.
35. Fahrner, R. L.; Dieckmann, T.; Harwig, S. S. L.; Lehrer, R. I.; Eisenberg, D.; Feigon, J. *Chem. Biol.* **1996**, *3*, 543–550.
36. Aumelas, A.; Mangoni, M.; Roumestand, C.; Chiche, L.; Despaux, E.; Grassay, G.; Calas, B. *Eur. J. Biochem.* **1996**, *237*, 575–583.
37. Hancock, R. E.; Lehrer, R. *Trends Biotechnol.* **1998**, *16*, 82–88.
38. Harwig, S. S. L.; Waring, A.; Yang, H. J.; Cho, Y.; Tan, L. *Eur. J. Biochem.* **1996**, *240*, 352–357.
39. Chen, J.; Falla, T. J.; Liu, H.; Hurst, M. A.; Fujii, C. A.; Mosca, D. A.; Embree, J. R.; Loury, D. J.; Radel, P. A.; Chang, C. C.; Gu, L.; Fiddes, J. C. *Biopolymers* **2000**, *55*, 88–98.
40. Mangoni, M. E.; Aumelas, A.; Charnet, P.; Roumestand, C.; Chiche, L.; Despaux, E.; Grassy, G.; Calas, B. *FEBS Lett.* **1996**, *383*, 93–98.
41. Villalain, J. *Eur. J. Biochem.* **1996**, *241*, 586–593.
42. Houdai, T.; Matsuoka, S.; Murata, M.; Satake, M.; Ota, S.; Oshima, Y.; Rhodes, L. L. *Tetrahedron* **2001**, *57*, 5551–5555.
43. Kimura, A.; Kuni, N.; Fujiwara, H. *Chem. Pharm. Bull.* **1997**, *45*, 431–436.
44. Chu, S. C.; Pike, M. M.; Fossel, E. T.; Smith, T. W.; Balschi, J. A. *J. Magn. Reson.* **1984**, *56*, 33–47.
45. Nilges, M.; Clore, G. M.; Gronenborn, A. M. *FEBS Lett.* **1988**, *229*, 317–324.
46. Scherrer, R.; Gerhardt, P. *J. Bacteriol.* **1971**, *107*, 718–735.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2803–2814

Hydrogen atom transfer methodology for the synthesis of C-22, C-23, and C-25 stereoisomers of cephalostatin north 1 side chain from spirostan sapogenins

Carmen Betancor,[b] Raimundo Freire,[a] Inés Pérez-Martín,[a] Thierry Prangé[c] and Ernesto Suárez[a,*]

[a]*Instituto de Productos Naturales y Agrobiología del C.S.I.C., Carretera de La Esperanza 3, 38206 La Laguna, Tenerife, Spain*
[b]*Departamento de Química Orgánica, Universidad de La Laguna, Tenerife, Spain*
[c]*LURE, Université Paris-Sud, Paris, 91405 Orsay Cedex, France*

Received 12 November 2004; revised 18 January 2005; accepted 20 January 2005

Abstract—A simple synthesis of all eight C-22, C-23, and C-25 diastereoisomers of the cephalostatin north 1 side chain has been accomplished from (25*R*)-5α-spirostan-3β-ol (tigogenin). The synthesis involves selective hydroxylations at C-23 and C-25 and reductive opening of the 1,6-dioxaspiro[4.5]decane spirostan system to give a conveniently protected 5α-furostan-3β,23,25,26-tetrol. The construction of the required 1,6-dioxaspiro[4.4]nonane system entailed an intramolecular hydrogen abstraction reaction promoted by the C-25 alkoxyl radical as the key step. Acid-catalyzed isomerization of the spiroketal unit suggested that 22*R* isomers are the thermodynamic products while the 22*S* isomers are the result of kinetic control. The acid-catalyzed equilibrium between 1,6-dioxaspiro[4.4]nonane and 1,6-dioxaspiro[4.5]decane systems was also studied. In the 1,6-dioxaspiro[4.4]nonane units, the observed $^{3}J_{23,24}$ coupling constants suggest that the five-membered puckered ring-F undergoes substantial conformational changes on going from 22*S* to 22*R* isomers.

1. Introduction

Cephalostatins[1] and the structurally related ritterazines[2] comprise a group of secondary metabolites isolated from marine invertebrates (*Cephalodiscus gilchristi* and *Ritterella tokioka*, respectively) which have attracted considerable attention from synthetic organic chemists and pharmacologists due to their complex structures and significant biological properties.[3] They are alkaloids constituted by two steroidal units linked through a pyrazine ring involving the C2-C3 position of each monomeric unit and are among the most potent cytotoxins ever isolated from a natural source. In most of these substances the steroidal eight-carbon side chain has been transformed into a 1,6-dioxaspiro [4.4]nonane system. In particular, a polyoxygenated (2*S*,4*R*,5*S*,9*S*)-2-hydroxymethyl-2,9-dimethyl-1,6-dioxaspiro [4.4]nonan-4-ol substructure is found in the side chain of the north unit in many cephalostatins (17 out of 19), and the majority of ritterazines have a 2,2,9-trimethyl-1,6-dioxaspiro[4.4]nonane system on one or other side of their skeletons (Fig. 1).

The syntheses of several of these natural products and analogues have been achieved[4] and during these studies very interesting methodologies have been brought to light.[5] Nevertheless, despite efforts by several research groups, the mechanism of biological action remains unknown.[6] The structure-activity relationship between cephalostatins and OSW-1 (Fig. 1), a related cholestane glycoside isolated from a terrestrial plant (*Ornithogalum saundersiae*),[7] supports the hypothesis that the bioactive intermediate might be an oxocarbenium ion located at rings E or F and originated by opening the dioxaspiro grouping.[6,7b,8] We can deduce from this that the stereochemistries at C-22, C-23, and C-25, which doubtless have a strong influence on the conformation and stability of the dioxaspiro[4.4]nonane system, may also influence the activity of cephalostatins.

With these ideas in mind, we decided to develop a simple methodology to permit the synthesis of all eight possible isomers of this system by modification of the steroidal side chain of a commercially available spirostan sapogenin,[9] the key step being the formation of the spiroketal system by an intramolecular hydrogen abstraction reaction (IHA) promoted by alkoxyl radicals.[10] In previous papers from this laboratory we have demonstrated the utility of IHA reactions in the synthesis of dioxaspiro[4.4]nonane ring systems in the carbohydrate field.[11] From this previous

Keywords: Cephalostatin; Radical reaction; Hydrogen abstraction; Alkoxyl radical; Steroid; Spirostan sapogenin.
* Corresponding author. Tel.: +34 922 251004; fax: +34 922 260135; e-mail: esuarez@ipna.csic.es

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.077

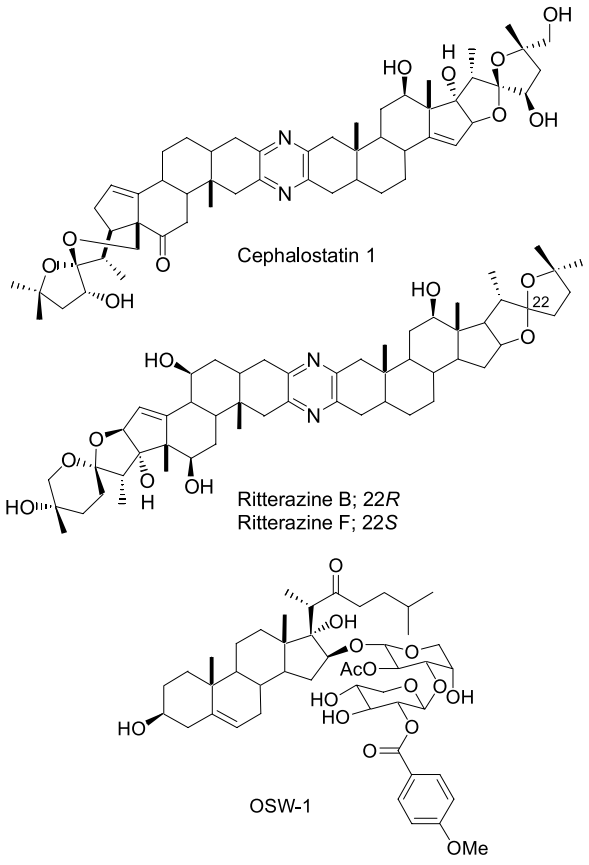
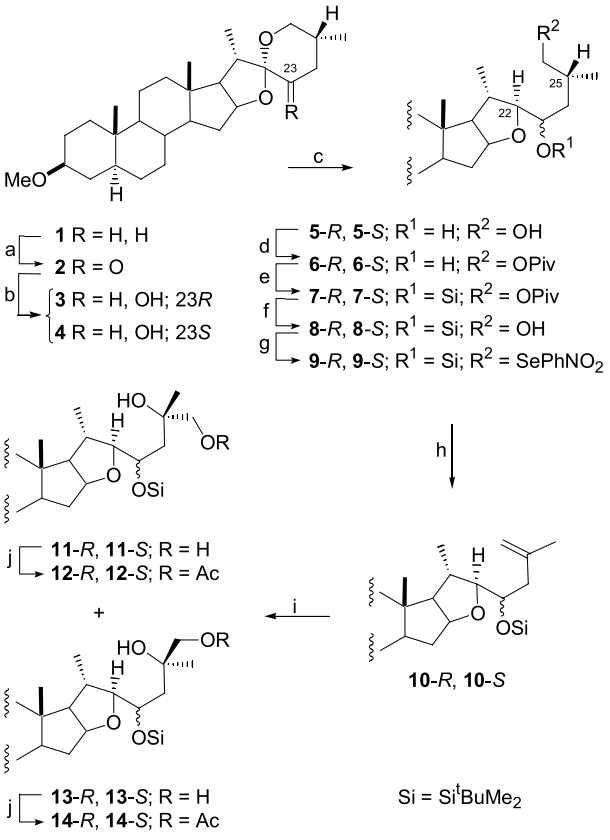


Figure 1. Examples of representative cephalostatins and reitterazines.



Scheme 1. Reagents and conditions: (a) $NaNO_2$, $BF_3{\cdot}Et_2O$, AcOH, rt, 1 h, 68%; (b) $NaBH_4$, EtOH, rt, 1 h, 91% (**3**/**4** ratio 5:95) or L-selectride, THF, −20 °C, 1.5 h, 72% (**3**/**4** ratio 63:37); (c) Ph_2SiH_2, $TiCl_4$, CH_2Cl_2, −20 °C; (d) pivaloyl chloride, Py, CH_2Cl_2, rt, **6**-*R* 95%, **6**-*S* 97%; (e) ^{t}BuMe$_2$SiOTf, CH_2Cl_2, Et_3N, rt, **7**-*R* 81%, **7**-*S* 98%; (f) KOH, MeOH, rt, **8**-*R* 92%, **8**-*S* 91%; (g) *o*-NO_2PhSeCN, *n*-Bu_3P, THF, rt, **9**-*R* 99%, **9**-*S* 97%; (h) H_2O_2, THF, rt, **10**-*R* 92%, **10**-*S* 82%; (i) OsO_4, Py, CH_2Cl_2, rt; (j) Ac_2O, Py, rt. [For yields of the (i) and (j) reactions, see supplementary data section]. The (*R*,*S*) designs the stereochemistry at C-23.

experience we were confident that both spiroketal isomers could be obtained using this methodology. This is synthetically important because in most of the ritterazines both stereoisomers at the spiroketal center were obtained from the natural source.[12]

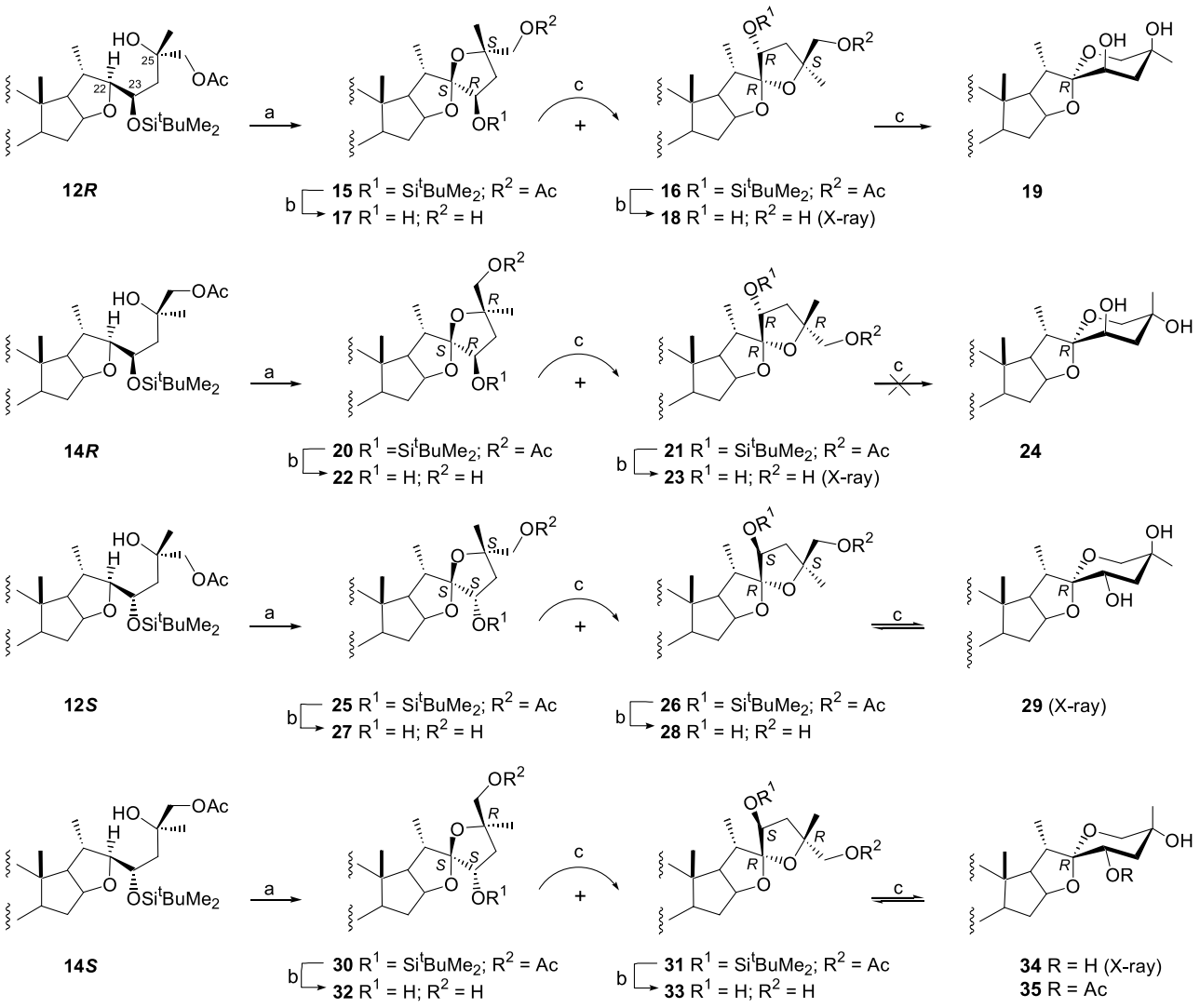
2. Results and discussion

The synthesis began with 3-methoxy-23-oxotigogenin (**2**) (Scheme 1) prepared by using a previously described procedure via oxidation of 3-methoxy-tigogenin (**1**) with $NaNO_2/BF_3{\cdot}Et_2O$.[13] The reduction of **2** with L-selectride furnished a mixture of alcohols **3** and **4** (72%, 1.7:1) from which the alcohol **3** with the correct natural orientation (23*R*) could be obtained in moderate yield. The reduction of **2** with $NaBH_4$ afforded preferentially the alcohol **4** (23*S*) with the non-natural stereochemistry (91%, 19:1).

The two C-23 diastereoisomers **3** and **4** were taken through the following steps of the synthesis separately (Scheme 1). The tigogenin dioxaespiro[4.5]decane system present in **3** was regio- and stereoselectively reduced with Ph_2SiH_2/$TiCl_4$ to give the diol **5**-*R*.[14] Conversion of **5**-*R* to the monoprotected secondary alcohol **8**-*R* was accomplished by a three-step protection-deprotection sequence involving formation of the primary pivalate **6**-*R*, silylation of the 23-alcohol with TBDMSOTf, and hydrolysis of pivalate **7**-*R* with KOH in methanol. Nitrophenylselenenylation of the primary alcohol in **8**-*R* followed by oxidative elimination furnished alkene **10**-*R*.[15] In a series of reactions identical to those described (Scheme 1), the 23*S* isomer **4** was converted into **10**-*S* via **5**-*S*, **6**-*S*, **7**-*S*, **8**-*S*, and **9**-*S*. Stoichiomeric osmylation of the **10**-*R* olefin afforded an inseparable mixture of diols **11**-*R* and **13**-*R* which could be separated after acetylation of the primary alcohol **12**-*R* and **14**-*R* in a 1:2 ratio (99%). In contrast, the osmylation of the **10**-*S* isomer afforded a separable mixture of diols **11**-*S* and **13**-*S* in a 2:1 ratio (98%), which were subsequently and separately acetylated to give **12**-*S* and **14**-*S*.

Initials attempts to asymmetrically dihydroxylate the 25-olefin were unsuccessful.[16] Using the Corey (1*S*,2*S*)-N^1,N^2-bis(mesitylmethyl)-1,2-diphenyl-1,2-ethanediamine reagent,[17] the **10**-*R* olefin gave the diols with similar yield and diastereomeric ratio (**11**-*R*/**13**-*R*, 1:2, 97%) compared with the uncatalyzed reaction. As both isomeric diols were required for this study the uncatalyzed osmylation reaction was preferred.

The IHA reaction was carried out by separately treating compounds **12**-*R*, **12**-*S*, **14**-*R*, and **14**-*S* with (diacetoxyiodo) benzene and iodine under irradiation with two 80 W tungsten-filament lamps at 50 °C (Scheme 2). The alcohols that possess the natural stereochemistry at C-23 (*R*) **12**-*R* and **14**-*R* gave 1,6-dioxaspiro[4.4]nonane compounds **15**



Scheme 2. Reagents and conditions: (a) $PhI(OAc)_2$, I_2, cyclohexane, *hν*, 70 °C, **15** 23%, **16** 60%; **20** 28%, **21** 55%; **25** 23%, **26** 74%; **30** 28%, **31** 47%; (b) (i) TBAF, THF, rt, (ii) KOH, MeOH, rt (yields too steps: **17** 62%, **18** 73%, **22** 74%, **23** 70%, **27** 67%, **28** 87%, **32** 99%, **33** 91%); (c) H^+, CH_2Cl_2, rt.

and **16** (83%, 28:72) and **20** and **21** (83%, 33:67), respectively. On the other hand, the alcohols with the inverted stereochemistry at C-23 (*S*) **12**-*S* and **14**-*S* afforded after the IHA reaction dioxaspiro compounds **25**, and **26** (97%, 24:76) and **30** and **31** (75%, 37:63), respectively. The protecting groups of the alcohols at C-23 and C-25 were selected to favor the 1,5-hydrogen atom transfer reaction. A weak electron-withdrawing group (EWG) at C-23 (silyl ether) should favor the hydrogen abstraction and the subsequent oxidation of the C-22 radical to the oxocarbenium ion intermediate.[18] Also the stronger EWG at C-26 (acetyl ester) should prevent the competitive β-fragmentation of the alkoxyl radical. The choice of the protecting group could be critical and a hypothetical model where the two protecting groups have been interchanged (acetyl at C-23 and silyl at C-26) should give significant amounts of the methyl ketone from β-fragmentation.[19] The desired diols **17**, **18**, **22**, **23**, **27**, **28**, **32**, and **33** were obtained by hydrolysis of the silyl and acetyl protective groups. The structures of these eight stereoisomers of the cephalostatine north 1 side chain were determined by extensive ^1H and ^{13}C NMR 1D and 2D studies including DEPT, COSY, HMBC, and HSQC experiments. Using 2D NOESY and DNOE, the relative stereochemistry of the newly created stereogenic centers (C-22 and C-25) with respect to the known stereochemistry of the alcohol at C-23 may be assigned in each case.[20] As the flexibility of the 1,6-dioxaspiro system (vide infra) may introduce some uncertainty in the NOE results, the structure and stereochemistry were subsequently confirmed by X-ray crystallographic analysis of compounds **18** and **23**.[21] The (22*S*,23*R*,25*S*)-diol **17** possesses the stereochemistry of the natural cephalostatins. Compounds **17**, **22**, **27**, and **32** appear to be the products of kinetic control whereas **18**, **23**, **28**, and **33** are the thermodynamic products. The relative stability of these compounds was determined by following the evolution of the acid-catalyzed rearrangement through a C-22 oxocarbenium ion. Due to the presence of the 25,26-glycol, dioxaspiro compounds of the 1,6-dioxaspiro[4.5]decane type (e.g. **19**) may also be formed.[22]

In a preliminary experiment, diol **17** was transformed into the 22*R*-isomer **18** and both **17** and **18** finally led to the dioxaspiro[4.5]decane **19** under prolonged acid treatment

(Scheme 2). Subsequently, it was established that, in the 1,6-dioxaspiro[4.4]nonane system the 22*S* isomers **17**, **22**, **27**, and **32** are easily transformed, under mild acid conditions, into the 22*R* isomers **18**, **23**, **28**, and **33**, respectively, confirming that the 22*R* are the most stable compounds.

The transformation from the dioxaspiro [4.4]nonane to the [4.5]decane system deserves further comment.[23] Although compound **19** is obtained in moderate yield by acid-catalyzed isomerization of diol **18**, we observed that even under prolonged reaction times neither **22** nor **23** yielded the corresponding dioxaspiro[4.5]-compound **24** to any appreciable extent. Furthermore, the reactions of **28** and **33** under similar conditions reach an equilibrium (**28/29**, 60:40 and **33/35**, 66:34) after several hours at room temperature.

Aware that iodine is a Lewis acid, we also explored the iodine-catalyzed isomerization of 22*R*-isomers and similar results to those obtained with protic acids were achieved. For example, reaction of diol **27** in cyclohexane with iodine (10 mM) under the same conditions of the IHA reaction afforded after 1 h at 70 °C the equilibrium mixture of **28** and **29** in a ratio of 60:40. Analogously, **32** was isomerized to a mixture of **33** and **34** (60:40) under the same conditions. These findings suggest that a possible iodine-catalyzed partial isomerization between 22*S* and 22*R* isomers may well have occurred during the IHA reaction. Partial isomerization at the spirocenter may indeed be accomplished by treatment of fully protected compound **25** with iodine under conditions emulating the IHA reaction, to give a mixture of **25** and **26** (2:8) after 4 h at 70 °C.

These finding are in agreement with the results of a MM2 study (Table 1), compounds **17**, **24**, **27**, and **32** being the highest energy isomers in the respective series while **19**, **23**, **28**, and **33** are the most stable.[24] The isomeric pairs **28** and **29**, and **33** and **34** have similar energy ($\Delta E = 0.4$–0.6 Kcal/mol) and in consequence, an acid-catalyzed equilibrium (ca. 60:40) is reached after extended periods of time.

Several other interesting features in the structure of these compounds are shown in Table 1. For instance, compounds with the same stereochemistry at C-23 display significantly different coupling constants between the protons H_{23} and H_{24} on changing from the 22*R* to the 22*S* series of compounds (compare the coupling constant of **17** with **18** or **22** with **23** in which the stereochemistry at C-23 is always *R*, or **27** with **28** and **32** with **33** where the stereochemistry is 23*S*). The small couplings (0, 5 Hz) suggest a pseudoaxial orientation of the C-23 alcohol (e.g. **18**) while the larger couplings (8, 10 Hz) are more consistent with a pseudo-equatorially disposed alcohol (e.g. **17**).[25] Nevertheless, a reasonable explanation for this phenomenon is necessarily associated with a change of the conformation of the tetrahydrofuran F-ring on going from the 22*S* to 22*R* series of compounds (Fig. 2). The study of the conformation of this 1,6-dioxaspiro ring system may not be an easy task due to the significant flexibility of the puckered five-membered rings, although, in this case, some conformational constraint, exerted by the substituents and the fused D-ring, may be expected.[26] In this approach we have determined the conformations of the E- and F-rings of the eight different

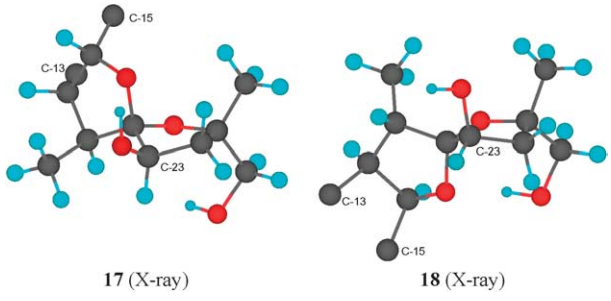


Figure 2. Ring F conformations of **17** and **18**, taken from the X-ray crystal structures. For the sake of clarity only E and F rings are shown.

Table 1. Structural characteristics of dioxaspiro compounds

Compound	ΔE[a] (kcal/mol)	P[b] (conformation)[c]		$^3J_{23,24}$[d] (Hz)
		E-ring	F-ring	
17	6.8	134 (E_{16})	146 ($^{23}T_{22}$)	8.1, 8.4
17 (X-ray)[e]	—	127 (E_{16})	145 ($^{23}T_{22}$)	—
18	3.4	102 ($^{o}T_{16}$)	339 (E_{23})	0.0, 5.7
18 (X-ray)	—	93 (^{o}E)	332 ($^{22}T_{23}$)	—
19	0.0	96 (^{o}E)	— ($^{22}C_{25}$)	2.8, 2.8
22	2.9	141 ($^{17}T_{16}$)	155 (^{23}E)	8.1, 9.8
23	0.0	85 (^{o}E)	320 ($^{22}T_{23}$)	0.0, 5.6
23 (X-ray)	—	90 (^{o}E)	324 ($^{22}T_{23}$)	—
24	4.3	99 (^{o}E)	— ($^{22}C_{25}$)	—
27	4.4	148 ($^{17}T_{16}$)	144 ($^{23}T_{22}$)	0.0, 4.9
28	0.0	89 (^{o}E)	346 (E_{23})	8.3, 10.5
29	0.4	89 (^{o}E)	— ($^{22}C_{25}$)	5.3, 11.6
29 (X-ray)	—	80 ($^{o}T_{22}$)	— ($^{22}C_{25}$)	—
32	4.6	145 ($^{17}T_{16}$)	153 ($^{23}T_{22}$)	0.0, 4.6
33	0.0	85 (^{o}E)	320 ($^{22}T_{23}$)	8.5, 9.8
34	0.6	91 (^{o}E)	— ($^{22}C_{25}$)	5.3, 11.7
34 (X-ray)	—	72 ($^{o}T_{22}$)	— ($^{22}C_{25}$)	—

[a] Changes of the relative MM2 energy (in kcal/mol) with respect to the lowest energy isomer in the respective series.
[b] Altone-Sundaralingam phase angle (in degrees) as defined in Ref. 29c.
[c] An adaptation of the IUPAC nomenclature of carbohydrates is used (Ref. 30).
[d] Experimental $^3J_{HH}$ coupling from 500 MHz spectra.
[e] Data were taken from the X-ray analysis of (22*S*,23*R*,25*S*)-3β,12β-diacetoxy-22,25-epoxy-5α-furostan-23,26-diol (Ref. 27).

isomers over minimized structures (MM2) using the X-ray structures **17**,[27] **18**, and **23** (X-ray) as starting geometry.[28] With this study we are not attempting to make a complete conformational analysis of the 1,6-dioxaspiro system, but simply to explain the apparently anomalous coupling constants observed for the proton at C-23.

The structures of lowest energy calculated by this methodology have E- and F-ring conformations that were very similar to those found in the crystallographic structures. A comparison of the conformations from the crystal structure with those established by molecular mechanics calculations is presented in Table 1 [compare **17** with **17** (X-ray), **18** with **18** (X-ray), and **23** with **23** (X-ray)]. The ring conformations have been described by the Altone-Sundaralingam phase angle[29] and the IUPAC conformational nomenclature for the furanose form of monosaccharides has been adapted to these rings.[30] The E-ring of the 22*S*-isomers (**17**, **22**, **27**, and **32**) adopts a preferred conformation E_{16} or $^{17}T_{16}$ ($P=134$–$148°$) in the southern hemisphere of the pseudorotational itinerary of the ring (Table 1).[29] The E-ring conformation in the 22*R* series of isomers (**18**, **23**, **28**, and **33**) is located in the east ^{o}E ($P=102$–$85°$) of the pseudorotational wheel. On the other hand, the F-ring of the 22*S*-isomers adopts a preferred conformation $^{23}T_{22}$ or ^{23}E ($P=144$–$155°$) in the southern hemisphere, while, conformations $^{22}T_{23}$ or E_{23} ($P=320$–$346°$) in the northern hemisphere are found for the F-ring of the 22*R*-isomers.

The experimental $^{3}J_{23,24}$ H-H coupling constants were measured in 500 MHz spectra (Table 1), and are in agreement with those calculated over minimized structures using the HLA equation,[31] the largest individual discrepancy between experimental and calculated constants being 1.4 Hz.

In the 1,6-dioxa[4.5]decane compounds **19**, **29** and **34**, firm evidence in favor of a $^{22}C_{25}$ conformation for the F-ring was obtained by the $^{3}J_{23,24}$ coupling constants. The alternative $^{25}C_{22}$ chair conformation can be ruled out on the basis of the same measurements (Table 1).[32] X-ray diffraction analysis confirmed the $^{22}C_{25}$ conformation in the solid state for **29** and **34**.[21]

3. Conclusion

In summary, we have demonstrated the usefulness of the IHA reaction in the construction of the steroidal 1,6-dioxaspiro[4.4]nonane ring system.[33] Since thermodynamically less stable isomers at the spirocenter can be obtained, this methodology should be especially useful in the synthesis of the natural products when both isomers are isolated from nature, as described for several ritterazines.[12]

The preparation of all eight possible isomers has led to the discovery that the spirocenter stereochemistry can profoundly influence the conformation of the F-ring. Taking such an effect into account, the apparently anomalous coupling constant for the proton at C-23, observed in the NMR spectra of these compounds, can be readily explained. From these findings the question that now arises is whether the conformation of the F-ring might influence the biological activity, as occurs in other types of tetrahydrofuran derivatives.[29c,34] In any case, this should be taken into consideration in the development of new biologically active cephalostatin and ritterazin analogs.

Although we are aware that our conclusions regarding the stability and conformation of the spiroketal side chain in the different series of these simple monomers may not be fully extrapolatable to the bioactive products, we believe that they could help in designing such compounds.

4. Experimental

4.1. General methods

Melting points were determined with a hot-stage apparatus and are uncorrected. Optical rotations were measured at the sodium line at ambient temperature in $CHCl_3$ solutions. IR spectra were recorded in $CHCl_3$ solutions unless otherwise stated. NMR spectra were determined at 500 MHz for ^{1}H and 125.7 MHz for ^{13}C in $CDCl_3$ unless otherwise stated, in the presence of TMS as internal standard. Mass spectra were determined at 70 eV. Merck silica gel 60 PF (0.063–0.2 mm) was used for column chromatography. Circular layers of 1 mm of Merck silica gel 60 PF_{254} were used on a Chromatotron for centrifugally assisted chromatography. Commercially available reagents and solvents were analytical grade or were purified by standard procedures prior to use. All reactions involving air- or moisture-sensitive materials were carried out under a nitrogen atmosphere. The spray reagents for TLC analysis were conducted with 0.5% vanillin in H_2SO_4–EtOH (4:1) and further heating until development of color.

4.1.1. (22*S*,23*R*,25*S*)-3β-Methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (15) and (22*R*,23*R*,25*S*)-3β-methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (16). A solution of the alcohol **12**-*R* (60 mg, 0.096 mmol) in cyclohexane (10 mL) containing (diacetoxyiodo)benzene (40 mg, 0.124 mmol) and iodine (25 mg, 0.098 mmol) was irradiated with two 80 W tungsten-filament lamps at 50 °C for 3.5 h. The reaction mixture was then poured into aqueous solution of sodium thiosulfate (10%) and extracted with Et_2O. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 95:5) of the residue afforded compound compound **15** (14 mg, 0.022 mmol, 23.4%) and **16** (36 mg, 0.058 mmol, 60%). Compound **15**: amorphous; $[\alpha]_D$ −18 (*c* 0.23); IR 1746 cm^{-1}; ^{1}H NMR 0.07 (3H, s), 0.09 (3H, s), 0.64 (1H, m), 0.81 (3H, s), 0.87 (3H, s), 0.90 (9H, s), 1.06 (3H, d, $J=7.2$ Hz), 1.32 (3H, s), 1.91 (1H, dd, $J=11.0$, 11.4 Hz), 2.03 (1H, dd, $J=7.6$, 11.6 Hz), 2.08 (3H, s), 2.30 (1H, dddd, $J=7.0$, 7.0, 7.0, 7.0 Hz), 3.12 (1H, dddd, $J=4.6$, 4.6, 10.9, 10.9 Hz), 3.35 (3H, s), 3.88 (2H, s), 4.30 (1H, dd, $J=7.8$, 10.4 Hz), 4.62 (1H, ddd, $J=7.1$, 7.1, 7.1 Hz); ^{13}C NMR −5.4 (CH_3), −4.0 (CH_3), 12.3 (CH_3), 16.1 (CH_3), 16.6 (CH_3), 17.8 (C), 20.9 (CH_2), 21.1 (CH_3), 25.8 (4×CH_3), 27.9 (CH_2), 28.8 (CH_2), 32.3 (CH_2), 32.4 (CH_2), 34.3 (CH_2), 35.0 (CH), 35.9 (C), 36.9 (CH_2), 37.5 (CH), 40.2 (CH_2), 40.3 (CH_2), 41.1 (C), 44.8 (CH), 54.5 (CH), 55.5 (CH_3), 55.6 (CH), 61.6 (CH),

70.3 (CH_2), 73.2 (CH), 79.0 (C), 79.8 (CH), 81.7 (CH), 117.9 (C), 170.6 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 561 (6), 475 (30), 287 (23); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4255. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.01; H, 10.17. Compound **16**: amorphous; $[\alpha]_D$ −45 (*c* 0.24); IR 1745 cm^{-1}; ^1H NMR 0.07 (3H, s), 0.08 (3H, s), 0.62 (1H, m), 0.76 (3H, s), 0.80 (3H, s), 0.90 (9H, s), 1.06 (3H, d, *J*=6.9 Hz), 1.33 (3H, s), 1.59 (1H, dd, *J*=0.0, 13.3 Hz), 1.93 (1H, ddd, *J*=5.7, 7.5, 12.4 Hz), 2.05 (3H, s), 2.22 (1H, dd, *J*=5.4, 13.3 Hz), 2.32 (1H, dddd, *J*=6.1, 6.1, 6.1, 6.1 Hz), 3.11 (1H, dddd, *J*=4.5, 4.5, 10.8, 10.8 Hz), 3.33 (3H, s), 3.88 (1H, d, *J*=10.9 Hz), 4.10 (1H, d, *J*=10.9 Hz), 4.14 (1H, d, *J*=4.6 Hz), 4.44 (1H, ddd, *J*=5.6, 7.8, 7.8 Hz); ^{13}C NMR −5.1 (CH_3), −5.0 (CH_3), 12.3 (CH_3), 16.3 (CH_3), 16.8 (CH_3), 17.9 (C), 20.9 (CH_2), 21.0 (CH_3), 25.0 (CH_3), 25.7 (3×CH_3), 27.9 (CH_2), 28.7 (CH_2), 32.0 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 35.3 (CH), 35.9 (C), 36.2 (CH), 36.9 (CH_2), 39.8 (CH_2), 41.0 (C), 42.3 (CH_2), 44.7 (CH), 54.4 (CH), 55.5 (CH_3), 56.3 (CH), 63.1 (CH), 70.8 (CH_2), 78.5 (CH), 79.8 (CH), 81.3 (CH), 82.0 (C), 120.9 (C), 171.0 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 561 (2), 545 (7), 475 (32), 287 (43); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4238. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.93; H, 10.22.

4.1.2. (22*S*,23*R*,25*S*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (17). To a solution of compound **15** (13 mg, 0.021 mmol) in THF (3 mL) was added TBAF (0.1 mL, 0.1 mmol, 1.0 M in THF) and stirred at room temperature for 2 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with AcOEt. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (benzene–EtOAc, 90:10) of the residue afforded (22*S*,23*R*,25*S*)-3β-methoxy-26-acetoxy-22,25-epoxy-5α-furostan-23-ol (8.6 mg, 0.017 mmol, 81%): mp 151–154 °C (from EtOAc); IR 3447, 1744 cm^{-1}; ^1H NMR 0.64 (1H, m), 0.81 (3H, s), 0.91 (3H, s), 1.13 (3H, d, *J*=7.5 Hz), 1.29 (3H, s), 2.01 (1H, ddd, *J*=5.7, 7.2, 12.3 Hz), 2.07 (3H, s), 2.27 (1H, dd, *J*=7.8, 12.6 Hz), 2.34 (1H, dddd, *J*=3.5, 7.4, 7.4, 7.4 Hz), 3.11 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.33 (3H, s), 3.86 (2H, s), 4.25 (1H, ddd, *J*=8.8, 8.8, 8.8 Hz), 4.54 (1H, ddd, *J*=7.0, 7.0, 7.0 Hz); ^{13}C NMR 12.3 (CH_3), 16.1 (CH_3), 17.5 (CH_3), 20.9 (CH_3), 20.9 (CH_2), 25.8 (CH_3), 27.9 (CH_2), 28.8 (CH_2), 32.4 (CH_2), 34.0 (CH_2), 34.3 (CH_2), 34.8 (CH), 35.9 (C), 36.9 (CH_2), 39.1 (CH), 40.1 (CH_2), 41.5 (C), 41.9 (CH_2), 44.8 (CH), 54.5 (CH), 55.48 (CH), 55.52 (CH_3), 63.3 (CH), 70.2 (CH_2), 73.0 (CH), 78.9 (C), 79.8 (CH), 83.7 (CH), 118.5 (C), 170.7 (C); MS *m/z* (rel intensity) 486 ($M^+ - H_2O$, 3), 471 (<1), 426 (4), 413 (4), 361 (39), 287 (100); HRMS calcd for $C_{30}H_{46}O_5$ 486.3345; found 486.3363. Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.51; H, 9.71. A solution of this acetate (8 mg, 0.0158 mmol) in MeOH (5 mL) containing KOH (0.15 g) was stirred at room temperature for 4 h. The mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 7:3) of the residue afforded compound **17** (5.6 mg, 0.012 mmol, 76%): mp 187.5–190 °C (from EtOAc-*n*-hexane); $[\alpha]_D$ +11 (*c* 0.19); IR 3417 cm^{-1}; ^1H NMR 0.64 (1H, m), 0.81 (3H, s), 0.92 (3H, s), 1.13 (3H, d, *J*=7.4 Hz), 1.27 (3H, s), 1.69 (1H, dd, *J*=8.4, 12.6 Hz), 2.02 (1H, ddd, *J*=5.6, 7.3, 12.4 Hz), 2.20 (1H, br d, *J*=10.0 Hz), 2.29 (1H, dd, *J*=8.1, 12.6 Hz), 2.34 (1H, dddd, *J*=4.0, 7.5, 7.5, 7.5 Hz), 3.12 (1H, dddd, *J*=4.7, 4.7, 11.0, 11.0 Hz), 3.29 (1H, d, *J*=11.3 Hz), 3.34 (3H, s), 3.38 (1H, d, *J*=11.3 Hz), 4.22 (1H, ddd, *J*=8.4, 8.4, 8.4 Hz), 4.56 (1H, ddd, *J*=7.0, 7.0, 8.7 Hz); ^{13}C NMR 12.3 (CH_3), 16.2 (CH_3), 17.5 (CH_3), 20.9 (CH_2), 25.3 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 32.3 (CH_2), 33.9 (CH_2), 34.3 (CH_2), 34.8 (CH), 35.9 (C), 36.9 (CH_2), 39.4 (CH), 40.1 (CH_2), 41.5 (C), 41.7 (CH_2), 44.8 (CH), 54.5 (CH), 55.4 (CH_3), 55.5 (CH), 63.4 (CH), 69.7 (CH_2), 73.3 (CH), 79.8 (CH), 81.3 (C), 83.7 (CH), 118.6 (C); MS *m/z* (rel intensity) 461 (M^+ −H, <1), 431 (4), 287 (100); HRMS calcd for $C_{28}H_{45}O_5$ 461.3267; found 461.3225. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.81; H, 10.19.

4.1.3. (22*R*,23*R*,25*S*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (18). To a solution of compound **16** (35 mg, 0.056 mmol) in THF (5 mL) was added TBAF (0.3 mL, 0.3 mmol, 1.0 M in THF) and stirred at room temperature for 3 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with Et_2O. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 80:20) of the residue afforded (22*R*,23*R*,25*S*)-3β-methoxy-26-acetoxy-22,25-epoxy-5α-furostan-23-ol (23 mg, 0.045 mmol, 81%): mp 208.5–209 °C (from EtOAc-*n*-hexane); $[\alpha]_D$ −57 (*c* 1.03); IR 3516, 1723 cm^{-1}; ^1H NMR 0.62 (1H, m), 0.77 (3H, s), 0.80 (3H, s), 1.09 (3H, d, *J*=6.9 Hz), 1.34 (3H, s), 1.93 (1H, ddd, *J*=5.8, 7.4, 12.6 Hz), 2.07 (3H, s), 2.28 (1H, dd, *J*=5.6, 13.7 Hz), 2.39 (1H, dddd, *J*=6.4, 6.4, 6.4, 6.4 Hz), 3.11 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.33 (3H, s), 3.93 (1H, d, *J*=10.9 Hz), 4.09 (1H, d, *J*=10.9 Hz), 4.18 (1H, br d, *J*=4.9 Hz), 4.44 (1H, ddd, *J*=5.7, 7.8, 7.8 Hz); ^{13}C NMR 12.3 (CH_3), 16.1 (CH_3), 16.8 (CH_3), 20.9 (CH_3), 21.0 (CH_2), 25.5 (CH_3), 27.9 (CH_2), 28.7 (CH_2), 31.9 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 35.2 (CH), 35.9 (C), 36.1 (CH), 36.9 (CH_2), 39.7 (CH_2), 41.0 (C), 42.0 (CH_2), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.3 (CH), 63.3 (CH), 70.9 (CH_2), 78.1 (CH), 79.8 (CH), 81.2 (CH), 81.6 (C), 120.2 (C), 171.0 (C); MS *m/z* (rel intensity) 504 (M^+ <1), 486 (11), 431 (6), 287 (100); HRMS calcd for $C_{30}H_{48}O_6$ 504.3451; found 504.3455. Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.06; H, 9.86. A solution of this acetate (20 mg, 0.0397 mmol) in MeOH (10 mL) containing KOH (0.35 g) was stirred at room temperature for 4 h. The mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 7:3) of the residue afforded compound **18** (16.5 mg, 0.036 mmol, 90%): mp 211.5–213.5 °C (from EtOAc-*n*-hexane); $[\alpha]_D$ −55 (*c* 0.108); IR 3426, 1453 cm^{-1}; ^1H NMR 0.63 (1H, m), 0.78 (3H, s), 0.81 (3H, s), 1.10 (3H, d, *J*=7.2 Hz), 1.30 (3H, s), 1.58 (1H, dd, *J*=0.0, 13.8 Hz), 1.96 (1H, m), 2.46 (1H, dddd, *J*=7.0, 7.0, 7.0, 7.0 Hz), 2.56 (1H, dd, *J*=5.7, 13.8 Hz), 3.11 (1H, dddd, *J*=4.5, 4.5, 10.7, 10.7 Hz), 3.31 (1H, d, *J*=9.0 Hz), 3.33 (3H, s), 3.49 (1H, d, *J*=9.0 Hz), 4.21 (1H, d, *J*=5.7 Hz), 4.53 (1H, ddd, *J*=5.6, 7.5, 7.5 Hz); ^{13}C NMR 12.3 (CH_3), 16.0 (CH_3), 17.0 (CH_3), 20.9 (CH_2), 25.2 (CH_3), 27.9 (CH_2), 28.7 (CH_2), 31.9 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 35.2 (CH), 35.5 (CH), 35.9 (C), 36.9 (CH_2), 39.5 (2×CH_2), 41.1 (C), 44.7 (CH), 54.4 (CH),

55.5 (CH_3), 56.2 (CH), 63.5 (CH), 68.5 (CH_2), 78.9 (CH), 79.8 (CH), 81.8 (CH), 85.3 (C), 120.3 (C); MS *m/z* (rel intensity) 462 (M^+, <1), 444 (1), 431 (28), 287 (100); HRMS calcd for $C_{28}H_{46}O_5$ 462.3345; found 462.3338. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.83; H, 9.78.

4.1.4. (22*S*,23*R*,25*S*)-3β-Methoxy-5α-spirostan-23,25-diol (19). A solution of compound **18** (10 mg, 0.02 mmol) in $CHCl_3$ (10 mL) was treated with an undetermined catalytic amount of HCl (some gas taken with a Pasteur pipet from of a concd HCl bottle) and stirred at room temperature for 24 h. The reaction mixture was poured into aqueous saturated $NaHCO_3$ and extracted with $CHCl_3$. Chromatotron chromatography (hexanes–EtOAc, 85:15) of the residue afforded compound **19** (5 mg, 0.01 mmol, 50%): mp 250.5–252.5 °C (from EtOAc-*n*-hexane); $[\alpha]_D$ −80 (*c* 0.45); IR 3601, 3492 cm^{-1}; ^1H NMR 0.63 (1H, m), 0.77 (3H, s), 0.80 (3H, s), 1.12 (3H, s), 1.15 (3H, d, *J*=6.9 Hz), 1.82 (1H, ddd, *J*=2.8, 2.8, 14.3 Hz), 1.96 (1H, dd, *J*=3.2, 14.3 Hz), 2.30 (1H, dddd, *J*=6.9, 6.9, 6.9, 6.9 Hz), 3.11 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.33 (3H, s), 3.39 (1H, dd, *J*=2.8, 11.8 Hz), 3.63 (1H, dd, *J*=2.8, 2.8 Hz), 3.77 (1H, d, *J*=11.8 Hz), 4.47 (1H, ddd, *J*=5.7, 7.8, 7.8 Hz); ^{13}C NMR 12.3 (CH_3), 16.2 (CH_3), 16.7 (CH_3), 20.9 (CH_2), 26.2 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 32.0 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 35.2 (CH), 35.9 (C), 36.9 (CH_2), 38.3 (CH_2), 39.6 (CH_2), 40.4 (CH), 41.0 (C), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.4 (CH), 64.1 (CH), 68.8 (C), 69.2 (CH_2), 70.9 (CH), 79.8 (CH), 81.8 (CH), 108.7 (C); MS *m/z* (rel intensity) 462 (M^+, 2), 444 (2), 426 (2), 411 (2), 361 (46), 287 (100); HRMS calcd for $C_{28}H_{46}O_5$ 462.3345; found 462.3401. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.71; H, 10.13.

4.1.5. (22*S*,23*R*,25*R*)-3β-Methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (20) and (22*R*,23*R*,25*R*)-3β-methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (21). A solution of the alcohol **14**-*R* (137 mg, 0.22 mmol) in cyclohexane (25 mL) containing (diacetoxyiodo)benzene (84 mg, 0.264 mmol) and iodine (56 mg, 0.22 mmol) was irradiated with two 80 W tungsten-filament lamps at 55 °C for 7 h. The reaction mixture was then poured into aqueous solution of sodium thiosulfate (10%) and extracted with Et_2O. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 95:5) of the residue afforded compound **20** (38 mg, 0.061 mmol, 27.8%) and compound **21** (76 mg, 0.122 mmol, 55.3%). Compound **20**: amorphous; $[\alpha]_D$ −27 (*c* 0.064); IR 1746 cm^{-1}; ^1H NMR 0.07 (3H, s), 0.10 (3H, s), 0.63 (1H, m), 0.80 (3H, s), 0.85 (3H, s), 0.90 (9H, s), 1.06 (3H, d, *J*=7.3 Hz), 1.20 (3H, s), 1.85 (1H, dd, *J*=7.5, 11.1 Hz), 1.90 (1H, ddd, *J*=6.0, 6.8, 12.3 Hz), 1.98 (1H, dd, *J*=11.2, 10.4 Hz), 2.05 (3H, s), 2.31 (1H, dddd, *J*=6.9, 6.9, 6.9, 6.9 Hz), 3.10 (1H, dddd, *J*=4.6, 4.6, 10.7, 10.7 Hz), 3.35 (3H, s), 4.01 (1H, d, *J*=10.9 Hz), 4.10 (1H, d, *J*=10.9 Hz), 4.22 (1H, dd, *J*=7.5, 10.4 Hz), 4.59 (1H, ddd, *J*=6.8, 6.8, 6.8 Hz); ^{13}C NMR −5.3 (CH_3), −4.0 (CH_3), 12.3 (CH_3), 16.0 (CH_3), 16.5 (CH_3), 17.8 (C), 21.0 (CH_2), 21.0 (CH_3), 25.0 (CH_3), 25.8 (3×CH_3), 27.9 (CH_2), 28.8 (CH_2), 32.3 (CH_2), 33.4 (CH_2), 34.3 (CH_2), 35.0 (CH), 35.9 (C), 36.9 (CH_2), 37.4 (CH), 40.1 (CH_2), 40.2 (CH_2), 41.2 (C), 44.8 (CH), 54.5 (CH), 55.5 (CH_3), 55.6 (CH), 61.9 (CH), 71.0 (CH_2), 72.7 (CH), 78.5 (C), 79.8 (CH), 81.8 (CH), 118.0 (C), 170.9 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 617 (<1), 561 (6), 475 (31), 287 (20); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4335. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.98; H, 10.22. Compound **21**: amorphous; $[\alpha]_D$ −58.2 (*c* 0.5); IR 1745 cm^{-1}; ^1H NMR 0.06 (3H, s), 0.07 (3H, s), 0.63 (1H, m), 0.76 (3H, s), 0.80 (3H, s), 0.89 (9H, s), 1.02 (3H, d, *J*=7.0 Hz), 1.29 (3H, s), 1.84 (1H, dd, *J*=0.0, 13.5 Hz), 1.96 (1H, ddd, *J*=5.7, 7.3, 12.7 Hz), 2.04 (1H, dd, *J*=5.1, 13.5 Hz), 2.06 (3H, s), 2.33 (1H, dddd, *J*=6.9, 6.9, 6.9, 6.9 Hz), 3.11 (1H, dddd, *J*=4.5, 4.5, 10.9, 10.9 Hz), 3.33 (3H, s), 4.04 (1H, d, *J*=10.6 Hz), 4.08 (1H, d, *J*=10.6 Hz), 4.17 (1H, d, *J*=4.4 Hz), 4.52 (1H, ddd, *J*=5.7, 7.9, 7.9 Hz); ^{13}C NMR −5.1 (CH_3), −5.0 (CH_3), 12.3 (CH_3), 16.4 (CH_3), 16.8 (CH_3), 17.9 (C), 20.91 (CH_2), 20.96 (CH_3), 25.7 (3×CH_3), 26.1 (CH_3), 27.9 (CH_2), 28.7 (CH_2), 32.1 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 35.3 (CH), 35.9 (C), 36.4 (CH), 36.9 (CH_2), 39.8 (CH_2), 41.1 (C), 42.7 (CH_2), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.3 (CH), 63.2 (CH), 70.4 (CH_2), 78.7 (CH), 79.8 (CH), 81.4 (CH), 81.8 (C), 122.0 (C), 170.9 (C); MS *m/z* (rel intensity) 618 (M^+, 1), 603 (<1), 561 (43), 545 (23), 287 (68); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4324. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.71; H, 9.98.

4.1.6. (22*S*,23*R*,25*R*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (22). To a solution of compound **20** (38 mg, 0.061 mmol) in THF (8 mL) was added TBAF (0.4 mL, 0.4 mmol, 1.0 M in THF) and stirred at room temperature for 1.5 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with Et_2O. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (benzene–EtOAc, 90:10) of the residue afforded (22*S*,23*R*,25*R*)-3β-methoxy-26-acetoxy-22,25-epoxy-5α-furostan-23-ol (28 mg, 0.055 mmol, 90%): amorphous; $[\alpha]_D$ +10 (*c* 0.39); IR 3443, 1745 cm^{-1}; ^1H NMR 0.62 (1H, m), 0.80 (3H, s), 0.89 (3H, s), 1.12 (3H, d, *J*=7.5 Hz), 1.19 (3H, s), 1.82 (1H, dd, *J*=9.1, 12.4 Hz), 1.87 (1H, ddd, *J*=5.7, 7.3, 12.2 Hz), 2.06 (3H, s), 2.36 (1H, dddd, *J*=3.5, 7.4, 7.4, 7.4 Hz), 3.11 (1H, dddd, *J*=4.5, 4.5, 10.6, 10.6, Hz), 3.33 (3H, s), 3.96 (1H, d, *J*=10.8 Hz), 4.06 (1H, d, *J*=10.8 Hz), 4.25 (1H, ddd, *J*=8.8, 8.8, 8.8 Hz), 4.54 (1H, ddd, *J*=7.0, 7.0, 7.0 Hz); ^{13}C NMR 12.2 (CH_3), 15.9 (CH_3), 17.3 (CH_3), 20.9 (CH_3), 20.9 (CH_2), 25.2 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 32.3 (CH_2), 32.8 (CH_2), 34.2 (CH_2), 34.7 (CH), 35.9 (C), 36.8 (CH_2), 39.1 (CH), 40.0 (CH_2), 41.4 (C), 41.7 (CH_2), 44.7 (CH), 54.5 (CH), 55.5 (CH), 55.5 (CH_3), 63.3 (CH), 70.4 (CH_2), 72.4 (CH), 78.5 (C), 79.7 (CH), 83.7 (CH), 128.3 (C), 170.8 (C); MS *m/z* (rel intensity) 505 (M^+ + H, < 1), 486 (< 1), 471 (<1), 413 <1), 361 (83), 287 (100); HRMS calcd for $C_{30}H_{46}O_5$ 486.3345; found 486.3335. Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.52; H, 9.71. A solution of this acetate (24 mg, 0.047 mmol) in MeOH (15 mL) containing KOH (0.3 g) was stirred at room temperature for 8 h. The mixture was poured into water and extracted with AcOEt. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 7:3) of the residue afforded compound **22** (18 mg, 0.039 mmol, 82%): mp 166.5–168 °C (from EtOAc-*n*-hexane); $[\alpha]_D$ −4

(*c* 0.55); IR 3408 cm^{-1}; ^1H NMR 0.62 (1H, m), 0.80 (3H, s), 0.89 (3H, s), 1.13 (3H, s), 1.16 (3H, d, $J=7.5$ Hz), 1.96 (1H, dd, $J=8.1$, 12.3 Hz), 2.04 (1H, ddd, $J=7.3$, 7.3, 7.3 Hz), 2.15 (1H, dd, $J=9.8$, 12.4 Hz), 2.39 (1H, dddd, $J=3.3$, 7.3, 7.3, 7.3 Hz), 3.11 (1H, dddd, $J=4.5$, 4.5, 10.7, 10.7 Hz), 3.25 (1H, d, $J=11.4$ Hz), 3.33 (3H, s), 3.45 (1H, d, $J=11.5$ Hz), 4.26 (1H, dd, $J=9.0$, 9.0 Hz), 4.58 (1H, ddd, $J=7.3$, 7.3, 7.3 Hz); ^{13}C NMR 12.2 (CH_3), 16.1 (CH_3), 17.2 (CH_3), 20.8 (CH_2), 24.9 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 32.2 (CH_2), 34.0 (CH_2), 34.2 (CH_2), 34.8 (CH), 35.9 (C), 36.9 (CH_2), 38.5 (CH), 38.5 (CH_2), 39.7 (CH_2), 41.5 (C), 44.7 (CH), 54.4 (CH), 55.4 (CH_3), 55.6 (CH), 63.8 (CH), 68.1 (CH_2), 73.4 (CH), 79.7 (CH), 81.7 (C), 83.8 (CH), 118.2 (C); MS *m/z* (rel intensity) 444 (M^+-H_2O, 2), 431 (6), 426 (9), 411 (4), 287 (100); HRMS calcd for $C_{28}H_{44}O_4$ 444.3240; found 444.3221. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.71; H, 10.14.

4.1.7. (22*R*,23*R*,25*R*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (23). To a solution of compound **21** (23 mg, 0.037 mmol) in THF (5 mL) was added TBAF (0.2 mL, 0.2 mmol, 1.0 M in THF) and stirred at room temperature for 3 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with Et_2O. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. The crude residue was saponified with methanolic KOH (10 mL, 3%) for 4 h at room temperature. Chromatotron chromatography (benzene–EtOAc, 75:25) of the residue afforded compound **23** (12 mg, 0.026 mmol, 70%): mp 269.5–270 °C (from MeOH); $[\alpha]_D$ −72 (*c* 0.122); IR 3317 cm^{-1}; ^1H NMR 0.63 (1H, m), 0.79 (3H, s), 0.80 (3H, s), 1.11 (3H, d, $J=6.7$ Hz), 1.27 (3H, s), 1.93 (1H, dd, $J=0.0$, 14.0 Hz), 1.96 (1H, ddd, $J=5.8$, 7.4, 12.4 Hz), 2.28 (1H, dd, $J=5.5$, 14.0 Hz), 2.53 (1H, dddd, $J=7.0$, 7.0, 7.0, 7.0 Hz), 3.11 (1H, dddd, $J=4.5$, 4.5, 10.7, 10.7 Hz), 3.33 (3H, s), 3.39 (1H, d, $J=11.2$ Hz), 3.65 (1H, d, $J=11.2$ Hz), 4.02 (1H, d, $J=5.5$ Hz), 4.51 (1H, ddd, $J=5.7$, 7.9, 7.9 Hz); ^{13}C NMR 12.3 (CH_3), 16.1 (CH_3), 17.1 (CH_3), 20.9 (CH_2), 26.2 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 32.0 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 35.1 (CH), 35.9 (C), 36.3 (CH), 36.9 (CH_2), 39.8 (CH_2), 41.1 (C), 42.4 (CH_2), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.3 (CH), 63.6 (CH), 68.9 (CH_2), 77.0 (CH), 79.8 (CH), 81.0 (CH), 83.2 (C), 120.6 (C); MS *m/z* (rel intensity) 444 (M^+-H_2O, <1), 413 (2), 287 (45); HRMS calcd for $C_{28}H_{44}O_4$ 444.3240; found 444.3268. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.69; H, 9.90.

4.1.8. (22*S*,23*S*,25*S*)-3β-Methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (25) and (22*R*,23*S*,25*S*)-3β-methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (26). A solution of the alcohol **12**-*S* (420 mg, 0.68 mmol) in cyclohexane (70 mL) containing (diacetoxyiodo)benzene (437 mg, 1.36 mmol) and iodine (172 mg, 0.68 mmol) was irradiated with a 80 W tungsten-filament lamp at 70 °C for 1 h. The reaction mixture was then poured into aqueous solution of sodium thiosulfate (10%) and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Flash-chromatography (hexanes–EtOAc, 93:7) of the residue afforded compound **25** (96 mg, 0.15 mmol, 23%) and compound **26** (310 mg, 0.50 mmol, 74%). Compound **25**: mp 62.4–63.7 °C (from EtOAc); $[\alpha]_D$ +21.2 (*c* 0.08); IR 1745 cm^{-1}; ^1H NMR 0.07 (3H, s), 0.08 (3H, s), 0.64 (1H, m), 0.81 (3H, s), 0.86 (3H, s), 0.90 (9H, s), 1.13 (3H, d, $J=7.2$ Hz), 1.33 (3H, s), 1.89 (1H, dd, $J=3.3$, 13.1 Hz), 2.01 (1H, dd, $J=5.2$, 13.1 Hz), 2.07 (3H, s), 3.11 (1H, dddd, $J=4.7$, 4.7, 11.0, 11.0 Hz), 3.33 (3H, s), 4.02 (1H, d, $J=10.7$ Hz), 4.11 (1H, d, $J=10.4$ Hz), 4.26 (1H, dd, $J=3.4$, 5.1 Hz), 4.38 (1H, ddd, $J=7.5$, 7.5, 9.4 Hz); NOE correlation from 23-H to 21-Me and to 27-Me; ^{13}C NMR (100.6 MHz) −5.2 (CH_3), −3.8 (CH_3), 12.3 (CH_3), 16.2 (CH_3), 17.0 (CH_3), 17.8 (C), 20.9 (CH_3), 21.2 (CH_2), 25.6 (CH_3), 25.9 (3×CH_3), 27.9 (CH_2), 28.8 (CH_2), 32.5 (CH_2), 33.1 (CH_2), 34.3 (CH_2), 34.4 (CH), 36.0 (C), 36.9 (CH_2), 39.4 (CH), 41.0 (CH_2), 41.1 (C), 42.6 (CH_2), 44.9 (CH), 54.6 (CH), 54.9 (CH), 55.5 (CH_3), 62.1 (CH), 70.5 (CH_2), 77.7 (CH), 79.8 (CH), 80.5 (C), 81.7 (CH), 120.6 (C), 170.8 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 561 (3), 545 (2), 198 (100); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4317. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.92; H, 10.04. Compound **26**: mp 135.0–136.0 °C (from EtOAc); $[\alpha]_D$ −34 (*c* 0.10); IR 1745 cm^{-1}; ^1H NMR 0.06 (3H, s), 0.07 (3H, s), 0.60 (1H, m), 0.77 (3H, s), 0.78 (3H, s), 0.88 (9H, s), 0.92 (3H, d, $J=6.9$ Hz), 1.17 (3H, s), 1.89 (1H, dd, $J=7.9$, 11.8 Hz), 2.05 (3H, s), 2.14 (1H, m), 3.10 (1H, dddd, $J=4.6$, 4.6, 10.9, 10.9 Hz), 3.32 (3H, s), 3.97 (1H, d, $J=10.8$ Hz), 4.01 (1H, dd, $J=7.8$, 10.6 Hz), 4.04 (1H, d, $J=10.8$ Hz), 4.43 (1H, ddd, $J=7.4$, 7.4, 7.4 Hz); NOE correlation from 23-H to 21-Me and to 27-Me; ^{13}C NMR (100.6 MHz) −4.8 (CH_3), −4.7 (CH_3), 12.3 (CH_3), 14.0 (CH_3), 16.6 (CH_3), 18.2 (C), 20.9 (CH_2), 20.9 (CH_3), 25.1 (CH_3), 25.8 (3× CH_3), 27.9 (CH_2), 28.7 (CH_2), 31.8 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 34.9 (CH), 35.0 (CH), 35.8 (C), 36.9 (CH_2), 40.1 (CH_2), 40.5 (CH_2), 40.9 (C), 44.7 (CH), 54.4 (CH), 55.5 (CH_3), 56.2 (CH), 61.4 (CH), 70.6 (CH), 71.1 (CH_2), 78.0 (C), 79.8 (CH), 80.8 (CH), 116.7 (C), 170.8 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 562 (3), 545 (2), 287 (100); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4317. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.84; H, 10.26.

4.1.9. (22*S*,23*S*,25*S*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (27). To a solution of compound **25** (96 mg, 0.15 mmol) in THF (22 mL) was added TBAF (0.75 mL, 0.75 mmol, 1.0 M in THF) and stirred at room temperature for 2 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. A solution of the residue in MeOH (46 mL) containing KOH (1.5 g) was stirred at room temperature for 0.5 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 7:3) afforded compound **27** (48 mg, 0.10 mmol, 67%): mp 172.5–173.2 °C (from *n*-hexane-EtOAc); $[\alpha]_D$ +30.8 (*c* 0.12); IR 3352 cm^{-1}; ^1H NMR 0.62 (1H, m), 0.79 (3H, s), 0.94 (3H, s), 1.19 (3H, d, $J=7.3$ Hz), 1.25 (3H, s), 1.89 (1H, dd, $J=0.0$, 13.6 Hz), 2.19 (1H, dd, $J=4.9$, 13.7 Hz), 2.36 (1H, dddd, $J=2.0$, 7.2, 7.2, 7.2 Hz), 3.11 (1H, dddd, $J=4.6$, 4.6, 11.0, 11.0 Hz), 3.32 (3H, s), 3.33 (1H, d, $J=11.2$ Hz), 3.53 (1H, d, $J=11.2$ Hz), 3.93 (1H, dd, $J=0.0$, 4.8 Hz), 4.54 (1H, ddd, $J=7.8$, 7.8, 7.8 Hz); NOE correlation from 23-H to 21-Me; ^{13}C NMR (100.6 MHz) 12.2 (CH_3), 16.1

(CH_3), 19.3 (CH_3), 21.1 (CH_2), 26.1 (CH_3), 27.8 (CH_2), 28.8 (CH_2), 32.5 (CH_2), 33.7 (2×CH_2), 34.3 (CH), 35.9 (C), 36.8 (CH_2), 38.6 (CH), 41.0 (CH_2), 41.4 (C), 42.2 (CH_2), 44.8 (CH), 54.6 (2×CH), 55.5 (CH_3), 64.2 (CH), 68.3 (CH_2), 74.8 (CH), 79.8 (CH), 82.4 (C), 83.8 (CH), 121.7 (C); MS *m/z* (rel intensity) 462 (M^+, <1), 444 (7), 426 (16), 287 (100); HRMS calcd for $C_{28}H_{46}O_5$ 462.3345; found 462.3326. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.77; H, 10.03.

4.1.10. (22*R*,23*S*,25*S*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (28). To a solution of compound **26** (83 mg, 0.13 mmol) in THF (19 mL) was added TBAF (0.65 mL, 0.65 mmol, 1.0 M in THF) and stirred at room temperature for 1 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with EtOAc. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. A solution of the residue in MeOH (40 mL) containing KOH (1.3 g) was stirred at room temperature for 0.5 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 6:4) afforded compound **28** (54 mg, 0.12 mmol, 87%): mp 183.8–184.2 °C (from *n*-hexane-EtOAc); $[\alpha]_D$ −46.7 (*c* 0.06); IR 3571, 3466 cm^{-1}; ^1H NMR 0.63 (1H, m), 0.77 (3H, s), 0.78 (3H, s), 0.94 (3H, d, *J*=7.0 Hz), 1.14 (3H, s), 1.97 (1H, dd, *J*=8.3, 12.3 Hz), 2.12 (1H, dd, *J*=10.5, 12.4 Hz), 2.31 (1H, dddd, *J*=6.9, 6.9, 6.9, 6.9 Hz), 3.10 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.29 (1H, d, *J*=10.3 Hz), 3.32 (3H, s), 3.44 (1H, d, *J*=11.3 Hz), 4.04 (1H, m), 4.55 (1H, ddd, *J*=7.1, 7.1, 8.7 Hz); NOE correlation from 23-H to 21-Me, to 20-H and to 27-Me; ^{13}C NMR (100.6 MHz) 12.2 (CH_3), 14.3 (CH_3), 16.4 (CH_3), 20.9 (CH_2), 24.6 (CH_3), 27.8 (CH_2), 28.6 (CH_2), 31.8 (CH_2), 32.2 (CH_2), 34.2 (CH_2), 34.9 (CH), 35.0 (CH), 35.8 (C), 36.8 (CH_2), 38.1 (CH_2), 39.9 (CH_2), 41.1 (C), 44.7 (CH), 54.3 (CH), 55.5 (CH_3), 56.0 (CH), 61.7 (CH), 68.3 (CH_2), 72.2 (CH), 79.7 (CH, C-3), 82.0 (C), 82.3 (CH), 116.8 (C); MS *m/z* (rel intensity) 431 ($M^+ - CH_2OH$, 26), 413 (11), 287 (100); HRMS calcd for $C_{27}H_{43}O_4$ 431.3161; found 431.3106. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.75; H, 9.92.

4.1.11. (22*S*,23*S*,25*S*)-3β-Methoxy-5α-spirostan-23,25-diol (29). A solution of compound **28** (47 mg, 0.1 mmol) in $CHCl_3$ (2.4 mL) was treated with *p*-TsOH (5 mg dissolved in 0.5 mL $CHCl_3$) and stirred at room temperature for 1 h. The reaction mixture was neutralized with Amberjet 4400 OH, filtered and concentrated. Chromatotron chromatography (toluene-EtOAc, 60:40) of the residue afforded starting material **28** (30 mg, 0.06 mmol, 60%) and compound **29** (15 mg, 0.03 mmol, 30%). Compound **29**: mp 214–216 °C (from acetone); $[\alpha]_D$ −92.2 (*c* 0.09); IR 3579 cm^{-1}; ^1H NMR 0.64 (1H, m), 0.80 (6H, s), 1.00 (3H, d, *J*=7.0 Hz), 1.13 (3H, s), 1.56 (1H, dd, *J*=12.3, 12.3 Hz), 2.05 (1H, ddd, *J*=2.7, 5.3, 12.7 Hz), 2.56 (1H, dddd, *J*=6.9, 6.9, 6.9, 6.9 Hz), 3.11 (1H, dddd, *J*=4.7, 4.7, 10.9, 10.9 Hz), 3.23 (1H, dd, *J*=2.7, 11.6 Hz), 3.33 (3H, s), 3.62 (1H, d, *J*=11.5 Hz), 3.75 (1H, dd, *J*=5.3, 11.6 Hz), 4.45 (1H, ddd, *J*=7.1, 7.1, 7.1 Hz); NOE correlation from 23-H to 20-H, to 21-H_3 and from 26-H_b to 24-H_a; ^{13}C NMR (100.6 MHz) 12.3 (CH_3), 14.0 (CH_3), 16.6 (CH_3), 21.0 (CH_2), 24.8 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 31.7 (CH_2), 32.3 (CH_2), 34.3 (CH_2), 34.9 (CH), 35.7 (CH), 35.9 (C), 36.9 (CH_2), 40.1 (CH_2), 41.1 (C), 42.6 (CH_2), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.3 (CH), 61.5 (CH), 64.3 (CH), 68.4 (CH_2), 70.3 (C), 79.8 (CH), 82.1 (CH), 110.5 (C); MS *m/z* (rel intensity) 462 (M^+, 6), 444 (3), 426 (3), 287 (100); HRMS calcd for $C_{28}H_{46}O_5$ 462.3345; found 462.3324. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.67; H, 10.17.

4.1.12. (22*S*,23*S*,25*R*)-3β-Methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (30) and (22*R*,23*S*,25*R*)-3β-methoxy-23-*tert*-butyldimethylsilyloxy-26-acetoxy-22,25-epoxy-5α-furostan (31). A solution of the alcohol **14**-*S* (49 mg, 0.08 mmol) in cyclohexane (8.6 mL) containing (diacetoxyiodo)benzene (52 mg, 0.16 mmol) and iodine (21 mg, 0.08 mmol) was irradiated with a 80 W tungsten-filament lamp at 70 °C for 1 h. The reaction mixture was then poured into aqueous solution of sodium thiosulfate (10%) and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 93:7) of the residue afforded compound **30** (14 mg, 0.02 mmol, 28%) and compound **31** (23 mg, 0.04 mmol, 47%). Compound **30**: mp 144.0–144.7 °C (from EtOAc); $[\alpha]_D$ +24 (*c* 0.10); IR 1745 cm^{-1}; ^1H NMR 0.07 (3H, s), 0.08 (3H, s), 0.62 (1H, m), 0.80 (3H, s), 0.89 (3H, s), 0.91 (9H, s), 1.13 (3H, d, *J*=7.3 Hz), 1.34 (3H, s), 1.67 (1H, dd, *J*=2.3, 13.1 Hz), 2.06 (3H, s), 2.12 (1H, dd, *J*=5.12, 13.0 Hz), 2.33 (1H, m), 3.10 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.33 (3H, s), 3.98 (1H, d, *J*=10.7 Hz), 4.03 (1H, d, *J*=10.7 Hz), 4.18 (1H, dd, *J*=2.3, 5.0 Hz), 4.37 (1H, ddd, *J*=7.4, 7.4, 7.4 Hz); NOE correlation from 23-H to 21-Me and to 26-H_2; ^{13}C NMR (100.6 MHz) −5.2 (CH_3), −3.7 (CH_3), 12.3 (CH_3), 16.1 (CH_3), 17.7 (CH_3), 17.8 (C), 20.9 (CH_3), 21.1 (CH_2), 25.2 (CH_3), 25.9 (3× CH_3), 27.9 (CH_2), 28.8 (CH_2), 32.5 (CH_2), 33.1 (CH_2), 34.3 (CH_2), 34.5 (CH), 35.1 (C), 36.9 (CH_2), 39.2 (CH), 40.9 (CH_2), 41.2 (C), 42.7 (CH_2), 44.8 (CH), 54.6 (CH), 54.9 (CH), 55.5 (CH_3), 62.8 (CH), 71.4 (CH_2), 76.9 (CH), 79.8 (CH), 80.5 (C), 81.9 (CH), 121.1 (C), 171.0 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 561 (5), 198 (100); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4321. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.80; H, 10.07. Compound **31**: mp 157.0–157.8 °C (from EtOAc); $[\alpha]_D$ −37.5 (*c* 0.12); IR 1746 cm^{-1}; ^1H NMR 0.04 (6H, s), 0.60 (1H, m), 0.76 (3H, s), 0.77 (3H, s), 0.85 (3H, d, *J*=5.3 Hz), 0.87 (9H, s), 1.28 (3H, s), 1.91 (1H, dd, *J*=10.6, 11.9 Hz), 2.02 (3H, s), 2.11 (1H, m), 3.07 (1H, dddd, *J*=4.5, 4.5, 10.8, 10.8 Hz), 3.29 (3H, s), 3.77 (1H, d, *J*=11.2 Hz), 3.93 (1H, d, *J*=11.2 Hz), 4.04 (1H, dd, *J*=8.1, 10.4 Hz), 4.48 (1H, ddd, *J*=7.3, 7.3, 7.3 Hz); NOE correlation from 23-H to 21-Me and to 20-H; ^{13}C NMR (100.6 MHz) −4.9 (CH_3), −4.8 (CH_3), 12.2 (CH_3), 13.9 (CH_3), 16.6 (CH_3), 18.2 (C), 20.8 (CH_3), 20.9 (CH_2), 25.8 (3×CH_3), 26.0 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 31.8 (CH_2), 32.2 (CH_2), 34.2 (CH_2), 35.0 (2×CH), 35.8 (C), 36.9 (CH_2), 40.1 (CH_2), 40.4 (CH_2), 40.9 (C), 44.7 (CH), 54.3 (CH), 55.4 (CH_3), 56.2 (CH), 61.6 (CH), 70.3 (CH_2), 71.2 (CH), 78.3 (C), 79.7 (CH), 80.8 (CH), 116.5 (C), 170.5 (C); MS *m/z* (rel intensity) 618 (M^+, <1), 561 (6), 545 (1), 75 (100); HRMS calcd for $C_{36}H_{62}O_6Si$ 618.4316; found 618.4303. Anal. Calcd for $C_{36}H_{62}O_6Si$: C, 69.86; H, 10.10. Found: C, 69.95; H, 10.13.

4.1.13. (22*S*,23*S*,25*R*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (32). To a solution of compound **30** (55 mg, 0.09 mmol) in THF (12.6 mL) was added TBAF (0.42 mL, 0.42 mmol, 1.0 M in THF) and stirred at room temperature for 3 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. A solution of the residue in MeOH (26 mL) containing KOH (0.9 g) was stirred at room temperature for 1.5 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 7:3) afforded compound **32** (41 mg, 0.09 mmol, 99%): mp 145.1–146.7 °C (from AcOEt); $[\alpha]_D$ +7.8 (*c* 0.09); IR 3629, 3448 cm^{-1}; ^1H NMR 0.62 (1H, m), 0.79 (3H, s), 0.93 (3H, s), 1.25 (3H, d, *J*=7.3 Hz), 1.31 (3H, s), 1.55 (1H, dd, *J*=0.0, 13.5 Hz), 2.46 (1H, dddd, *J*=3.1, 7.3, 7.3, 7.3 Hz), 2.56 (1H, dd, *J*=4.6, 13.6 Hz), 3.11 (1H, dddd, *J*=4.6, 4.6, 11.0, 11.0 Hz), 3.31 (1H, d, *J*=10.3 Hz), 3.32 (3H, s), 3.54 (1H, d, *J*=11.3 Hz), 4.15 (1H, dd, *J*=0.0, 4.5 Hz), 4.53 (1H, ddd, *J*=7.1, 7.1, 9.3 Hz); NOE correlation from 23-H to 21-H_3; ^{13}C NMR 12.2 (CH_3), 15.9 (CH_3), 18.7 (CH_3), 20.9 (CH_2), 25.8 (CH_3), 27.8 (CH_2), 28.6 (CH_2), 32.3 (CH_2), 33.5 (CH_2), 34.2 (CH_2), 34.4 (CH), 35.8 (C), 36.8 (CH_2), 38.4 (CH), 39.1 (CH_2), 40.5 (CH_2), 41.4 (C), 44.7 (CH), 54.5 (CH), 55.0 (CH), 55.4 (CH_3), 64.6 (CH), 68.3 (CH_2), 76.7 (CH), 79.8 (CH), 83.1 (CH), 84.4 (C), 120.6 (C); MS *m*/*z* (rel intensity) 444 (M^+-H_2O, 5), 426 (34), 287 (100); HRMS calcd for $C_{28}H_{44}O_4$ 444.3240; found 444.3239. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.85; H, 9.74.

4.1.14. (22*R*,23*S*,25*R*)-3β-Methoxy-22,25-epoxy-5α-furostan-23,26-diol (33). To a solution of compound **31** (108 mg, 0.17 mmol) in THF (25 mL) was added TBAF (0.84 mL, 0.84 mmol, 1.0 M in THF) and stirred at room temperature for 1 h. The mixture was then poured into aqueous saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried (Na_2SO_4) and concentrated. A solution of the residue in MeOH (50 mL) containing KOH (1.7 g) was stirred at room temperature for 5 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4) and concentrated. Chromatotron chromatography (hexanes–EtOAc, 7:3) afforded compound **33** (74 mg, 0.16 mmol, 91%): mp 198.5–199.7 °C (from *n*-hexane-EtOAc); $[\alpha]_D$ −47.7 (*c* 0.13); IR 3567 mmolcm^{-1}; ^1H NMR 0.64 (1H, m), 0.77 (3H, s), 0.79 (3H, s), 0.95 (3H, d, *J*=7.0 Hz), 1.26 (3H, s), 1.69 (1H, dd, *J*=9.8, 12.4 Hz), 2.32 (1H, dd, *J*=8.5, 12.4 Hz), 3.10 (1H, dddd, *J*=4.6, 4.6, 11.0, 11.0 Hz), 3.30 (1H, d, *J*=11.0 Hz), 3.32 (3H, s), 3.39 (1H, d, *J*=11.3 Hz), 4.03 (1H, ddd, *J*=9.0, 9.0, 9.0 Hz), 4.55 (1H, ddd, *J*=6.6, 6.6, 8.7 Hz); NOE correlation from 23 to 21-Me, to 20-H and to 26-H_2; ^{13}C NMR (100.6 MHz) 12.2 (CH_3), 14.3 (CH_3), 16.4 (CH_3), 20.9 (CH_2), 25.6 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 31.8 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 34.9 (CH), 35.5 (CH), 35.8 (C), 36.8 (CH_2), 40.0 (CH_2), 41.0 (C), 41.3 (CH_2), 44.7 (CH), 54.3 (CH), 55.5 (CH_3), 56.1 (CH), 61.8 (CH), 69.8 (CH_2), 72.2 (CH), 79.7 (CH), 81.3 (CH), 81.3 (C), 116.7 (C); MS *m*/*z* (rel intensity) 444 (M^+-H_2O, 5), 426 (27), 287 (100); HRMS calcd for $C_{28}H_{44}O_4$ 444.3240; found 444.3252. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.72; H, 9.98.

4.1.15. (22*S*,23*S*,25*R*)-3β-Methoxy-22,26-epoxy-5α-spirostan-23,25-diol (34). A solution of compound **33** (46 mg, 0.1 mmol) in $CHCl_3$ (2.5 mL) was treated with *p*-TsOH (5 mg dissolved in 0.5 mL $CHCl_3$) and stirred at room temperature for 24 h. The reaction mixture was neutralized with Amberjet 4400 OH, filtered and concentrated. Chromatotron chromatography (toluene-EtOAc, 65:35) of the residue afforded starting material **33** (31 mg, 0.06 mmol, 60%) and compound **34** (13 mg, 0.03 mmol, 30%). Compound **34**: mp 263–263.5 °C (from acetone); $[\alpha]_D$ −132.8 (*c* 0.07); IR 3576 cm^{-1}; ^1H NMR 0.64 (1H, m), 0.79 (3H, s), 0.80 (3H, s), 0.95 (3H, d, *J*=7.0 Hz), 1.30 (3H, s), 1.99 (1H, ddd, *J*=5.5, 7.6, 12.3 Hz), 2.07 (1H, ddd, *J*=2.5, 5.2, 11.6 Hz), 2.50 (1H, dddd, *J*=7.0, 7.0, 7.0, 7.0 Hz), 3.11 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.19 (1H, dd, *J*=2.5, 10.4 Hz), 3.33 (3H, s), 3.49 (1H, dd, *J*=5.3, 11.7 Hz), 3.50 (1H, d, *J*=10.2 Hz), 4.46 (1H, ddd, *J*=7.1, 7.1, 8.7 Hz); NOE correlation from 23-H to 20-H, to 21-H_3; ^{13}C NMR (100.6 MHz) 12.3 (CH_3), 13.9 (CH_3), 16.6 (CH_3), 21.0 (CH_2), 25.0 (CH_3), 27.8 (CH_2), 28.7 (CH_2), 31.6 (CH_2), 32.3 (CH_2), 34.3 (CH_2), 34.9 (CH), 35.6 (CH), 35.9 (C), 36.9 (CH_2), 40.1 (CH_2), 41.0 (C), 44.6 (CH_2), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.2 (CH), 61.5 (CH), 65.8 (CH), 68.2 (CH_2), 68.9 (C), 79.8 (CH), 82.0 (CH), 110.0 (C); MS *m*/*z* (rel intensity) 462 (M^+, 1), 444 (2), 426 (5), 287 (100); HRMS calcd for $C_{28}H_{46}O_5$ 462.3345; found 462.3336. Anal. Calcd for $C_{28}H_{46}O_5$: C, 72.69; H, 10.02. Found: C, 72.89; H, 10.13.

4.1.16. (22*S*,23*S*,25*R*)-3β-Methoxy-23-acetoxy-5α-spirostan-23,25-diol (35). The compound **34** (4 mg, 8.6×10^{-3} mmol) was acetylated with Ac_2O and pyridine to give after chromatography (hexanes–EtOAc, 85:15) compound **35** (3 mg, 5.9×10^{-3} mmol, 68%): amorphous; $[\alpha]_D$ −72.0 (*c* 0.05); IR 3434, 1745 cm^{-1}; ^1H NMR 0.64 (1H, m), 0.78 (3H, s), 0.80 (3H, s), 0.96 (3H, d, *J*=7.0 Hz), 1.37 (3H, s), 2.04 (3H, s), 2.07 (1H, m), 3.12 (1H, dddd, *J*=4.6, 4.6, 10.9, 10.9 Hz), 3.22 (1H, dd, *J*=2.0, 10.5 Hz), 3.33 (3H, s), 3.60 (1H, d, *J*=10.6 Hz), 4.45 (1H, ddd, *J*=7.7, 7.7, 7.7 Hz), 4.84 (1H, dd, *J*=5.3, 11.9 Hz); NOE correlation from 23-H to 20-H, to 21-H_3 and to 27-H_3; ^{13}C NMR (100.6 MHz) 12.3 (CH_3), 13.9 (CH_3), 16.1 (CH_3), 20.9 (CH_2), 21.0 (CH_3), 25.3 (CH_3), 27.9 (CH_2), 28.7 (CH_2), 31.6 (CH_2), 32.3 (CH_2), 34.3 (CH_2), 35.2 (CH), 35.9 (C), 36.0 (CH), 36.9 (CH_2), 39.9 (CH_2), 40.3 (CH_2), 41.1 (C), 44.8 (CH), 54.4 (CH), 55.5 (CH_3), 56.3 (CH), 61.5 (CH), 66.7 (CH), 68.1 (CH_2), 69.0 (C), 79.8 (CH), 81.6 (CH), 108.2 (C), 170.4 (C); MS *m*/*z* (rel intensity) 504 (M^+, 3), 486 (6), 444 (31), 287 (100); HRMS calcd for $C_{30}H_{48}O_6$ 504.3451; found 504.3418. Anal. Calcd for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59. Found: C, 71.27; H, 9.84.

Acknowledgements

This work was supported by the Investigation Programmes nos. BQU2000-0650 and BQU2001-1665 of the Dirección General de Investigación Científica y Técnica, Spain. I.P.-M. thanks the I3P-CSIC Program for a fellowship.

Supplementary data

Detailed experimental procedures, and spectral and analytical data for compounds **6**-*R*, **6**-*S*, **7**-*R*, **7**-*S*, **8**-*R*, **8**-*S*, **9**-*R*, **9**-*S*, **10**-*R*, **10**-*S*, **11**-*S*, **12**-*R*, **12**-*S*, **13**-*S*, **14**-*R*, and **14**-*S* (15 pages) are provided. A figure with the pseudorotational wheels for E and F-rings is also included.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.01.077

References and notes

1. (a) Pettit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. *J. Am. Chem. Soc.* **1988**, *110*, 2006–2007. (b) Recent publication: Pettit, G. R.; Tan, R.; Xu, J.-P.; Ichihara, Y.; Williams, M. D.; Boyd, M. R. *J. Nat. Prod.* **1998**, *61*, 955–958 and references cited therein.
2. (a) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1994**, *59*, 6164–6166. (b) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 608–614. (c) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *Tetrahedron* **1995**, *51*, 6707–6716. (d) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1997**, *62*, 4484–4491.
3. (a) Pettit, G. R.; Xu, J.-P.; Williams, M. D.; Christie, N. D.; Doubek, D. L.; Schmidt, J. M.; Boyd, M. R. *J. Nat. Prod.* **1994**, *57*, 52–63. (b) LaCour, T. G.; Guo, C.; Ma, S.; Jeong, J. U.; Boyd, M. R.; Matsunaga, S.; Fusetani, N.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2587–2592. (c) Dirsch, V. M.; Muller, I. M.; Eichhorst, S. T.; Pettit, G. R.; Kamano, Y.; Inoue, M.; Xu, J. P.; Ichihara, Y.; Wanner, G.; Vollmar, A. M. *Cancer Res.* **2003**, *63*, 8869–8876. (d) Komiya, T.; Fusetani, N.; Matsunaga, S.; Kubo, A.; Kaye, F. J.; Kelley, M. J.; Tamura, K.; Yoshida, M.; Fukuoka, M.; Nakagawa, K. *Cancer Chemother. Pharm.* **2003**, *51*, 202–208.
4. (a) Heathcock, C. H.; Smith, S. C. *J. Org. Chem.* **1994**, *59*, 6828–6839. (b) Drögemüller, M.; Jautelat, R.; Winterfeldt, E. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1572–1574. (c) LaCour, T. G.; Guo, C.; Bhandaru, S.; Boyd, M. R.; Fuchs, P. L. *J. Am. Chem. Soc.* **1998**, *120*, 692–707. (d) Drögemüller, M.; Flessner, T.; Jautelat, R.; Scholz, U.; Winterfeldt, E. *Eur. J. Org. Chem.* **1998**, 2811–2831. (e) Jautelat, R.; Müller--Fahrnow, A.; Winterfeldt, E. *Chem. Eur. J.* **1999**, *5*, 1226–1233. (f) Kim, S.; Sutton, S. C.; Guo, C.; LaCour, T. G.; Fuchs, P. L. *J. Am. Chem. Soc.* **1999**, *121*, 2056–2070. (g) Jeong, J. U.; Guo, C.; Fuchs, P. L. *J. Am. Chem. Soc.* **1999**, *121*, 2071–2084. (h) Li, W.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 2849–2852. (i) Jeong, J. U.; Sutton, S. C.; Kim, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **1995**, *117*, 10157–10158. (j) For a short review see: Ganesan, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 611–615.
5. (a) Li, W.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 2853–2856. (b) Lee, J. S.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 3619–3622. (c) Lee, J. S.; Fuchs, P. L. *Org. Lett.* **2003**, *5*, 2247–2250. (d) Lee, S.; Fuchs, P. L. *J. Am. Chem. Soc.* **2002**, *124*, 13978–13979. (e) Flessner, T.; Ludwing, V.; Siebeneicher, H.; Winterfeldt, E. *Synthesis* **2002**, 1373–1378. (f) Li, W.; LaCour, T. G.; Fuchs, P. L. *J. Am. Chem. Soc.* **2002**, *124*, 4548–4549. (g) Lee, S.; Fuchs, P. L. *Org. Lett.* **2002**, *3*, 317–318. (h) LaCour, T. G.; Tong, Z.; Fuchs, P. L. *Org. Lett.* **1999**, *1*, 1815–1818.
6. LaCour, T. G.; Guo, C.; Boyd, M. R.; Fuchs, P. L. *Org. Lett.* **2000**, *2*, 33–36.
7. Isolation (a) : Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969–3973. Synthesis (b): Guo, C.; Fuchs, P. L. *Tetrahedron Lett.* **1998**, *39*, 1099–1102. (c) Deng, S.; Yu, B.; Lou, Y.; Hui, Y. J. *Org. Chem.* **1999**, *64*, 202–208. (d) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzebska, I. *Tetrahedron Lett.* **2000**, *41*, 3751–3754. (e) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2001**, *123*, 3369–3370. (f) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576–6583.
8. Guo, C.; LaCour, T. G.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 419–424.
9. Part of this work was previously published as a preliminary communication: Betancor, C.; Freire, R.; Pérez-Martín, I.; Prangé, T.; Suárez, E. *Org. Lett.* **2002**, *4*, 1295–1297.
10. (a) Majetich, G.; Wheless, K. *Tetrahedron* **1995**, *51*, 7095–7129. (b) Feray, L.; Kuznetsov, N.; Renaud, P. In *Hydrogen Atom Abstraction*; Renaud, P., Sibi, M. P., Eds.; Radicals in Organic Synthesis; Wiley-VCH: Weinheim, 2001; Vol. 2, pp 246–278. (c) Robertson, J.; Pillai, J.; Lush, R. K. *Chem. Soc. Rev.* **2001**, *30*, 94–103. (d) Togo, H.; Katohgi, M. *Synlett* **2001**, 565–581.
11. (a) Martín, A.; Salazar, J. A.; Suárez, E. *J. Org. Chem.* **1996**, *61*, 3999–4006. (b) Dorta, R. L.; Martín, A.; Salazar, J. A.; Suárez, E.; Prangé, T. *J. Org. Chem.* **1998**, *63*, 2251–2261.
12. See for example: pairs of ritterazines A/D, B/F (Ref. 2b) and N/O, P/Q, R/S and W/X (Ref. 2c).
13. (a) Barton, D. H. R.; Sammes, P. G.; Taylor, M. V.; Werstiuk, E. *J. Chem. Soc. (C)* **1970**, 1977–1981. (b) González, A. G.; Freire, R.; García-Estrada, M. G.; Salazar, J. A.; Suárez, E. *Tetrahedron* **1972**, *28*, 1289–1297. (c) González, A. G.; Freire, R.; García-Estrada, M. G.; Salazar, J. A.; Suárez, E. *Anal. Quim.* **1971**, *67*, 903–905.
14. Oikawa, M.; Oikawa, H.; Ichihara, A. *Tetrahedron* **1995**, *51*, 6237–6254.
15. (a) Grieco, P. A.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1976**, *41*, 1485–1486. (b) Grieco, P. A.; Takigawa, T.; Schillinger, W. J. *J. Org. Chem.* **1980**, *45*, 2247–2251.
16. For similar unsuccessful results on a related olefin using $(DHQ)_2PHAL$ as ligand Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M.; Xu, D.; Zhang, X. L. *J. Org. Chem.* **1992**, *57*, 2768–2771 see: Refs. 5c,17b.
17. Corey, E. J.; Jardine, P. D.; Virgil, S.; Yuen, P.-W.; Connell, R. D. *J. Am. Chem. Soc.* **1989**, *111*, 9243–9244.
18. For studies of the influence of polar factors on intermolecular hydrogen abstraction reactions see: (a) Beckwith, A. L. J.; Zavitsas, A. A. *J. Am. Chem. Soc.* **1995**, *117*, 607–614. (b) Zavitsas, A. A.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **1995**, *117*, 10645–10654. (c) Kaushal, P.; Mok, P. L. H.; Roberts, B. P. *J. Chem. Soc., Perkin Trans. 2* **1990**, 1663–1670.
19. Although we have no experimental support for this reasoning, it is strongly supported by previous results on related models. For examples of the influence of the polarity of the substituents on the 1,5-hydrogen atom tranfer reaction compared with competing β-fragmentation of alkoxyl radicals, see: (a) Allen, P. R.; Brimble, M. A.; Farès, F. A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2403–2411. (b) Lee, S.; LaCour, T. G.; Lantrip, D.; Fuchs, P. L. *Org. Lett.* **2002**, *4*, 313–316.
20. For example, irradiation at H-23 in each of the different

diastereoisomers results in enhancement of the following protons: **17** (21-Me and 26-H_2), **18** (18-Me and 20-H), **22** (21-Me and 27-Me), **23** (18-Me, 20-H, and 27-Me), **27** (21-Me), **28** (20H, 21-Me, and 27-Me), **32** (21-Me), **33** (21-Me and 26-H_2).

21. Crystallographic data (excluding structure factors) for the structures **18**, **23**, **29**, and **34** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-176076, CCDC-176077, CCDC-243668, CCDC-243669 respectively. 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].
22. Acid-catalyzed spiroketal equilibration in 23-deoxy compounds: (a) Thompson, M. J.; Scheer, I.; Mosettig, E. *J. Am. Chem. Soc.* **1959**, *81*, 5222–5224. (b) Thompson, M. J.; Scheer, I.; Mosettig, E. *J. Am. Chem. Soc.* **1959**, *81*, 5225–5230. (c) González, A. G.; Freire, R.; Salazar, J. A.; Suárez, E. *Phytochemistry* **1971**, *10*, 1339–1346. (d) González, A. G.; Freire, R.; Salazar, J. *Anal. Quim.* **1972**, *68*, 1021–1027. (e) Jeong, J. U.; Fuchs, P. L. *Tetrahedron Lett.* **1994**, *35*, 5385–5388.
23. No compounds of the 1,6-dioxaspiro[4.5]decane type belonging to the (22*S*)-spirostan, (20*R*,22*R*)-spirostan (cyclopseudoiso), or (20*R*,22*S*)-spirostan (cyclopseudoneo) series of spirostan sapogenins, which are theoretically possible through an oxocarbenium ion via an intermediate of furost-20(22)-en sapogenin, were detected during these acid-catalyzed experiments. (a) Fieser, L. F.; Fieser, M. In *Steroids*; Reinhold Publishing Corp: New York, 1959. (b) IUPAC-IUB Revised Tentative Rule for Nomenclature of Steroids Verkade, P. E.; Hoffmann-Ostenhof, O. *J. Org. Chem.* **1969**, *34*, 1517–1532.
24. CS Chem3D Ultra v. 6.0, CambridgeSoft Corp.: Cambridge, MA.
25. Dihedral angles consistent with these configurations are observed in the X-ray crystallographic structures of **17** (X-ray) (H_{23}–C_{23}–C_{24}–H_{24a}=154.4 and H_{23}–C_{23}–C_{24}–H_{24b}=34.8°) and **18** (H_{23}–C_{23}–C_{24}–H_{24a}=29.7 and H_{23}–C_{23}–C_{24}–H_{24b}=90.6°).
26. (a) Fuchs, B. *Top. Stereochem.* **1978**, *10*, 1–94. (b) Romers, C.; Altona, C.; Buys, H. R.; Havinga, E. *Top. Stereochem.* **1969**, *4*, 36–97.
27. Data were taken from the X-ray analysis of (22*S*,23*R*,25*S*)-3β,12β-diacetoxy-22,25-epoxy-5α-furostan-23,26-diol.Jeong, J. U.; Fuchs, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 773–774.
28. The five endocyclic dihedral angles ϕ_j (J=0–4) were obtained from the X-ray cif files using Mercury v. 1.2.1 (Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK) (http://www.ccdc.cam.ac.uk/mercury/).
29. (a) Houseknecht, J. B.; Altona, C.; Hadad, C. M.; Lowary, T. L. *J. Org. Chem.* **2002**, *67*, 4647–4651. (b) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344. (c) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8212.
30. IUPAC: Nomenclature of Carbohydrates, IUPAC, (http://www.chem.qmul.ac.uk/iupac/2carb/). Rule 2-Carb-7.1.
31. Coupling constants were measured using the HLA equation as implemented in MacroModel v. 7.0: Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
32. For an early conformational study of the F-ring of natural spirostan sapogenins deoxygenated at C-23 see: Callow, R. K.; James, V. H. T.; Kennard, O.; Page, J. E.; Paton, P. N.; Riva di Sanseverino, L. *J. Chem. Soc. (C)* **1966**, 288–297.
33. Shortly before submitting our preliminary communication (Ref. 9) we became aware of the studies by Prof. P.L. Fuchs in the ASAP section of Organic Letters (Refs. 5g, 19b, see also Ref. 5c) where IHA reactions are used to prepare related dioxaspiro compounds.
34. (a) Westhof, E.; Sundaralingam, M. *J. Am. Chem. Soc.* **1983**, *105*, 970–976. (b) Levitt, M.; Warshel, A. *J. Am. Chem. Soc.* **1978**, *100*, 2607–2613.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2815–2830

Synthesis of functionalized α-amino-phosphine oxides and -phosphonates by addition of amines and aminoesters to 4-phosphinyl- and 4-phosphonyl-1,2-diaza-1,3-butadienes

Francisco Palacios,* Domitila Aparicio, Yago López and Jesús M. de los Santos

Departamento de Química Orgánica I, Facultad de Farmacia. Universidad del País Vasco, Apartado 450, 01080 Vitoria, Spain

Received 27 October 2004; revised 14 January 2005; accepted 20 January 2005

Abstract—1,2-Diaza-1,3-butadienes derived from phosphine oxides and phosphonates and with optically active substituents on N-1 and C-3 are obtained by 1,4-elimination from chlorohydrazonoalkyl-phosphine oxides and -phosphonates in the presence of bases. Michael addition (1,4-addition) of ammonia, aliphatic and aromatic amines and aminoesters to these azo-alkenes gives functionalized α-amino-phosphine oxides and -phosphonates.

1. Introduction

Hydrazones constitute an important class of compounds due to the rich chemistry of the hydrazono group and have attracted a great deal of attention in recent years because of their range of applications.[1] They form part of the structure of new azapeptides,[2] biologically active antibiotic compounds,[3a,b] as well as potent anticancer[3c] and antimalarial agents[3d] and have been also extensively used as versatile precursors in acyclic[4] and heterocyclic synthesis.[5] 1,2-Diaza-1,3-butadienes **I** (Fig. 1) are widely used intermediates in organic synthesis[6,7] because they offer easy access to a broad range of heterocycles.[8] Many of these heterodienes containing alkyl[9a] (**I**, R^1=Me), aryl[9b,c] (**I**, R^1=Ar), tosyl[9c] (**I**, R^1=$ArSO_2$), or carbonyl[6a] (**I**, R^1=COR, COOR, $CONR_2$) derivatives as substituents in the terminal nitrogen (N-1) and with alkyl[9d] (**I**, R^3=Me), halogen[9e] (**I**, R^3=Br, Cl), carbonyl[6a,9f] (**I**, R^3=COR, COOR, $CONR_2$) or carbohydrate[7d] (**I**, R^3=D-*arabino*-$(CHOAc)_3CH_2OAc$) derivatives as well as without substituents[9c] in the terminal carbon (C-4) have been described. However, very little information about the behaviour of 1,2-diaza-1,3-butadienes **II** (Fig. 1) containing phosphorus substituents have been reported.[10] Furthermore, it is known that phosphorus substituents regulate important biological functions,[11] and that molecular modifications involving the introduction of organophosphorus functionalities in simple *synthons* could be very interesting from a synthetic point of view because they can be useful substrates for the preparation of biologically active compounds.

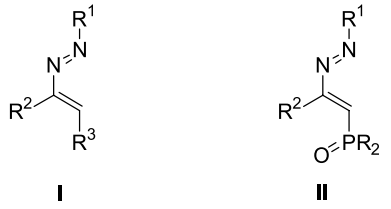
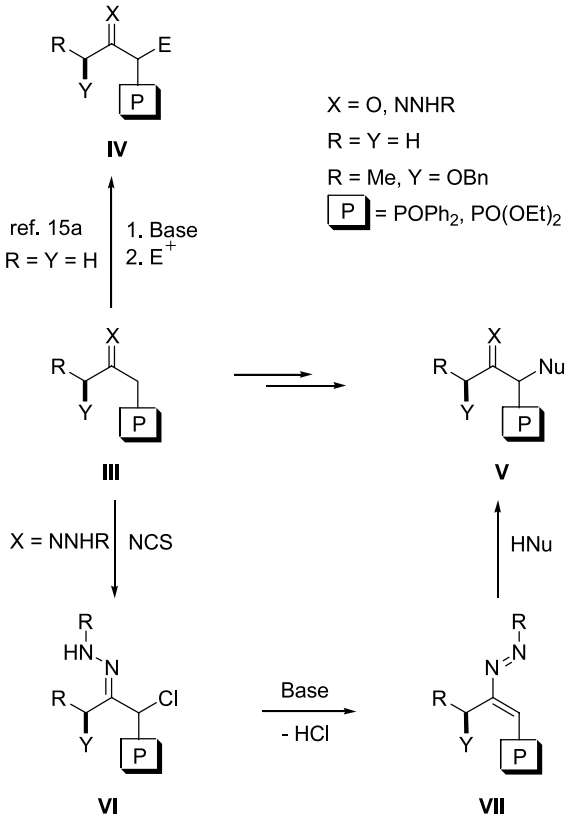


Figure 1.

We have previously described the synthesis of 2-aza-dienes[12] and the application of phosphorus substituted enamines,[13] oximes[14] and hydrazones[15] as starting materials for the preparation of acyclic and cyclic compounds. In this context, the usefulness of carbanions derived from hydrazones for carbon–carbon bond formation reaction, has been well documented.[1,16] Besides enantioselective α-alkylations of aldehydes and ketones, the carbanion hydrazone method can be successfully applied to the introduction of electrophilic reagents in the Cα–carbon atom (Scheme 1) and we have used this strategy for the functionalization of hydrazonoalkyl-phosphine oxides and -phosphonates[15a] **IV** (Scheme 1). Hydrazones can be considered as protected carbonyl compounds and can also be used as starting materials for the preparation of 1,2-diaza-1,3-butadienes.[6] These heterodienes react very easily with nucleophilic reagents through a conjugate addition (1,4-addition).[6] We

Keywords: α-Amino-phosphine oxides; α-Amino-phosphonates; 4-Phosphinyl-1,2-diaza-1,3-butadienes; 4-Phosphonyl-1,2-diaza-1,3-butadienes; Hydrazones; Michael addition.
* Corresponding author. Tel.: +34 945 013103; fax: +34 945 013049; e-mail: qoppagaf@vc.ehu.es

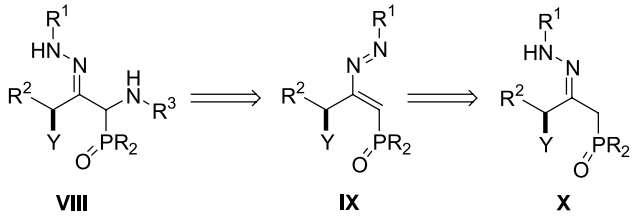
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.081



Scheme 1.

envisaged the use of hydrazonoalkyl-phosphine oxides and -phosphonates (**III**, X=NNHR, R=Y=H) and derivatives containing a chiral substituent at 3-position (**III**, X=NNHR, R=Me, Y=OBn) as starting materials for the preparation of functionalized hydrazones containing a nucleophilic substituent **V**. The sequence involve halogenation with formation of an α-halogenated hydrazone **VI**, subsequent formation of aza-alkene **VII**, and Michael addition of nucleophiles to the heterodiene.[17] Therefore with this strategy it could be possible to reverse the polarity of Cα–carbon atom of the phosphonate (or phosphine oxide) group favouring the introduction of nucleophiles in order to prepare functionalized hydrazones or their synthetic equivalent carbonyl compounds **V** (Scheme 1).

This strategy could present special interest for the introduction of amino substituents ($NuH=RNH_2$) in the Cα–carbon atom of hydrazones, because we could obtain azapeptides[2] or α-aminophosphonates. It is noteworthy that α-aminophosphonates[18,19] are important substrates in organic and medicinal chemistry because they can be considered as surrogates for α-aminoacids,[20a] and have been used as haptens for the generation of catalytic antibodies,[20b,c] as antibacterial agents,[20d,e] and as nucleoside,[20f] or as phosphapeptide enzyme inhibitors.[20g–k] For this reason, here we wish to describe a novel strategy for the preparation of functionalized α-amino-phosphine oxides **VIII** (R=Ph) and -phosphonates **VIII** (R=OEt) from hydrazones **X** (R=Ph, OEt) involving nucleophilic addition of racemic and optically pure amines to 1,2-diaza-1,3-butadienes **IX** as shown in Scheme 2. The effect of optically active substituents[21] on the terminal nitrogen (N-1) **VIII-X**



Scheme 2.

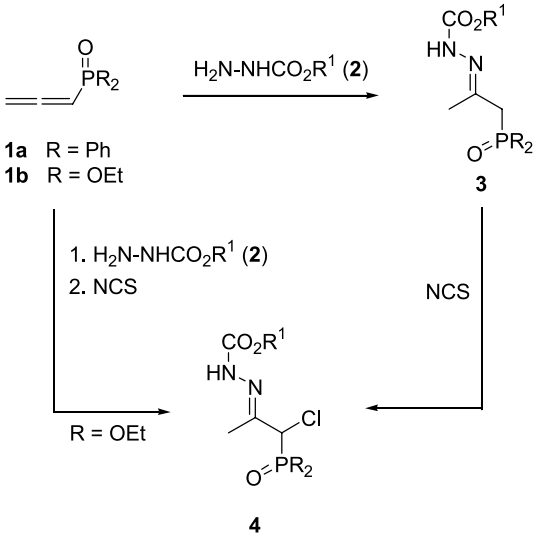
($R^1=CO_2(-)$-Ment, $R^2=Y=H$) and on the carbon (C-3) atoms **VIII-X** ($R^1=CO_2R$, $R^2=Me$, Y=OBn) in the Michael addition of amine derivatives to phosphorylated 1,2-diaza-1,3-butadienes **IX** (R=Ph, OEt) is also reported.

2. Results and discussion

2.1. Preparation of 1,2-diaza-1,3-butadienes 5

The required functionalized β-hydrazones **X** (R=Ph, OEt; $R^2=Y=H$) were prepared by the reaction of allenic phosphine oxide **1a** (R=Ph) or phosphonate **1b** (R=OEt) with carbazates **2**, in a similar way to that previously reported for simple hydrazines.[15e] Addition of ethoxycarbonylhydrazide **2a** ($R^1=Et$) to allene **1a** in refluxing chloroform (TLC control) led to the formation of β-hydrazono phosphine oxide **3aa** (R=Ph, $R^1=Et$) (Scheme 3, Table 1, entry 1). This compound **3aa** was characterized by its spectroscopic data, which indicated that it was isolated as the *anti*-hydrazone **3aa**. ^{31}P NMR spectrum of **3aa** showed one absorption at δ_P 32.3 ppm. Likewise, the ^1H NMR spectra of **3aa** gave a well resolved doublet for the methylene proton at δ_H 3.33 ppm ($^2J_{PH}=14.8$ Hz) and a singlet at 10.63 ppm for the NH group,[22] while in ^{13}C NMR a doublet appeared at δ_C 36.2 ppm ($^1J_{PC}=63.0$ Hz) for the carbon bonded to the phosphorus atom.

The process was extended to allenes derived from phosphonate, and this allene **1b** (R=OEt) reacted also with hydrazide **2a** and gave β-functionalized phosphonate **3ba** (R=OEt, $R^1=Et$) (Scheme 3, Table 1, entry 2),



Scheme 3.

Table 1. Hydrazones **3** and **4** and 1,2-diaza-1,3-butadienes **5** obtained

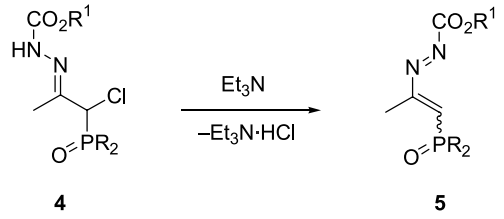
Entry	Compound	R	R^1	Yield (%)
1	**3aa**	Ph	Et	92[a]
2	**3ba**	OEt	Et	76[b]
3	**3bb**	OEt	Bn	95[b]
4	**3bc**	OEt	(−)-Ment	93[b]
5	**4aa**	Ph	Et	70[a]
6	**4ba**	OEt	Et	47[c]
7	**4bb**	OEt	Bn	55[c]
8	**4bc**	OEt	(−)-Ment	43[c]
9	**5aa**	Ph	Et	>98[b,d]
10	**5ba**	OEt	Et	>98[b,d]
11	**5bb**	OEt	Bn	>98[b,d]
12	**5bc**	OEt	(−)-Ment	>98[d]

[a] Yield of isolated purified compounds.
[b] Conversion calculated by ^{31}P NMR on the crude reaction mixture.
[c] Yield of isolated purified compounds **4** in a 'one pot' reaction from allene **1b**.
[d] Non-isolated compounds **5**.

although in this case hydrazone **3ba** was isolated as a mixture of the *syn*- and *anti*-isomers in a ratio of 57:43 (δ_P 23.5 and 24.1 ppm). Likewise, the ^1H and ^{13}C NMR spectra gave singlets for the NH at 7.67 (*syn*-isomer) and 9.21 ppm (*anti*-isomer) and well resolved doublets for the methylene proton at δ_H 2.79 ($^2J_{PH}=23.2$ Hz, *anti*) and 2.88 ($^2J_{PH}=22.1$ Hz, *syn*) as well as for the carbon at δ_C 28.6 ($^1J_{PC}=132.4$ Hz, *syn*) and 35.1 ($^1J_{PC}=135.0$ Hz, *anti*) of **3ba**. The same strategy was used for the preparation of the hydrazone with a benzyl carboxylate in the terminal nitrogen **3bb** (R=OEt, R^1=Bn) by using benzyl carbazate **2b** (R^1=Bn) (Scheme 3, Table 1, entry 3).

The preparation of 1,2-diaza-1,3-butadienes **5** involves base treatment of hydrazone derivatives bearing a leaving group in the Cα–carbon atom of the hydrazono carbon-nitrogen double bond.[6] For this reason, we explored the synthesis of Cα–chlorohydrazones derived from phosphine oxides and phosphonates **4** and their use for the preparation of the diaza-alkenes by treatment with bases. Functionalized chlorohydrazone (R=Ph, R^1=Et) **4aa** was prepared from β-hydrazone **3aa** by treatment with 1.1 equiv of *N*-chlorosuccinimide (NCS) in refluxing carbon tetrachloride (Scheme 3, Table 1, entry 5). This compound **4aa** was isolated as the *anti*-substituted hydrazone (δ_P 28.0 ppm). In the case of phosphonates **4ba** (R=OEt, R^1=Et) and **4bb** (R=OEt, R^1=Bn), these hydrazones were directly prepared from allene **1b** and crude products **3ba** or **3bb**, obtained from allene **1b** and carbazates **2a** or **2b**, were treated without isolation with 1.1 equiv of NCS in CCl_4 at room temperature to give *anti*-chlorohydrazone derived from phosphonate **4ba** or **4bb** in moderate yield (Scheme 3, Table 1, entries 6 and 7).

Heterodienes **5** were synthesized from chlorohydrazones **4** in the presence of bases (Scheme 4). The addition of an excess (1.5 equiv) of triethylamine to a solution of functionalized chlorohydrazone **4aa** (R=Ph, R^1=Et) in dichloromethane underwent 1,4-elimination of HCl and led to the formation of the red coloured 4-phosphinyl-1-ethoxycarbonyl-1,2-diaza-1,3-butadiene **5aa** (R=Ph, R^1=Et) in almost quantitative yield (Scheme 4, Table 1, entry 9). Diaza-alkene **5aa** proved to be not enough stable for chromatography, yet not purified crude mixtures could be satisfactorily used for the next step. The presence of the diazabutadiene **5aa** in the crude reaction mixture was confirmed by NMR spectroscopy and showed as a mixture of the *E*- and *Z*-isomers in a ratio of 85:15 (δ_P 20.5 and 26.4). Likewise, the ^1H NMR spectrum showed a well resolved doublet at δ_H 2.18 ppm ($^4J_{PH}=1.5$ Hz) for the methyl group corresponding to the *Z*-isomer, while in the case of the *E*-isomer this signal appeared at lower field at δ_H 2.29 ppm (doublet, $^4J_{PH}=2.3$ Hz).[9a,9f] A similar behaviour was observed for chlorohydrazones **4ba** (R=OEt, R^1=Et) and **4bb** (R=OEt, R^1=Bn) and after addition of triethylamine 4-phosphonyl-1-alcoxycarbonyl-1,2-diaza-1,3-butadiene **5ba** and **5bb** were obtained (Scheme 4, Table 1, entries 10 and 11) as a mixture of the *E*- and *Z*-isomers in a ratio of 90:10. 1,2-Diaza-1,3-butadienes **5** were used in situ without isolation in subsequent Michael additions.



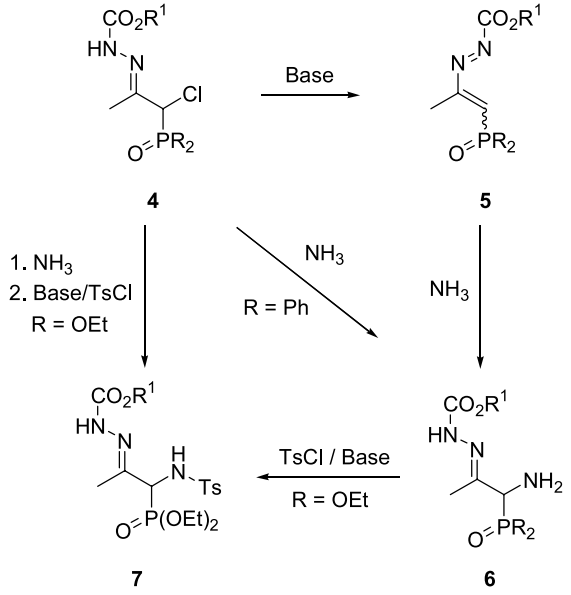
4 **5**

Scheme 4.

2.2. Michael addition of ammonia and amines to 1,2-diaza-1,3-butadienes 5

The presence of electron-poor groups on the terminal nitrogen atom of the azo-ene system of 1,2-diaza-1,3-butadienes favoured the Michael addition of nucleophilic reagents on the terminal carbon atom (1,4-addition). On the other hand, electron-withdrawing groups enhance the electrophilic character of this atom.[6] Some conjugate additions of amines to 1,2-diaza-1,3-butadienes have been reported.[22,23] However, both Michael additions of ammonia and amines to phosphorus substituted 1,2-diazadienes, and the effect of an optically active group[21] on the N-1 and C-3 atoms of 1,2-diazadienes towards nucleophilic additions have not been reported. For these reasons, we explored the addition of amine derivatives to phosphorus substituted 1,2-diaza-1,3-butadienes **5**. In addition, this strategy could be useful for the preparation of functionalized α-aminophosphorus derivatives.

Initially, we explored the addition of ammonia to 1,2-diazadiene **5aa** (R=Ph) and generated in situ from chlorohydrazone **4aa**. When ammonia was bubbled through a solution of chlorohydrazone **4aa** in chloroform, the reaction mixture became red, showing the formation of 1,2-diazadiene **5aa**. The red colour disappeared very fast (<10 min) and TLC control showed the end of the reaction with the formation of only the *anti*-α-amino phosphine oxide **6aa** (R=Ph, R^1=Et) in good yield (Scheme 5, Table 2, entry 1). Mass spectrometry of **6aa** supported the molecular ion peak, while in the ^{31}P NMR spectrum the phosphonate group resonated at δ_P 31.3 ppm. The ^{1}H NMR spectrum showed an absorption at δ_H 4.53 ppm as a doublet with coupling constant $^2J_{PH}$=10.4 Hz for the methine proton and ^{13}C NMR spectrum showed an absorption at δ_C 58.6 ppm as a doublet with coupling constant $^1J_{PC}$=74.0 Hz for the carbon atom directly bonded to the phosphorus atom. The formation of *anti*-α-amino phosphine oxide **6aa** could be explained by selective Michael addition (1,4-addition) of ammonia to conjugated diaza-alkene **5aa** (Scheme 5).



Scheme 5.

The process was extended to 4-phosphonyl-1-benzyloxycarbonyl-1,2-diaza-1,3-butadiene **5bb** (R=OEt, R^1=Bn). However, in this case after the addition of ammonia to hydrazone **4bb**, α-amino phosphonate **6bb** (R=OEt, R^1=Bn) was not isolated, being very unstable, although its protection with tosyl chloride in the presence of base, made possible the isolation of the corresponding *anti*-α-tosyl-amino phosphonate **7bb** (R=OEt, R^1=Bn) (Scheme 5, Table 2, entry 2).

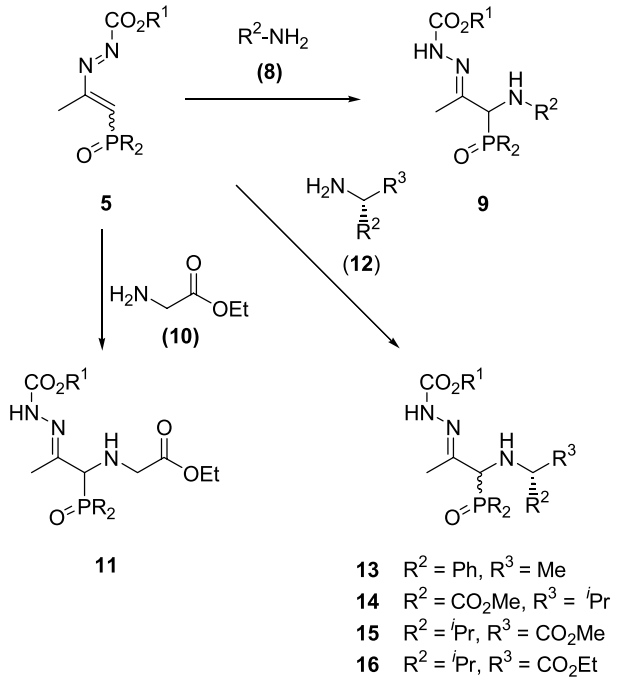
We also studied the addition of primary amines. The addition of aryl **8a** (R^2=*p*-MeO-C_6H_4) or propargyl **8b** (R^2=$CH_2C{\equiv}CH$) amines to 4-phosphinyl-1,2-diaza-1,3-butadiene **5aa**, generated in situ from chlorohydrazone **4aa** and triethylamine, led to the formation of functionalized *anti*-α-amino phosphine oxide **9aaa** (R=Ph, R^1=Et, R^2=*p*-MeO-C_6H_4) and **9aab** (R=Ph, R^1=Et, R^2=$CH_2C{\equiv}CH$) in good yields (Scheme 6, Table 2, entries 3 and 4). The reaction can be extended to functionalized amines such as ethyl glycinate **10**. Treatment of α-aminoester **10** with 1,2-diaza-1,3-butadiene **5aa** (R=Ph) afforded functionalized *anti*-α-amino phosphine oxide **11aaa** (R=Ph, R^1=Et) derived from glycine in excellent yield (Scheme 6, Table 2, entry 7).

In a similar way, the treatment not only of aryl **8a** (R^2=*p*-MeO-C_6H_4) and alkyl amines **8c** (R^2=Bn) but also of functionalized amines such as α-aminoester **10** to 4-phosphonyl-diaza-1,3-butadienes **5ba** and **5bb** gave functionalized *anti*-α-aminophosphonates **9** (R=OEt) and **11** (R=OEt) in excellent yields (Scheme 6, Table 2, entries 5, 6 and 8, 9). The *anti*-configuration is supported by the chemical shift of the hydrazone protons (δ_H=7.69–8.10 ppm) and NOE experiments on **9baa** showed that selective saturation of the NH singlet at 7.77 ppm afforded positive NOE (7.8%) over the adjacent methyl group. It is noteworthy that these new functionalized α-aminophosphonates **11baa** and **11bba** derived from α-aminoesters can be considered as 'depsi-phosphapeptides' and could be interesting substrates in medicinal chemistry.[18a,b]

Table 2. Michael addition of ammonia and amine derivatives to azo-alkenes **5**

Entry	Compound	R	R^1	R^2	Yield (%)[a]
1	**6aa**	Ph	Et	—	68
2	**7bb**	OEt	Bn	—	38[b]
3	**9aaa**	Ph	Et	*p*-MeO-C_6H_4	86
4	**9aab**	Ph	Et	$CH_2C{\equiv}CH$	78
5	**9baa**	OEt	Et	*p*-MeO-C_6H_4	92
6	**9bbc**	OEt	Bn	Bn	98
7	**11aaa**	Ph	Et	—	89
8	**11baa**	OEt	Et	—	99
9	**11bba**	OEt	Bn	—	93
10	**18**	OEt	(−)-Ment		43
11	**19**	OEt	(−)-Ment		82
12	**20**	OEt	(−)-Ment		92
13	**21**	OEt	(−)-Ment		83[c,d]
14	**22**	OEt	(−)-Ment		79[e,d]

[a] Yield of isolated purified compounds.
[b] Yield of isolated purified compound **7bb** obtained in a two-step procedure (addition of ammonia and protection of primary amine with TsCl).
[c] d.e. 10.
[d] d.e. was determined by ^{31}P NMR on the crude reaction mixture.
[e] d.e. 15.



Scheme 6.

Then, we studied the diastereoselective addition of optically active amines **12** to 1,2-diaza-1,3-butadienes **5**. Very low diastereoselectivity (<10% d.e.) was obtained when (*R*)-benzylmethylamine **12a** (R^2=Ph, R^3=Me) was treated at 0 °C with 1-ethoxycarbonyl-4-phosphinyl-1,2-diaza-1,3-butadiene **5aa** (R=Ph, R^1=Et). Functionalized α-aminophosphine oxide **13aa** (R=Ph, R^1=Et, R^2=Ph, R^3=Me) was obtained as a non-separable diastereoisomeric mixture and very low diastereoselective excess (<10% d.e.) was observed[24] (Scheme 6, Table 3, entry 1).

A similar behaviour was observed when 1,2-diaza-1,3-butadienes containing a phosphonate group was used and, as before, a very low diastereoselectivity was obtained when (*R*)-benzylmethylamine **12a** (R^2=Ph, R^3=Me), methyl (*R*)-valinate **12b** (R^2=CO_2Me, R^3=^{i}Pr) or (*S*)-valinate **12c** (R^2=^{i}Pr, R^3=CO_2Me) as well as ethyl (*S*)-valinate **12d** (R^2=^{i}Pr, R^3=CO_2Et) were treated at 0 °C with 1-ethoxycarbonyl-4-phosphonyl-1,2-diaza-1,3-butadiene **5ba** (R=OEt, R^1=Et) and benzyloxycarbonyl-4-phosphonyl-1,2-diaza-1,3-butadienes **5bb** (R=OEt, R^1=Bn). Adducts **13ba** and **14–16** were obtained as non-separable diastereoisomeric mixtures and very low diastereoselective excess[24] (Scheme 6, Table 3, entries 2–6). At low temperatures (−40 °C) the ratio (d.e.) (Table 3, entries 5 and 6) can be slightly enhanced.

2.3. Michael addition of amine derivatives to 1,2-diaza-1,3-butadiene 5bc

Then, we explored the influence in this process of the presence of an optically active group ((−)-menthylcarboxylate substituent)[21] in the terminal nitrogen atom (N-1) of 1,2-diazadienes. (−)-Menthyl carbazate **2c** was prepared by addition of (−)-menthyl chloroformiate in dichloromethane to hydrazine hydrate in the presence of carbon tetrachloride, potassium carbonate and triethylbenzylamonium chloride. Treatment of (−)-menthylcarbonylhydrazide **2c** with allene derived from phosphonate **1b** in refluxing chloroform (TLC control) afforded *anti*- and *syn*-β-hydrazono phosphonate **3bc** (62:38) (Scheme 3, Table 1, entry 4). Functionalized chlorohydrazone **4bc** was directly prepared from allene **1b** and crude product **3bc** was treated without isolation with 1.1 equiv of NCS in refluxing CCl_4 to give *anti*-chlorohydrazone derived from phosphonate **4bc** in moderate yield (Scheme 3, Table 1, entry 8). 4-Phosphonyl-1,2-diaza-1,3-butadiene **5bc** (*E*/*Z* ratio 83:17) was synthesized from chlorohydrazones **4bc** by addition of triethylamine in almost quantitative yield (Scheme 4, Table 1, entry 12). Diaza-alkene **5bc** is unstable and crude reaction mixture without purification was used.

Conjugate additions of ammonia and amine derivatives to azo-alkene **5bc** were then explored. Ammonia was bubbled through a solution of 1,2-diazadiene **5bc** in chloroform and *anti*-α-amino phosphonate **17** was obtained. As before, this α-amino phosphonate **17** could not be isolated, although its protection with tosyl chloride gave the corresponding *anti*-α-tosylamino phosphonate **18** as a diastereisomeric ratio of 1:1 (Scheme 7, Table 2, entry 10). The addition of aryl amine **8** (R^2=*p*-MeO–C_6H_4) or ethyl glycinate **10** (R^2=CH_2CO_2Et) to 1,2-diaza-1,3-butadiene **5bc** gave functionalized *anti*-α-amino phosphonates **19** and **20**, respectively, in good yields (Scheme 7, Table 2, entries 11 and 12). Likewise, optically active amines (*R*)-benzylmethylamine **12a** (R^2=Ph, R^3=Me) or ethyl (*S*)-valinate **12d** (R^2=^{i}Pr, R^3=CO_2Et) were used and adducts **21** and **22** were obtained with very low diastereoselectivity (Scheme 7, Table 2, entries 13 and 14). These results suggest that the chiral group on the nitrogen atom of the azo-alkene is too distant from reaction centre (C-4 atom) with negligible influence on it.

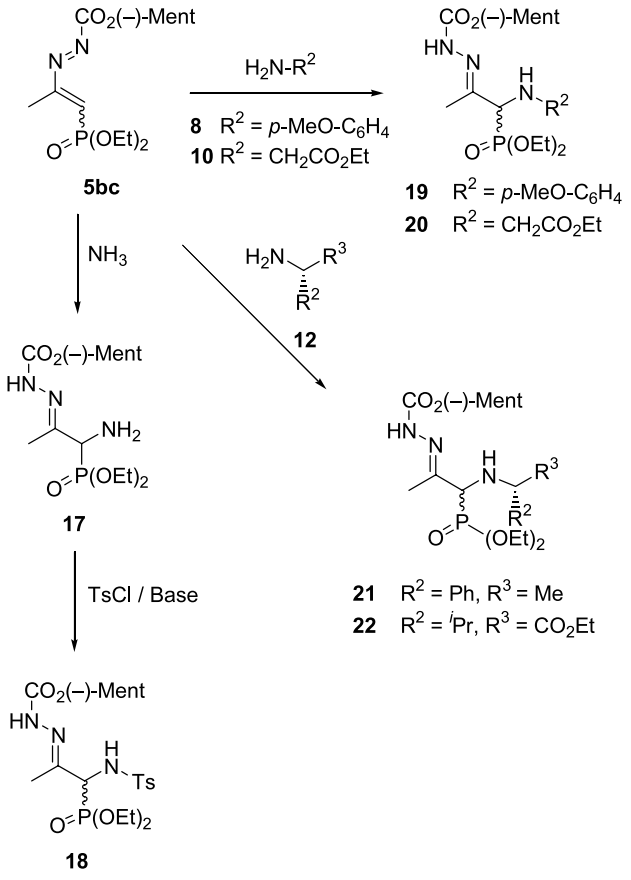
2.4. Michael addition of amine derivatives to 1,2-diaza-1,3-butadiene 26

Finally, we explored the effect of the presence of an optically active group[21] at C-3 of 1,2-diazadienes **II** (Fig. 1) in the Michael addition of these substrates. The preparation

Table 3. Diastereoselective addition of optically active amines to azo-alkenes **5**

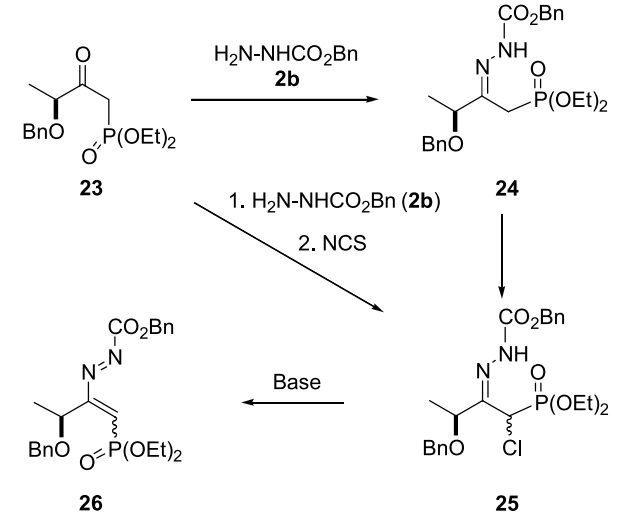
Entry	Compound	R	R^1	R^2	R^3	T (°C)	d.e. (%)[a]	Yield (%)[b]
1	**13aa**	Ph	Et	Ph	Me	0	<10	79
2	**13ba**	OEt	Et	Ph	Me	0	<10	88
3	**14**	OEt	Bn	CO_2Me	^{i}Pr	0	12	91
4	**15**	OEt	Bn	^{i}Pr	CO_2Me	0	12	96
5	**16ba**	OEt	Et	^{i}Pr	CO_2Et	−40	20	95
6	**16bb**	OEt	Bn	^{i}Pr	CO_2Et	−40	18	91

[a] d.e. was calculated by ^{31}P NMR on the crude reaction mixture.
[b] Yield of isolated purified compounds.



Scheme 7.

of phosphorus substituted 1,2-diaza-1,3-butadiene **IX** (Scheme 2, R^2=Me, Y=OBn) derived from lactate was performed in several steps. Initially, we synthesized optically pure β-ketophosphonate **23** by addition of (2*S*)-ethyl lactate benzyl ester to lithium salts derived from diethyl methylphosphonate through a modified procedure of the Shapiro method[25] (Scheme 8, Table 4, entry 1). Condensation reaction of β-ketophosphonate **23** with benzyl carbazate **2b** in refluxing methanol led to the formation of unstable *syn*-β-hydrazone **24** (Scheme 8, Table 4, entry 2).



Scheme 8.

Phosphonate **25** was directly prepared from carbonyl compound **23**, and crude product **24** were treated without isolation with 1.1 equiv of NCS in carbon tetrachloride at room temperature to give *syn*-chlorohydrazone **25** in moderate yield (Scheme 8, Table 4, entry 3). Addition of triethylamine to a solution of chlorohydrazone **25** in dichloromethane gave the red coloured heterodiene **26** (Scheme 8, Table 4, entry 4). Diaza-alkene **26** is unstable to chromatography and crude reaction mixture without purification was satisfactorily used for the next step. As before, the presence of the diazabutadiene **26** in the crude reaction mixture was confirmed by ^{31}P NMR spectroscopy (*E*/*Z* ratio 83:17).

Michael addition of primary amines to heterodienes **26** was explored. The addition of benzyl amine **8c** (R^2=Bn) or ethyl 3-aminopropanoate **8d** (R^2=$CH_2CH_2CO_2Et$) to 1,2-diaza-1,3-butadiene **26** derived from lactate gave functionalized *syn*-α-aminohydrazone **27a** and **27b**, respectively, (Scheme 9, Table 4, entries 5 and 6) with moderate diastereoisomeric excess. The hydrazonic protons appeared in ^{1}H NMR at higher shifts than 10 ppm, and is consistent with the *syn*-configuration, because in the case of α-aminohydrazones the hydrazonic protons were found between 7.5 and 8.5 ppm.[22] However, a considerable increase of diastereoselectivity was observed when aryl amines **8a** (R^2=*p*-MeO–C_6H_4) or **8e** (R^2=*p*-EtO_2C–C_6H_4) was treated with heterodiene **26** to give *syn*-α-aminohydrazones **27c** and **27d**, respectively, with quite good d.e. (70–84%) (Scheme 9, Table 4, entries 7 and 8). The high diastereoselectivity observed in the case of aryl amines could be explained by means of a π-stacking interaction between the phenyl group of aromatic amines and the benzyl group of the substituent at C-3 in aza-alkene.

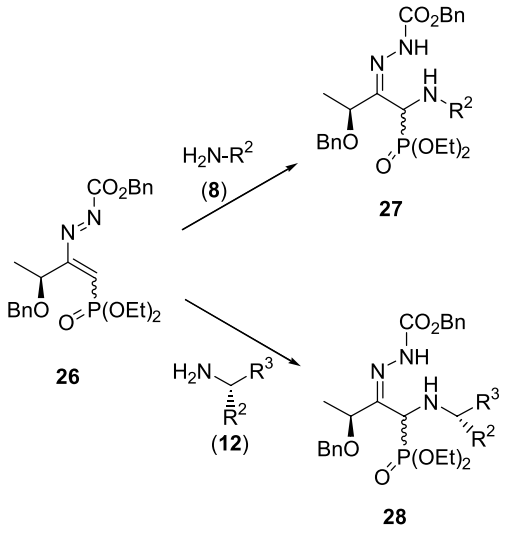
Finally, we studied the diastereoselective addition of optically active amino esters **12** to 4-phosphonyl-1,2-diaza-1,3-butadiene **26** to give *syn*-α-aminohydrazones **28a** (R^2=^{i}Pr, R^3=CO_2Me) obtained also as a non-separable diastereoisomeric mixture and moderate diastereoselectivity (40% d.e.) when (*S*)-valine ester **12c** (R^2=^{i}Pr, R^3=CO_2Me) was used (Scheme 9, Table 4, entry 9). Aryl substituents in the amino esters did not seem to play such an important role as before in the case of aromatic amines, because when (*S*)-phenylalanine ester **12e** (R^2=Bn, R^3=CO_2Me) was used moderate diastereoselectivity (50% d.e.) in the preparation of α-aminophosphonate **28b** (R^2=Bn, R^3=CO_2Me) was observed (Scheme 9, Table 4, entry 10). The diastereoselectivity increases in the case of methyl (*R*)-valinate **12c** (R^2=CO_2Me, R^3=^{i}Pr) and even in this case major diastereoisomer **28c** (R^2=CO_2Me, R^3=^{i}Pr) was isolated (Scheme 9, Table 4, entry 11).

In conclusion, the synthesis of 1-alcoxycarbonyl-1,2-diaza-1,3-butadienes containing a phosphine oxide group **5aa** or a phosphonate group **5ba** and **5bb** at 4-position as well as 4-phosphonyl-1,2-diaza-1,3-butadienes containing optically active substituents at the terminal nitrogen (N-1) **5bc** and at C-3 (derived from lactate) **26**, is described. The process implies 1,4-elimination of HCl from chlorohydrazones in the presence of amines. Michael addition of ammonia, alkyl and aryl amines and aminoesters on phosphorylated 1,2-diaza-1,3-butadienes **5** and **26** is reported and functionalized

Table 4. Hydrazone compounds derived from lactate **23–28**

Entry	Compound	R^2	R^3	d.e. (%)[a]	Yield (%)[b]
1	**23**	—			79[b]
2	**24**	—			>98[c,d]
3	**25**	—			56[e]
4	**26**	—			>98[c,d]
5	**27a**	Bn		40	75[b]
6	**27b**	$CH_2CH_2CO_2Et$		30	64[b]
7	**27c**	*p*-MeO-C_6H_4		70	83[b]
8	**27d**	*p*-EtO_2C-C_6H_4		84	77[b]
9	**28a**	^{i}Pr	CO_2Me	40	87[b]
10	**28b**	Bn	CO_2Me	50	81[b]
11	**28c**	CO_2Me	^{i}Pr	65	57[b,f]

[a] d.e. was calculated by ^{31}P NMR on the crude reaction mixture.
[b] Yield of isolated purified compounds.
[c] Non-isolated compound.
[d] Conversion calculated by ^{31}P NMR of the crude reaction mixture.
[e] Yield of isolated purified compound **25** from ketone **23**.
[f] Yield of isolated major diastereoisomer.



Scheme 9.

α-amino-phosphine oxide and -phosphonates are obtained in very good yields. These hydrazonoalkyl-α-aminophosphonate derivatives may be important synthons in organic synthesis and for the preparation of biologically active compounds of interest to medicinal chemistry.[2,18–20]

3. Experimental

3.1. General

Solvents for extraction and chromatography were of technical grade. All solvents used in reactions were freshly distilled. All other reagents were recrystallized or distilled as necessary. All reactions were performed under an atmosphere of dry nitrogen. Analytical TLC's were performed with silica gel 60 F_{254} plates. Spot visualization was accomplished by UV light or $KMnO_4$ solution. Flash chromatography was carried out using silica gel 60 (230–400 mesh). Melting points were determined with a Electrothermal IA9100 digital apparatus and are uncorrected. ^{1}H (300 MHz), ^{13}C (75 MHz) and ^{31}P NMR (120 MHz) spectra were recorded on a Varian Unity Plus 300 MHz spectrometer using tetramethylsilane (TMS) (0.00 ppm) or chloroform (7.24 ppm) as an internal reference in $CDCl_3$ solutions for ^{1}H NMR spectra, or chloroform (77.0 ppm) as an internal reference in $CDCl_3$ solutions for ^{13}C NMR spectra, and phosphoric acid (85%) for ^{31}P NMR spectra. Chemical shifts (δ) are given in ppm; multiplicities are indicated by s (singlet), bs (broad singlet), d (doublet), dd (double-doublet), t (triplet), q (quadruplet) or m (multiplet). Coupling constants (J) are reported in Hertz. Low-resolution mass spectra (MS) were obtained on a Hewlett Packard 5971 MSD Series spectrometer at 50–70 eV by electron impact (EI) or on a Hewlett Packard 1100 MSD Series spectrometer by chemical ionization (CI). Data are reported in the form m/z (intensity relative to base = 100). Infrared spectra (IR) were taken on a Nicolet FTIR Magna 550 spectrometer, and were obtained as solids in KBr or as neat oils in NaCl. Peaks are reported in cm^{-1}. Elemental analyses were performed in a Perkin Elmer Model 240 instrument. $[\alpha]_D^{20}$ were taken on a Perkin Elmer Model 341 polarimeter using a Na/HaI lamp. Methyl phosphonic acid diethyl ester[26] and 2-benzyloxy propionic acid ethyl ester[25] were synthesized according to literature procedures.

3.1.1. Synthesis of (−)-menthyl carbazate (2c). Following the literature procedure[27] with some modifications: to a stirred solution of benzyltriethylammonium chloride (0.02 g, 0.1 mmol) and K_2CO_3 (2.07 g, 15 mmol) in CCl_4 (10 mL) and CH_2Cl_2 (40 mL), was added hydrazine hydrate 80% (2.0 g, 50 mmol) at room temperature. After 15 min at room temperature a solution of (−)-menthyl chloroformate (2.14 mL, 10 mmol) in CH_2Cl_2 (10 mL), was added dropwise. Then, the mixture was stirred at room temperature for 3 h. The crude mixture was washed with H_2O (2× 20 mL) and extracted with CH_2Cl_2 (10 mL), and the solvent was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by crystallization from hexanes to give **2c** (1.88 g, 88%) as a white solid: mp 97–98 °C. Anal. Calcd. for $C_{11}H_{22}N_2O_2$: C, 61.65; H, 10.35; N, 13.07. Found C, 61.73; H, 10.32; N, 13.05.

3.2. General procedure for the preparation of functionalized β-hydrazones derived from phosphine oxides and phosphonates (3)

To a stirred solution of phosphorylated allene **1a** or **1b** (10 mmol) in dry chloroform (50 mL), was added carbazate **2** (12 mmol) under a nitrogen atmosphere. Then, the mixture was stirred and heated at reflux for 16–24 h. The solvent was evaporated under vacuum, and the crude product was precipitated with diethyl ether and recrystallized from a mixture of hexanes/CH_2Cl_2 (for β-hydrazones derived from phosphine oxides **3aa**); while β-hydrazones derived from phosphonates **3ba**, **3bb** and **3bc** were purified by flash-chromatography (silica gel).

3.2.1. *Anti*-ethyl *N*-[2-(diphenylphosphinoyl)-1-methylethylidene] hydrazinecarboxylate (3aa). (3.16 g, 92%) obtained as a white solid from allene **1a** (2.40 g, 10 mmol) and ethyl carbazate (1.25 g, 12 mmol) as described in the general procedure: mp 168–169 °C; ^{1}H NMR ($CDCl_3$) δ 10.63 (s, 1H), 7.74–7.21 (m, 10H), 4.20 (q, $^3J_{HH}$=7.0 Hz, 2H), 3.33 (d, $^2J_{PH}$=14.8 Hz, 2H), 1.58 (s, 3H), 1.25 (t, $^3J_{HH}$=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 155.8, 146.8, 132.8–128.6 (m), 61.5, 36.2 (d, $^1J_{PC}$=63.0 Hz), 26.0, 14.6; ^{31}P NMR ($CDCl_3$) δ 32.3; IR (KBr) 3186, 2989, 2957, 1741, 1521, 1244, 1164, 1043; MS (EI) *m/z* 344 (M^+, 8). Anal. Calcd. for $C_{18}H_{21}N_2O_3P$: C, 62.78; H, 6.15; N, 8.14. Found C, 62.58; H, 6.17; N, 8.17.

3.2.2. *Syn*- and *anti*-diethyl [2-(ethoxycarbonyl hydrazono) propyl] phosphonate (3ba). 76% conversion calculated by ^{31}P NMR on the crude reaction mixture. Obtained as an oil from allene **1b** (1.76 g, 10 mmol) and ethyl carbazate **2a** (1.25 g, 12 mmol) as described in the general procedure. The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.23, AcOEt): ^{1}H NMR ($CDCl_3$) δ 9.21 (s, 1H, *anti*), 7.67 (s, 1H, *syn*), 4.13 (m, 6H *syn* and *anti*), 2.88 (d, $^2J_{PH}$=22.1 Hz, 2H, *syn*), 2.79 (d, $^2J_{PH}$=23.2 Hz, 2H, *anti*), 2.06 (d, $^4J_{PH}$=2.9 Hz, 3H, *syn*), 1.92 (d, $^4J_{PH}$=2.9 Hz, 3H, *anti*), 1.26 (m, 9H, *syn* and *anti*); ^{13}C NMR ($CDCl_3$) δ 157.2 (*syn*), 153.0 (*anti*), 144.5 (*syn*), 143.7 (*anti*), 61.1–59.4 (m), 35.1 (d, $^1J_{PC}$=135.0 Hz, *anti*), 28.6 (d, $^1J_{PC}$=132.4 Hz, *syn*), 14.5, 15.1 (*syn*), 12.8 (*anti*); ^{31}P NMR ($CDCl_3$) δ 24.1 (*anti*), 23.5 (*syn*); IR (NaCl) 3264, 2986, 1726, 1527, 1249; MS (CI) *m/z* 281 (M^++1, 100). Anal. Calcd. for $C_{10}H_{21}N_2O_5P$: C, 42.86; H, 7.55; N, 10.00. Found C, 42.77; H, 7.56; N, 9.96.

3.2.3. *Syn*- and *anti*-diethyl [2-(benzyloxycarbonyl hydrazono)propyl] phosphonate (3bb). 95% conversion calculated by ^{31}P NMR on the crude reaction mixture. Obtained as an oil from allene **1b** (1.76 g, 10 mmol) and benzyl carbazate (1.99 g, 12 mmol) as described in the general procedure. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 70:30, R_f=0.14, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 9.36 and 8.06 (s, 1H, *syn* and *anti*), 7.41–7.31(m, 5H, *anti* and *syn*), 5.22 (s, 2H, *syn* and *anti*), 4.18–4.05 (m, 4H, *anti* and *syn*), 2.92 (d, 2H, $^2J_{PH}$=22.1 Hz, *anti*), 2.83 (d, 2H, $^2J_{PH}$=23.2 Hz, *syn*), 2.12 (d, 3H, $^4J_{PH}$=2.9 Hz, *syn*), 1.95 (d, 3H, $^4J_{PH}$=3.1 Hz, *anti*), 1.30 (t, 6H, $^3J_{HH}$=7.0 Hz, *anti* and *syn*); ^{13}C NMR ($CDCl_3$) δ 154.4 (*syn*), 153.7 (*anti*), 145.9 (*syn*), 145.7 (*anti*), 135.8–127.6 (m), 66.7, 62.5 (d, $^2J_{PC}$=6.6 Hz, *syn*), 61.7 (d, $^2J_{PC}$=6.6 Hz, *anti*), 36.5 (d, $^1J_{PC}$=136.0 Hz, *anti*), 30.5 (d, $^1J_{PC}$=135.0 Hz, *syn*), 25.0, 15.8; ^{31}P NMR ($CDCl_3$) δ 24.1 (*anti*), 23.4 (*syn*); IR (NaCl) 3230, 2979, 1739, 1513, 1228; MS (CI) *m/z* 343 (M^++1, 67). Anal. Calcd. for $C_{15}H_{23}N_2O_5P$: C, 52.63; H, 6.77; N, 8.18. Found C, 52.80; H, 6.75; N, 8.21.

3.2.4. *Syn*- and *anti*-diethyl [2-((−)-menthyloxycarbonyl hydrazono)propyl] phosphonate (3bc). 93% conversion calculated by ^{31}P NMR on the crude reaction mixture. Obtained as an oil from allene **1b** (1.76 g, 10 mmol) and (−)-menthyl carbazate **2c** (2.57 g, 12 mmol) as described in the general procedure. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 70:30, R_f=0.24 and 0.38 AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 9.06 (s, 1H, *syn*), 7.63 (s, 1H, *anti*), 4.76–4.67 (m, 1H, *syn* and *anti*), 4.22–4.11 (m, 4H, *syn* and *anti*), 2.95 (d, $^2J_{PH}$=21.8 Hz, 2H, *anti*), 2.85 (d, $^2J_{PH}$=23.2 Hz, 2H, *syn*), 2.14–0.78 (m, 27H, *syn* and *anti*); ^{13}C NMR ($CDCl_3$) δ 154.2 (*syn*), 153.4 (*anti*), 145.2 (*syn*), 144.7 (*anti*), 75.3 (*anti*), 74.6 (*syn*), 62.5–62.0 (m), 47.1 (*anti*), 46.7 (*syn*), 40.9 (*anti*), 40.7 (*syn*), 36.3 (d, $^1J_{PC}$=136.0 Hz, *anti*), 34.0 (*anti*), 33.8 (*syn*), 31.1 (*anti*), 30.9 (*syn*), 30.5 (d, $^1J_{PC}$=135.0 Hz, *syn*), 25.9 (*anti*), 25.6 (*syn*), 23.2 (*anti*), 23.0 (*syn*), 21.7 (*anti*), 21.6 (*syn*), 20.5 (*anti*), 20.3 (*syn*), 16.1 (*syn*), 16.0 (*anti*), 15.8 (*syn*), 15.9 (*anti*), 15.8 (*syn*), 15.7 (*anti*); ^{31}P NMR ($CDCl_3$) δ 24.2 (*anti*), 23.4 (*syn*); IR (NaCl) 3230, 2952, 1725, 1699, 1540, 1235; MS (CI) *m/z* 391 (M^++1, 100). Anal. Calcd. for $C_{18}H_{35}N_2O_5P$: C, 55.37; H, 9.04; N, 7.17. Found C, 55.56; H, 9.01; N, 7.17. $[\alpha]_D^{22}$ −36.0 (*c* 0.78, CH_2Cl_2).

3.3. General procedure for the synthesis of functionalized chlorohydrazones derived from phosphine oxides and phosphonates (4)

Method A. To a stirred solution of phosphorylated β-hydrazones **3** (10 mmol) in dry carbon tetrachloride (150 mL), was added dropwise NCS (1.50 g, 11 mmol) under a nitrogen atmosphere. Then, the mixture was stirred at room temperature for 16 h. The crude mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (2×20 mL) and the solvent was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by precipitation with diethyl ether. *Method B*. To a stirred solution of phosphorylated allene **1** (10 mmol) in dry chloroform (50 mL), was added carbazate **2** (12 mmol) under a nitrogen atmosphere. Then, the mixture was stirred and heated at reflux for 16–24 h. The solvent was evaporated under vacuum, and crude products **3** were diluted in dry carbon tetrachloride (150 mL). NCS (1.50 g, 11 mmol) was added dropwise under a nitrogen atmosphere and the mixture was stirred at room temperature for 16 h. The crude mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (2× 20 mL) and the solvent was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by flash-chromatography (silica gel).

3.3.1. Ethyl *N*-[2-chloro-2-(diphenylphosphinoyl)-1-methylethylidene] hydrazinecarboxylate (4aa). (2.65 g, 70%) obtained as a white solid from hydrazone **3aa** (3.44 g, 10 mmol) as described in the general procedure (method A). The crude product was recrystallized from CH_2Cl_2/hexanes:

mp 161–162 °C; ^{1}H NMR ($CDCl_3$) δ 7.74–7.39 (m, 11H), 5.30 (s, 1H), 4.20 (q, $^3J_{HH}$=7.1 Hz, 2H), 1.96 (s, 3H), 1.22 (t, $^3J_{HH}$=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 153.8, 145.7, 134.0–128.0 (m), 62.1, 59.5 (d, $^1J_{PC}$=68.5 Hz), 14.4, 12.6; ^{31}P NMR ($CDCl_3$) δ 28.0; IR (KBr) 3238, 2933, 1700, 1441, 1262, 1189; MS (CI) *m/z* 379 (M^++1, 100). Anal. Calcd. for $C_{18}H_{20}ClN_2O_3P$: C, 57.07; H, 5.32; N, 7.40. Found C, 56.84; H, 5.34; N, 7.36.

3.3.2. Diethyl [1-chloro-2-(ethoxycarbonyl hydrazono)-propyl] phosphonate (4ba). (1.48 g, 47%) obtained as an oil in a 'one pot' reaction from allene **1b** (1.76 g, 10 mmol) as described in the general procedure (method B). The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.43, AcOEt): ^{1}H NMR ($CDCl_3$) δ 8.07 (s, 1H), 4.78 (d, $^2J_{PH}$=13.6 Hz, 1H), 4.32–4.09 (m, 6H), 2.00 (d, $^4J_{PH}$=2.1 Hz, 3H), 1.39–1.23 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 154.5, 145.0, 65.2–61.3 (m), 55.5 (d, $^1J_{PC}$=158.6 Hz), 15.8, 13.9, 11.9; ^{31}P NMR ($CDCl_3$) δ 15.5; IR (NaCl) 3230, 2979, 1725, 1533, 1208; MS (CI) *m/z* 315 (M^++1, 100). Anal. Calcd. for $C_{10}H_{20}ClN_2O_5P$: C, 38.17; H, 6.41; N, 8.90. Found C, 38.05; H, 6.44; N, 8.89.

3.3.3. Diethyl [1-chloro-2-(benzyloxycarbonyl hydrazono) propyl] phosphonate (4bb). (2.07 g, 55%) obtained as an oil in a 'one pot' reaction from allene **1b** (1.76 g, 10 mmol) as described in the general procedure (method B). The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 30:70, R_f=0.45, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H). 7.39–7.28 (m, 5H), 5.23 (s, 2H), 4.77 (d, $^2J_{PH}$=13.4 Hz, 1H), 4.29–4.09 (m, 4H), 2.00 (s, 3H), 1.36–1.27 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 153.5, 145.7, 135.5–128.4 (m), 128.3, 67.6, 64.2 (d, $^2J_{PC}$=6.5 Hz), 63.7 (d, $^2J_{PC}$=7.1 Hz), 55.7 (d, $^1J_{PC}$=158.1 Hz), 16.2, 12.0; ^{31}P NMR ($CDCl_3$) δ 15.4; IR (NaCl) 3204, 2992, 1699, 1540, 1261; MS (CI) *m/z* 377 (M^++1, 45). Anal. Calcd. for $C_{15}H_{22}ClN_2O_5P$: C, 47.82; H, 5.89; N, 7.44. Found C, 47.98; H, 5.86; N, 7.47.

3.3.4. Diethyl [1-chloro-2-((−)-menthyloxycarbonyl hydrazono)propyl] phosphonate (4bc). (1.83 g, 43%) obtained as a white solid in a 'one pot' reaction from allene **1b** (1.76 g, 10 mmol) as described in the general procedure (method B). The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 30:70): mp 70–71 °C; ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 4.82–4.70 (m, 2H), 4.32–4.15 (m, 4H), 2.03 (d, $^4J_{PH}$=2.0 Hz, 3H), 1.94–0.79 (m, 24H); ^{13}C NMR ($CDCl_3$) δ 153.0, 144.8, 76.2, 64.4 (d, $^2J_{PC}$=6.6 Hz), 63.8 (d, $^2J_{PC}$=7.1 Hz), 55.8 (d, $^1J_{PC}$=158.1 Hz), 47.3, 41.1, 34.2, 31.4, 26.2, 23.4, 22.0, 20.7, 16.4, 16.3, 11.8; ^{31}P NMR ($CDCl_3$) δ 15.5; IR (KBr) 3217, 2965, 1706, 1533, 1261; MS (CI) *m/z* 425 (M^++1, 38). Anal. Calcd. for $C_{18}H_{34}ClN_2O_5P$: C, 50.88; H, 8.07; N, 6.59. Found C, 51.04; H, 8.09; N, 6.56. [α]$_D^{22}$ −32.0 (*c* 1.00, CH_2Cl_2).

3.4. Preparation of 1,2-diaza-1,3-butadienes (5)

To a room temperature solution of chlorohydrazone **4** (5 mmol) in dry CH_2Cl_2 (25 mL), was added dropwise triethylamine (0.85 mL, 6 mmol) under a nitrogen atmosphere. The mixture was stirred at this temperature for 45 min. The crude mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (2×20 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (10 mL). The solvent was dried over $MgSO_4$ and evaporated under vacuum. Diaza-alkenes **5** proved to be unstable to chromatography and were then used in the next steps without further purification.

3.4.1. 4-(Diphenylphosphinoyl)-1-ethoxycarbonyl-3-methyl-1,2-diaza-1,3-butadiene (5aa). >98% conversion calculated by ^{31}P NMR on the crude reaction mixture, from chlorohydrazone **4aa** (1.90 g, 5 mmol) as described in the general procedure: (R_f=0.81, AcOEt). ^{1}H NMR ($CDCl_3$) δ 7.89–7.40 (m, 11H), 4.48 (q, $^3J_{HH}$=7.2 Hz, 2H, *E*), 4.21 (q, $^3J_{HH}$=7.2 Hz, 2H, *Z*), 2.29 (d, $^4J_{PH}$=2.3 Hz, 3H, *E*), 2.18 (d, $^4J_{PH}$=1.5 Hz, 3H, *Z*), 1.43 (t, $^3J_{HH}$=7.2 Hz, 3H, *E*), 1.26 (t, $^3J_{HH}$=7.2 Hz, 3H, *Z*); ^{13}C NMR ($CDCl_3$) δ (*E*-isomer) 165.1 (d, $^2J_{PC}$=7.1 Hz), 161.8, 135.2 (d, $^1J_{PC}$=97.2 Hz), 134.7–127.8 (m), 64.1, 13.5 (d, $^3J_{PC}$=9.1 Hz), 11.3; ^{31}P NMR ($CDCl_3$) δ 26.4 (*Z*), 20.5 (*E*); IR (NaCl) 2979, 1732, 1533, 1440, 1228.

3.4.2. 4-(Diethoxyphosphoryl)-1-ethoxycarbonyl-3-methyl-1,2-diaza-1,3-butadiene (5ba). >98% conversion calculated by ^{31}P NMR on the crude reaction mixture, from chlorohydrazone **4ba** (1.57 g, 5 mmol) as described in the general procedure: (R_f=0.63, AcOEt). ^{1}H NMR ($CDCl_3$) δ 6.84 (d, $^2J_{PH}$=13.0 Hz, 1H, *E*), 6.70 (d, $^2J_{PH}$=13.0 Hz, 1H, *Z*), 4.52–4.10 (m, 6H), 2.04–2.00 (m, 3H), 1.47–1.24 (m, 9H); ^{13}C NMR ($CDCl_3$) δ (*E*-isomer) 164.7 (d, $^2J_{PC}$=15.1 Hz), 161.9, 129.8 (d, $^1J_{PC}$=187.9 Hz), 64.3–61.9 (m), 16.0–11.4 (m); ^{31}P NMR ($CDCl_3$) δ 14.2 (*E*), 13.0 (*Z*); IR (NaCl) 3482, 2979, 1752, 1255.

3.4.3. 4-(Diethoxyphosphoryl)-1-benzyloxycarbonyl-3-methyl-1,2-diaza-1,3-butadiene (5bb). >98% conversion calculated by ^{31}P NMR on the crude reaction mixture, from chlorohydrazone **4bb** (1.88 g, 5 mmol) as described in the general procedure: (R_f=0.78, AcOEt). ^{1}H NMR ($CDCl_3$) δ 7.46–7.37 (m, 5H), 6.84 (d, $^2J_{PH}$=13.0 Hz, 1H, *E*), 6.69 (d, $^2J_{PH}$=13.0 Hz, 1H, *Z*), 5.42 (s, 2H, *E*), 5.40 (s, 2H, *Z*), 4.22–4.06 (m, 4H), 2.21 (d, $^4J_{PH}$=2.90 Hz, 3H, *E*), 1.98 (s, 3H, *Z*), 1.38–1.21 (m, 6H); ^{13}C NMR ($CDCl_3$) δ (*E*-isomer) 164.4 (d, $^2J_{PC}$=15.2 Hz) 161.5, 130.2 (d, $^1J_{PC}$=187.9 Hz) 133.6–127.6 (m), 69.3, 61.6 (d, $^2J_{PC}$=5.5 Hz), 15.7 (d, $^3J_{PC}$=6.0 Hz), 11.1; ^{31}P NMR ($CDCl_3$) δ 14.2 (*E*), 12.8 (*Z*); IR (NaCl) 3456, 2979, 1765, 1235.

3.4.4. 4-(Diethoxyphosphoryl)-1-(−)-menthyloxycarbonyl-3-methyl-1,2-diaza-1,3-butadiene (5bc). >98% conversion calculated by ^{31}P NMR on the crude reaction mixture, from chlorohydrazone **4bc** (2.12 g, 5 mmol) as described in the general procedure: (R_f=0.78, AcOEt). ^{1}H NMR ($CDCl_3$) δ 6.75 (d, $^2J_{PH}$=13.0 Hz, 1H, *E*), 6.60 (d, $^2J_{PH}$=13.0 Hz, 1H, *Z*), 4.87–4.78 (m, 1H), 4.15–4.08 (m, 4H), 2.16–0.74 (m, 27H); ^{13}C NMR ($CDCl_3$) δ (*E*-isomer) 164.7 (d, $^2J_{PC}$=15.5 Hz), 161.7, 129.2 (d, $^2J_{PH}$=187.9 Hz), 78.7, 61.7 (d, $^2J_{PC}$=5.5 Hz), 46.0, 40.1, 33.5, 31.0, 25.7, 22.9, 21.4, 20.1, 15.8, 15.7–11.3 (m); ^{31}P NMR ($CDCl_3$) δ 14.5 (*E*), 13.1 (*Z*); IR (NaCl) 3482, 2959, 1752, 1248.

3.4.5. Synthesis of ethyl *N*-[2-amino-2-(diphenylphosphinoyl)-1-methylethylidene] hydrazinecarboxylate (6aa). To a stirred solution of chlorohydrazone **4aa**

(0.38 g, 1 mmol) in chloroform (10 mL), ammonia was bubbled for 10 min at room temperature. Then, the solvent was evaporated under vacuum and the crude product was purified by flash-chromatography (silica gel, CH_2Cl_2/MeOH 95:5) affording compound **6aa** (0.24 g, 68%) as a white solid: 174–176 °C; ^{1}H NMR ($CDCl_3$) δ 7.96–7.46 (m, 11H), 4.53 (d, $^2J_{PH}$=10.4 Hz, 1H), 4.24–4.17 (m, 2H), 2.19 (bs, 2H), 1.92 (s, 3H), 1.28–1.24 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 150.0, 144.5, 132.8–128.0 (m), 61.2, 58.6 (d, $^1J_{PC}$=74.0 Hz), 14.4, 14.0; ^{31}P NMR ($CDCl_3$) δ 31.3; IR (KBr) 3350, 2972, 1712, 1427, 1235; MS (CI) *m/z* 360 (M^++1, 7). Anal. Calcd. for $C_{18}H_{22}N_3O_3P$: C, 60.16; H, 6.17; N, 11.69. Found C, 60.48; H, 6.20; N, 11.69.

3.4.6. Synthesis of diethyl [2-(benzyloxycarbonyl hydrazono)-1-(toluene-4-sulfonylamino)propyl] phosphonate (7bb). To a stirred solution of chlorohydrazone **4bb** (0.38 g, 1 mmol) in chloroform (10 mL), ammonia was bubbled for 10 min at room temperature. Then, the solvent was evaporated under vacuum and the crude product was diluted in CH_2Cl_2 (5 mL). TsCl (0.23 g, 1.2 mmol) and Et_3N (0.21 mL, 1.5 mmol) were then added at room temperature. The mixture was stirred at this temperature for 6 h. The crude mixture was diluted with CH_2Cl_2 (10 mL), washed with H_2O (2×5 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (5 mL). The solvent was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 70:30) to obtain (0.19 g, 38%) of **7bb** as a white solid. mp 145–146 °C; ^{1}H NMR ($CDCl_3$) δ 7.82–7.27 (m, 10H), 6.28 (bs, 1H), 5.22 (s, 2H), 4.34–4.11 (m, 5H), 2.30 (s, 3H), 1.62 (d, $^4J_{PH}$=2.6 Hz, 3H), 1.31–1.26 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 153.6, 144.9, 143.6–126.4 (m), 67.6, 64.5 (d, $^2J_{PC}$=7.1 Hz), 63.7 (d, $^2J_{PC}$=7.1 Hz), 56.4 (d, $^1J_{PC}$=155.1 Hz), 21.4, 16.2, 14.2; ^{31}P NMR ($CDCl_3$) δ 16.7; IR (NaCl) 3350, 3297, 3138, 1745, 1454, 1195; MS (CI) *m/z* 512 (M^++1, 100). Anal. Calcd. for $C_{22}H_{30}N_3O_7PS$: C, 51.66; H, 5.91; N, 8.21. Found C, 51.70; H, 5.89; N, 8.25.

3.5. General procedure for the addition of primary amines to azo-alkenes (5)

Method A. To a stirred solution of chlorohydrazone **4** (1 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (0.21 mL, 1.5 mmol) at room temperature and under a nitrogen atmosphere. The mixture was stirred at room temperature for 15–45 min and a solution of primary amine (1.2 mmol) in CH_2Cl_2 (5 mL) was then added at the same temperature. The reaction mixture was stirred at room temperature for 20–120 min. The crude mixture was diluted with CH_2Cl_2 (10 mL), washed with H_2O (2×5 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (5 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum and the crude product was purified by flash-chromatography (silica gel). Method B: to a stirred solution of chlorohydrazone **4** (1 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (0.21 mL, 1.5 mmol) at room temperature and under a nitrogen atmosphere. The mixture was stirred at room temperature for 30–45 min. The crude mixture was diluted with CH_2Cl_2 (10 mL), washed with H_2O (2×5 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (5 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum. Diaza-alkenes **5** were diluted with CH_2Cl_2 (10 mL) and a solution of primary amine (1.2 mmol) in CH_2Cl_2 (5 mL) was then added at the same temperature. The reaction mixture was stirred at room temperature for 20–120 min. The solvent was evaporated under vacuum and the crude product was purified by flash-chromatography (silica gel).

3.5.1. Ethyl *N*-[2-(diphenylphosphinoyl)-2-(4-methoxyphenylamino)-1-methylethylidene] hydrazinecarboxylate (9aaa). (0.40 g, 86%) obtained as a white solid from **4aa** (0.38 g, 1 mmol) and 4-methoxyaniline (0.15 g, 1.2 mmol) as described in the general procedure (method A). The crude product was crystallized from ethyl ether: mp 183–184 °C; ^{1}H NMR ($CDCl_3$) δ 7.73–6.55 (m, 15H), 4.94 (dd, $^3J_{HH}$=9.2, $^2J_{PH}$=12.2 Hz, 1H), 4.76 (m, 1H), 4.06 (q, $^3J_{HH}$=7.0 Hz, 2H), 3.56 (s, 3H), 1.56 (s, 3H), 1.11 (t, $^3J_{HH}$=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 152.8, 149.1, 140.2–114.8 (m), 61.6, 60.8 (d, $^1J_{PC}$=74.0 Hz), 55.6, 14.4, 12.4; ^{31}P NMR ($CDCl_3$) δ 31.5; IR (NaCl) 3357, 3291, 1746, 1501, 1441, 1235; MS (CI) *m/z* 466 (M^++1, 100). Anal. Calcd. for $C_{25}H_{28}N_3O_4P$: C, 64.51; H, 6.06; N, 9.03. Found C, 64.29; H, 6.08; N, 9.00.

3.5.2. Ethyl *N*-[2-(diphenylphosphinoyl)-2-propargylamino-1-methylethylidene] hydrazinecarboxylate (9aab). (0.31 g, 78%) obtained as a white solid from **4aa** (0.38 g, 1 mmol) and propargilamine (0.066 g, 1.2 mmol) as described in the general procedure (method A). The crude product was crystallized from ethyl ether: mp 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 8.00–7.40 (m, 11H), 4.35 (d, $^3J_{PH}$=13.9 Hz, 1H), 4.23–4.16 (m, 2H), 3.35 (s, 2H), 2.45 (bs, 1H), 2.17 (s, 1H), 1.92 (s, 3H), 1.28–1.22 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 153.5, 148.7, 132.0–128.2 (m), 81.1, 72.1, 63.8 (d, $^1J_{PC}$=79.6 Hz,), 61.6, 37.1 (d, $^3J_{PC}$=14.6 Hz), 14.6, 14.5; ^{31}P NMR ($CDCl_3$) δ 29.5; IR (NaCl) 3330, 3197, 2979, 1739, 1527, 1447, 1241; MS (CI) *m/z* 398 (M^++1, 5). Anal. Calcd. for $C_{21}H_{24}N_3O_3P$: C, 63.47; H, 6.09; N, 10.57. Found C, 63.62; H, 6.10; N, 10.53.

3.5.3. Diethyl [2-(ethoxycarbonyl hydrazono)-1-(-4-methoxyphenylamino)propyl] phosphonate (9baa). (0.37 g, 92%) obtained as an oil from **4ba** (0.32 g, 1 mmol) and 4-methoxyphenilaniline (0.15 g, 1.2 mmol) as described in the general procedure (method A). The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.44, AcOEt): ^{1}H NMR ($CDCl_3$) δ 7.77 (s, 1H), 6.77–6.67 (m, 4H), 4.55–4.49 (m, 2H), 4.31–4.13 (m, 6H), 3.73 (s, 3H), 1.86 (d, $^4J_{PH}$=2.7 Hz, 3H), 1.34–1.28 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 152.6 148.2, 140.3–114.4 (m), 63.4 (d, $^2J_{PC}$=7.1 Hz), 62.9 (d, $^2J_{PC}$=7.1 Hz), 61.4, 58.9 (d, $^1J_{PC}$=151.6 Hz), 55.3, 16.0, 14.2, 12.5; ^{31}P NMR ($CDCl_3$) δ 20.8; IR (NaCl) 3323, 2998, 1739, 1513, 1235, 1049; MS (CI) *m/z* 402 (M^++1, 100). Anal. Calcd. for $C_{17}H_{28}N_3O_6P$: C, 50.87; H, 7.03; N, 10.47. Found C, 51.03; H, 7.00; N, 10.43.

3.5.4. Diethyl [2-(benzyloxycarbonyl hydrazono)-1-benzylaminopropyl] phosphonate (9bbc). (0.44 g, 98%) obtained as an oil in a reaction from **4bb** (0.38 g, 1 mmol) and benzylamine (0.13 g, 1.2 mmol) as described in the general procedure (method A). The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 30:70, R_f=0.32, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.85

(s, 1H), 7.42–7.22(m, 10H), 5.25 (s, 2H), 4.21–4.08 (m, 4H), 3.85 (d, $^2J_{PH}$=21.7 Hz, 1H), 3.78–3.67 (m, 2H), 2.27 (bs, 1H), 1.85 (d, $^4J_{PH}$=2.6 Hz, 3H), 1.32–1.26 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 153.7, 148.2, 138.8–126.6 (m), 66.6, 62.7 (d, $^2J_{PC}$=6.6 Hz), 62.2 (d, $^2J_{PC}$=7.5 Hz), 61.5 (d, $^1J_{PC}$=152.6 Hz), 51.7 (d, $^3J_{PC}$=17.8 Hz), 15.8, 13.1; ^{31}P NMR ($CDCl_3$) δ 22.2; IR (NaCl) 3211, 2965, 1752, 1527, 1208; MS (CI) *m/z* 448 (M^++1, 100). Anal. Calcd. for $C_{22}H_{30}N_3O_5P$: C, 59.05; H, 6.76; N, 9.39. Found C, 58.88; H, 6.73; N, 9.42.

3.5.5. Ethyl [1-(diphenylphosphinoyl)-2-(ethoxycarbonyl hydrazono)propylamino] acetate (11aaa). (0.40 g, 89%) obtained as a white solid from cholorohydrazone **4aa** (0.38 g, 1 mmol), glycine ethyl ester hydrochloride (0.17 g, 1.2 mmol) and Et_3N (0.42 mL, 3 mmol) as described in the general procedure (method A). The crude product was purified by flash-chromatography (silica gel, AcOEt): mp 147–149 °C; ^{1}H NMR ($CDCl_3$) δ 7.93–7.30 (m, 11H), 4.43 (d, $^2J_{PH}$=11.8 Hz, 1H), 4.22–4.07 (m, 4H), 3.33 (s, 2H), 2.56 (bs, 1H), 1.89 (s, 3H), 1.27–1.18 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 171.5, 153.4, 148.9, 132.0–128.2 (m), 64.7 (d, $^1J_{PC}$=78.6 Hz), 61.7, 60.7, 49.3 (d, $^3J_{PC}$=13.6 Hz), 14.4, 14.0, 13.5; ^{31}P NMR ($CDCl_3$) δ 29.5; IR (KBr) 2979, 1732, 1533, 1440, 1228; MS (CI) *m/z* 446 (M^++1, 100). Anal. Calcd. for $C_{22}H_{28}N_3O_5P$: C, 59.32; H, 6.34; N, 9.43. Found C, 59.11; H, 6.37; N, 9.41.

3.5.6. Ethyl [1-(diethoxyphosphoryl)-2-(ethoxycarbonyl hydrazono)propylamino] acetate (11baa). (0.38 g, 99%) obtained as an oil from hydrazone **4ba** (0.32 g, 1 mmol), glycine ethyl ester hydrochloride (0.17 g, 1.2 mmol) and Et_3N (0.42 mL, 3 mmol) as described in the general procedure (method A). The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.25, AcOEt): ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1H), 4.20–4.05 (m, 8H), 3.87 (d, $^2J_{PH}$=20.6 Hz, 1H), 3.33 (d, $^4J_{PH}$=1.8 Hz, 2H), 1.90 (d, $^4J_{PH}$=1.5 Hz, 3H), 1.34–1.18 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 171.6, 153.9, 148.4, 63.1 (d, $^2J_{PC}$=6.8 Hz), 62.9 (d, $^2J_{PC}$=7.0 Hz), 62.0 (d, $^1J_{PC}$=152.8 Hz), 61.6, 60.7, 49.0 (d, $^3J_{PC}$=16.3 Hz), 16.3–13.0 (m); ^{31}P NMR ($CDCl_3$) δ 20.9; IR (NaCl) 3237, 2979, 1732, 1546, 1367, 1222, 1029; MS (CI) *m/z* 382 (M^++1, 100). Anal. Calcd. for $C_{14}H_{28}N_3O_7P$: C, 44.09; H, 7.40; N, 11.02. Found C, 43.98; H, 7.43; N, 11.03.

3.5.7. Ethyl [1-(diethoxyphosphoryl)-2-(benzyloxycarbonyl hydrazono)propylamino] acetate (11bba). (0.41 g, 93%) obtained as an oil in a reaction from **4bb** (0.38 g, 1 mmol) and glycine ethyl ester hydrochloride (0.17 g, 1.2 mmol) as described in the general procedure (method A) by using Et_3N (0.42 mL, 3 mmol). The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.22, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.86 (s, 1H), 7.41–7.35 (m, 5H), 5.23 (s, 2H), 4.23–4.10 (m, 6H), 3.87 (d, $^2J_{PH}$=20.7 Hz, 1H), 3.38 (d, $^4J_{PH}$=2.8 Hz, 2H), 1.93 (d, $^4J_{PH}$=2.6 Hz, 3H), 1.34–1.20 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 171.2, 153.5, 148.2, 135.6–128.0 (m), 127.8, 66.7, 62.7 (d, $^2J_{PC}$=7.1 Hz), 62.5 (d, $^2J_{PC}$=7.0 Hz), 61.8 (d, $^1J_{PC}$=144.5 Hz,), 60.3, 45.6 (d, $^3J_{PC}$=16.1 Hz), 15.8, 13.6, 12.8; ^{31}P NMR ($CDCl_3$) δ 20.8; IR (NaCl) 3224, 2985, 1725, 1208, 1023,; MS (CI) *m/z* 444 (M^++1, 100). Anal. Calcd. for $C_{19}H_{30}N_3O_7P$: C, 51.46; H, 6.82; N, 9.48. Found C, 51.59; H, 6.80; N, 9.47.

3.5.8. Ethyl *N*-[2-(diphenylphosphinoyl)-1-methyl-2-(1-(*R*)-phenylethylamino)ethylidene] hydrazinecarboxylate (13aa). (0.37 g, 79%) obtained as an oil from hydrazone **4aa** (0.38 g, 1 mmol) and (*R*)-methylbenzylamine (0.16 mL, 1.2 mmol) as described in the general procedure (method A) and addition of primary amine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.60, AcOEt): ^{1}H NMR ($CDCl_3$) δ 7.95–7.03 (m, 16H), 4.40–4.09 (m, 3H), 3.69–3.51 (m, 1H), 2.48 (bs, 1H), 2.45 (bs, 1H), 1.87 (d, $^4J_{PH}$=2.1 Hz, 3H, *mayor*), 1.56 (d, $^4J_{PH}$=2.1 Hz, 3H, *minor*), 1.42–1.25 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 153.8 (*minor*), 149.2 (*mayor*), 144.6 (*minor*), 143.5 (*mayor*), 132.1–125.6 (m), 63.4 (d, $^1J_{PC}$=79.6 Hz, *minor*), 61.7 (d, $^1J_{PC}$=80.1 Hz, *mayor*), 61.1, 57.3 (d, $^3J_{PC}$=11.1 Hz, *minor*), 55.8 (d, $^3J_{PC}$=13.6 Hz, *mayor*), 24.3 (*mayor*), 22.5 (*minor*), 14.2, 13.6; ^{31}P NMR ($CDCl_3$) δ 30.5 (*mayor*), 29.7 (*minor*); IR (NaCl) 3217, 2985, 1706, 1447, 1222; MS (CI) *m/z* 464 (M^++1, 72). Anal. Calcd. for $C_{26}H_{30}N_3O_3P$: C, 67.37; H, 6.52; N, 9.07. Found C, 67.28; H, 6.51; N, 9.04.

3.5.9. Diethyl [2-(ethoxycarbonyl hydrazono)-1-(*R*)-(methylbenzylamino)propyl] phosphonate (13ba). (0.35 g, 88%) obtained as an oil in a reaction from **4ba** (0.32 g, 1 mmol) and (*R*)-methylbenzylamine (0.16 mL, 1.2 mmol) as described in the general procedure (method A) and addition of (*R*)-methylbenzylamine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt, R_f=0.50, AcOEt): ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1H), 7.34–7.20 (m, 5H), 4.28–4.02 (m, 6H), 3.89 (d, $^2J_{PH}$=21.7 Hz, 1H, *mayor*), 3.82–3.65 (m, 1H), 3.55 (d, $^2J_{PH}$=23.5 Hz, 1H, *minor*), 2.32 (bs, 1H), 1.89 (d, $^4J_{PH}$=2.8 Hz, 3H, *minor*), 1.59 (d, $^4J_{PH}$=2.6 Hz, 3H, *mayor*), 1.41–1.23 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 154.6 (*minor*), 149.1 (*mayor*), 145.2 (*minor*), 144.4 (*mayor*), 128.9–125.8 (m), 63.4–62.3 (m), 62.5 (d, $^1J_{PC}$=141.0 Hz, *mayor*), 60.2 (d, $^1J_{PC}$=154.6 Hz, *minor*), 57.3 (d, $^3J_{PC}$=13.6 Hz, *minor*), 56.4 (d, $^3J_{PC}$=17.1 Hz, *mayor*), 24.9 (*mayor*), 23.0 (*minor*), 16.6–13.5 (m); ^{31}P NMR ($CDCl_3$) δ 22.7 (*minor*), 22.2 (*mayor*); IR (NaCl) 3462, 2965, 1725, 1228, 1016; MS (CI) *m/z* 400 (M^++1, 100). Anal. Calcd. for $C_{18}H_{30}N_3O_5P$: C, 54.13; H, 7.57; N, 10.52. Found C, 54.24; H, 7.61; N, 10.55.

3.5.10. Methyl 2-(*R*)-[2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl)propylamino]-3-methyl butyrate (14) and methyl 2-(*S*)-[2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl)propylamino]-3-methyl butyrate (15). (0.43 g, 91%) and (0.45 g, 96%) obtained as an oil from **4bb** (0.38 g, 1 mmol) and D-valine methyl ester hydrochloride (0.20 g, 1.2 mmol) or L-valine methyl ester hydrochloride (0.20 g, 1.2 mmol), respectively, as described in the general procedure (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of amine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 70:30, R_f=0.32, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.79 (s, 1H), 7.69 (s, 1H), 7.24–7.20 (m, 5H), 5.16–5.08 (m, 2H), 4.09–3.93 (m, 4H), 3.74 (d, $^2J_{PH}$=20.0 Hz, 1H), 3.67 (d, $^2J_{PH}$=19.1 Hz, 1H), 3.55 (s, 3H), 3.41 (s, 3H), 2.98–2.93 (m, 1H), 2.76–2.71 (m, 1H), 2.27 (bs, 1H), 1.78 (d, $^4J_{PH}$=2.9 Hz, 3H), 1.75 (d, $^4J_{PH}$=2.3 Hz, 3H), 1.29–1.18 (m, 6H), 0.91–0.82 (m, 6H);

^{13}C NMR ($CDCl_3$) δ 175.0, 174.2, 154.2, 154.1, 149.4, 148.5, 135.4–127.1 (m), 66.2, 65.5 (d, $^3J_{PC}=16.1$ Hz), 64.1 (d, $^3J_{PC}=16.1$ Hz), 62.6–60.5 (m), 50.8, 50.6, 31.1, 30.6, 18.7–12.3 (m); ^{31}P NMR ($CDCl_3$) δ 21.1, 20.6; IR (NaCl) 3217, 2959, 1739, 1540, 1222; MS (CI) *m/z* 472 ($M^+ +1$, 100). Anal. Calcd. for $C_{21}H_{34}N_3O_7P$: C, 53.50; H, 7.27; N, 8.91. Found from L-Valine C, 53.31; H, 7.25; N, 8.94; from D-Valine C 53.38; H, 7.28; N, 8.92.

3.5.11. Methyl 2-(*S*)-[1-(diethoxyphosphoryl)-2-(ethoxycarbonyl hydrazono)propylamino]-3-methyl butyrate (16ba). (0.40 g, 95%) obtained as an oil from **4ba** (0.32 g, 1 mmol) and L-valine ethyl ester hydrochloride (0.22 g, 1.2 mmol) as described in the general procedure (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of amine at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt, $R_f=0.50$, AcOEt): ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1H, *mayor*), 7.71 (s, 1H, *minor*), 4.30–4.09 (m, 8H), 3.84 (d, $^2J_{PH}=20.1$ Hz, 1H, *mayor*), 3.11–3.06 (m, 1H), 2.88–2.83 (m, 1H), 2.34 (bs, 1H), 1.95 (d, $^4J_{PH}=2.8$ Hz, 3H, *mayor*), 1.91 (d, $^4J_{PH}=2.1$ Hz, 3H, *minor*), 1.41–1.19 (m, 12H) 0.99–0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 174.0 (*minor*), 173.1 (*mayor*), 153.6 (*minor*), 148.2 (*mayor*), 65.6 (d, $^3J_{PC}=16.1$ Hz, *minor*), 64.4 (d, $^3J_{PC}=16.1$ Hz, *mayor*), 63.9–59.1 (m), 31.4 (*minor*), 30.9 (*mayor*), 18.9–12.3 (m); ^{31}P NMR ($CDCl_3$) δ 21.1 (*mayor*), 20.7 (*minor*); IR (NaCl) 3224, 2985, 1725, 1235, 1016; MS (CI) *m/z* 424 ($M^+ +1$, 100). Anal. Calcd. for $C_{17}H_{34}N_3O_7P$: C, 48.22; H, 8.09, N, 9.92. Found C. 48.37; H. 8.06; N. 9.89.

3.5.12. Ethyl 2-(*S*)-[2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl)propylamino]-3-methyl butyrate (16bb). (0.44 g, 91%) obtained as an oil from **4bb** (0.38 g, 1 mmol) and L-valine ethyl ester hydrochloride (0.22 g, 1.2 mmol) as described in the general procedure (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of amine at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/ hexanes 70:30, $R_f=0.54$, AcOEt/ hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1H, *mayor*), 7.71 (s, 1H, *minor*), 7.63–7.27 (m, 5H), 5.27–5.22 (m, 2H), 4.26–3.99 (m, 6H), 3.82 (d, $^2J_{PH}=19.8$ Hz, 1H, *mayor*), 3.73–3.71 (m, 1H, *minor*), 3.16–3.10 (m, 1H), 2.85–2.79 (m, 1H), 2.33 (bs, 1H), 1.92 (d, $^4J_{PH}=2.9$ Hz, 3H, *mayor*), 1.89 (d, $^4J_{PH}=2.2$ Hz, 3H, *minor*), 1.35–1.13 (m, 9H), 0.99–0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 174.1, 173.2, 154.2, 153.8, 148.4 (*minor*), 147.9 (*mayor*), 135.6–127.7 (m), 66.6, 65.7 (d, $^3J_{PC}=16.1$ Hz, *minor*), 64.4 (d, $^3J_{PC}=17.0$ Hz, *mayor*), 62.8–62.0 (m), 60.9 (d, $^1J_{PC}=148.1$ Hz), 60.1, 59.9, 31.4 (*minor*), 30.9 (*mayor*), 19.0–12.4 (m); ^{31}P NMR ($CDCl_3$) δ 21.1 (*mayor*), 20.6 (*minor*); IR (NaCl) 3230, 2998, 1739, 1235, 1036; MS (CI) *m/z* 486 ($M^+ +1$, 100). Anal. Calcd. for $C_{22}H_{36}N_3O_7P$: C, 54.42; H, 7.47; N, 8.65. Found C, 54.21; H, 7.46; N, 8.63.

3.5.13. Synthesis of diethyl [2-((-)-menthyloxycarbonyl hydrazono)-1-(toluene-4-sulfonylamino)propyl] phosphonate (18). To a stirred solution of chlorohydrazone **4bc** (0.42 g, 1 mmol) in CH_2Cl_2 (5 mL) was added Et_3N (0.21 mL, 1.5 mmol) at room temperature and under nitrogen atmosphere. Then, the mixture was stirred at room temperature for 30 min. The crude mixture was diluted with CH_2Cl_2 (10 mL), washed with H_2O (2×5 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (5 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum. Diaza-alkene **5bc** was diluted with chloroform (10 mL) under a nitrogen atmosphere and ammonia was bubbled for 10 min. The solvent was evaporated under vacuum and the crude product was diluted with CH_2Cl_2 (5 mL). Et_3N (0.21 mL, 1.5 mmol) and tosyl chloride (0.23 g, 1.2 mmol) was added at room temperature. Then, the mixture was stirred at room temperature for 6 h and the crude mixture was diluted with CH_2Cl_2 (10 mL), washed with H_2O (2×5 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (5 mL). The organic layer was dried over $MgSO_4$ and evaporated under vacuum and the crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 70:30) affording compound **18** (0.24 g, 43%) as a white solid: 162–164 °C; ^{1}H NMR ($CDCl_3$) δ 7.72–7.69 (m, 2H), 7.35 (s, 1H), 7.21–7.20 (m, 2H), 6.32 (bs, 1H), 4.72–4.64 (m, 1H), 4.36–4.10 (m, 5H), 2.39 (s, 3H), 2.19–0.81 (m, 27H); ^{13}C NMR ($CDCl_3$) δ 152.8, 144.0, 143.3, 136.6–127.6 (m), 76.1, 75.8, 63.7–62.8 (m), 54.3 (d, $^1J_{PC}=155.1$ Hz), 47.0, 41.0, 34.0, 31.2, 26.2, 26.1, 23.3, 23.2, 21.9, 21.4, 20.6, 16.2, 16.1–13.4 (m); ^{31}P NMR ($CDCl_3$) δ 16.4, 16.2; IR (KBr) 3297, 3138, 2959, 2919, 1752, 1520, 1215; MS (CI) *m/z* 560 ($M^+ +1$, 100). Anal. Calcd. for $C_{25}H_{42}N_3O_7PS$: C, 53.65; H, 7.56; N, 7.51. Found C, 53.42; H, 7.59; N, 7.53.

3.5.14. Diethyl [2-((−)-menthyloxycarbonyl hydrazono)-1-(4-methoxyphenylamino)propyl] phosphonate (19). (0.42 g, 82%) obtained as an oil from **4bc** (0.42 g, 1 mmol) and 4-methoxyaniline (0.15 g, 1.2 mmol) as described in the general procedure (method A) and addition of amine at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, $R_f=0.48$, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.74 (bs, 1H), 6.77–6.66 (m, 4H), 4.76–4.67 (m, 1H), 4.50 (d, $^2J_{PH}=23.0$ Hz, 1H), 4.25–4.12 (m, 4H), 3.73 (s, 3H), 2.08–0.79 (m, 27H),; ^{13}C NMR ($CDCl_3$) δ 152.9, 153.4, 148.1, 140.5–114.7 (m), 75.8, 63.7–62.2, 59.2 (d, $^1J_{PC}=151.6$ Hz), 55.6, 47.3, 41.1, 34.1, 31.3, 26.3, 26.1, 23.5, 23.3, 21.9, 20.8, 20.7, 16.4, 16.3, 16.2, 16.1, 12.4; ^{31}P NMR ($CDCl_3$) δ 20.7, 20.6; IR (NaCl) 3230, 2965, 1712, 1513, 1235, 1036; MS (CI) *m/z* 512 ($M^+ +1$, 25). Anal. Calcd. for $C_{25}H_{42}N_3O_6P$: C, 58.69; H, 8.27; N, 8.21. Found C, 58.73; H, 8.24; N, 8.20.

3.5.15. Ethyl [1-(diethoxyphosphoryl)-2-((−)-menthyloxycarbonyl hydrazono)propylamino] acetate (20). (0.45 g, 92%) obtained as an oil from hydrazone **4bc** (0.42 g, 1 mmol), glycine ethyl ester hydrochloride (0.17 g, 1.2 mmol) and Et_3N (0.42 mL, 3 mmol) as described in the general procedure (method A) and addition of primary amine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, $R_f=0.57$, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.69 (s, 1H), 4.69–4.67 (m, 1H), 4.20–4.11 (m, 6H), 3.87 (d, $^2J_{PH}=20.6$ Hz, 1H), 3.37 (s, 2H), 2.14–0.78 (m, 30 H); ^{13}C NMR ($CDCl_3$) δ 170.8, 153.2, 147.1, 74.6, 62.3 (d, $^2J_{PC}=5.5$ Hz), 62.1 (d, $^2J_{PC}=6.5$ Hz), 61.5 (d, $^1J_{PC}=152.6$ Hz), 59.8, 48.3 (d, $^3J_{PC}=16.6$ Hz), 46.5, 40.5, 33.5, 30.6, 25.4, 22.8, 21.2, 19.9, 15.6, 13.4, 12.3; ^{31}P NMR ($CDCl_3$) δ 21.2, 21.1; IR (NaCl) 3217, 2972, 1739, 1387, 1228, 1016; MS (CI) *m/z* 492 ($M^+ +1$, 8). Anal. Calcd. for $C_{22}H_{42}N_3O_7P$: C, 53.75; H, 8.61; N, 8.55. Found C, 53.59; H, 8.62; N, 8.56.

3.5.16. Diethyl [2-(2-(−)-menthyloxycarbonyl hydrazono)-1-(1-(*R*)-phenylethylamino)propyl] phosphonate (21). (0.42 g, 83%) obtained as an oil from hydrazone **4bc** (0.42 g, 1 mmol) and (*R*)-methylbenzylamine (0.16 mL, 1.2 mmol) as described in the general procedure (method A) and addition of primary amine at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.52, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H, *minor*), 7.47 (s, 1H, *mayor*), 7.35–7.20 (m, 5H), 4.74–4.66 (m, 1H), 4.25–3.70 (m, 5H), 3.50 (d, $^2J_{PH}$=24.9 Hz, 1H, *mayor*), 2.80 (bs, 1H), 2.14–0.80 (m, 30H); ^{13}C NMR ($CDCl_3$) δ 153.9 (*minor*), 147.9 (*mayor*), 144.9 (*minor*), 144.1 (*mayor*), 128.3–125.9 (m), 75.7, 63.3–62.2 (m), 61.0 (d, $^1J_{PC}$=153.1 Hz, *minor*), 59.8 (d, $^1J_{PC}$=154.1 Hz, *mayor*), 57.2 (d, $^3J_{PC}$=14.1 Hz, *minor*), 56.2 (d, $^3J_{PC}$=17.1 Hz, *mayor*), 47.1, 41.0, 34.0, 31.2, 26.1 (*minor*), 26.0 (*mayor*), 24.6 (*minor*), 23.4 (*mayor*), 23.2, 21.8, 20.6 (*minor*), 20.5 (*mayor*), 16.2, 16.1–13.1 (m); ^{31}P NMR ($CDCl_3$) δ 22.7 (*minor*), 22.2 (*mayor*); IR (NaCl) 3217, 2965, 1712, 1235, 1023; MS (CI) *m/z* 510 (M^++1, 100). Anal. Calcd. for $C_{26}H_{44}N_3O_5P$: C, 61.28; H, 8.70; N, 8.25. Found C, 61.11; H, 8.74; N, 8.23.

3.5.17. Ethyl 2-(*S*)-[1-(Diethoxyphosphoryl)-2-((−)-menthyloxycarbonyl hydrazono)propylamino]-3-methyl butyrate (22). (0.42 g, 79%) obtained as an oil in a reaction from **4bc** (0.42 g, 1 mmol) and L-valine ethyl ester hydrochloride (0.22 g, 1.2 mmol) as described in the general procedure (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of amine at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.63, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 7.71 (s, 1H, *mayor*), 7.60 (s, 1H, *minor*), 4.71 (bs, 1H), 4.24–4.08 (m, 6H), 3.85 (d, $^2J_{PH}$=20.4 Hz, 1H, *mayor*), 3.29–3.12 (m, 1H, *mayor* and *minor*), 2.89 (bs, 1H), 2.36 (bs, 1H), 2.09–0.81 (m, 36H); ^{13}C NMR ($CDCl_3$) δ 175.2 (*minor*), 174.7 (*mayor*), 153.2 (*minor*), 148.2 (*mayor*), 75.5, 74.6, 66.2 (d, $^3J_{PC}$=16.1 Hz, *minor*), 64.9 (d, $^3J_{PC}$=16.1 Hz, *mayor*), 63.3–59.7 (m), 47.1, 41.0, 34.0, 31.2 (*minor*), 31.1 (*mayor*), 26.0, 23.3 (*minor*), 23.2 (*mayor*), 21.8, 20.5, 19.4–14.0 (m); ^{31}P NMR ($CDCl_3$) δ 22.1 (*minor*), 21.7 (*mayor*); IR (NaCl) 3211, 2939, 1719, 1367, 1235, 1036; MS (CI) *m/z* 543 (M^++1, 100). Anal. Calcd. for $C_{25}H_{48}N_3O_7P$: C, 56.27; H, 9.07; N, 7.87. Found C. 56.07; H. 9.10; N. 7.90.

3.5.18. Synthesis of diethyl (3-(*S*)-benzyloxy-2-oxobutyl) phosphonate (23). To a −78 °C stirred solution of methyl phosphonic acid diethyl ester[26] (1.82 g, 12 mmol) in THF (70 mL) was added a solution of MeLi (8.1 mL, 13 mmol) under a nitrogen atmosphere. The mixture was stirred at the same temperature for 1 h. Then, a solution of 2-benzyloxy propionic acid ethyl ester[25] in THF (5 mL) was added at −78 °C, and stirred for 16 h allowing to standing from −78 °C to room temperature. The solvent was evaporated under vacuum and the crude reaction mixture was hydrolyzed by adding 10% HCl solution (15 mL) for 1 h. The crude mixture was extracted with CH_2Cl_2 (3×10 mL), the organic phase was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 1:20, R_f=0.27, AcOEt/hexanes 50:50) affording compound **23** (2.98 g, 79%) as an oil; ^{1}H NMR ($CDCl_3$) δ 7.36–7.28 (m, 5H), 4.63–4.51 (m, 2H), 4.18–4.07 (m, 5H), 3.35–3.18 (m, 2H), 1.36 (d, $^3J_{HH}$=6.8 Hz, 3H), 1.34–1.28 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 203.6 (d, $^2J_{PC}$=6.6 Hz), 137.4–127.7 (m), 80.2, 71.8, 62.3 (d, $^2J_{PC}$=6.5 Hz), 36.5 (d, $^1J_{PC}$=131.3 Hz), 16.3, 16.1; ^{31}P NMR ($CDCl_3$) δ 20.4; IR (NaCl) 3462, 2992, 1719, 1454, 1387, 1255; MS (CI) *m/z* 315 (M^++1, 100). Anal. Calcd. for $C_{15}H_{23}O_5P$: C, 57.32; H, 7.38. Found C, 57.19; H, 7.36. $[\alpha]_D^{20}$ −31.4 (*c* 1.00, CH_2Cl_2).

3.5.19. Synthesis of *syn*-diethyl [3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)butyl] phosphonate (24). To a stirred solution of ketone **23** (1.57 g, 5 mmol) in MeOH (5 mL), was added benzyl carbazate **2b** (1.00 g, 6 mmol) at room temperature. The mixture was refluxed for 20 h and the solvent was evaporated under vacuum. The crude mixture was diluted in CH_2Cl_2 (5 mL) and dried over $MgSO_4$. Finally, the solvent was evaporated under vacuum to give crude product **24** which was not possible to purify and was then used in the next step without further purification. > 98% conversion calculated by ^{31}P NMR on the crude reaction mixture. (R_f=0.56, AcOEt/hexanes 50:50). ^{1}H NMR ($CDCl_3$) δ 10.3 (bs, 1H), 7.44–7.25 (m, 10H), 5.30–5.22 (m, 2H), 4.42 (dd, $^2J_{HH}$=21.9 Hz, $^4J_{HH}$=11.8 Hz, 2H), 4.35 (q, $^3J_{HH}$=6.7 Hz, 1H), 4.13 (q, $^3J_{HH}$=7.5 Hz, 4H), 1.36 (d, $^3J_{HH}$=6.7 Hz, 3H), 1.32–1.28 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 154.8, 138.0, 136.2–127.7, 78.6, 71.0, 67.2, 63.1–63.0 (m), 24.7 (d, $^1J_{PC}$=138.5 Hz), 18.9, 16.3, 16.2; ^{31}P NMR ($CDCl_3$) δ 24.8; IR (NaCl) 2972, 1752, 1241, 1049; MS (CI) *m/z* 463 (M^++1, 100).

3.5.20. Synthesis of *syn*-diethyl [3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-chlorobutyl] phosphonate (25). To a stirred solution β-hydrazone **24** (4.62 g, 10 mmol) in dry carbon tetrachloride (150 mL), was added dropwise NCS (1.50 g, 11 mmol) under a nitrogen atmosphere. Then, the mixture was stirred at room temperature for 16 h. The crude mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (2×20 mL) and the solvent was dried over $MgSO_4$ and evaporated under vacuum. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 30:70, R_f=0.27, AcOEt/hexanes 50:50) affording compound **25** (2.78 g, 56%) as an oil; ^{1}H NMR ($CDCl_3$) δ 10.50 (bs, 1H), 10.33 (bs, 1H), 7.54–7.26 (m, 10H), 5.42–5.12 (m, 2H), 4.85–4.02 (m, 8H), 1.59 (d, $^3J_{HH}$=6.9 Hz, 3H), 1.58 (d, $^3J_{HH}$=6.9 Hz, 3H), 1.46–1.18 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 154.8, 153.2, 146.4, 145.7, 138.0–127.3 (m), 74.8, 73.5, 72.0, 71.7, 67.6–63.8 (m), 55.0 (d, $^1J_{PC}$=160.0 Hz), 54.6 (d, $^1J_{PC}$=159.1 Hz), 18.6–16.2 (m); ^{31}P NMR ($CDCl_3$) δ 15.1, 14.6; IR (NaCl) 3297, 2972, 1752, 1500, 1222; MS (CI) *m/z* 497 (M^++1, 100). Anal. Calcd. for $C_{23}H_{30}ClN_2O_6P$: C, 55.59; H, 6.09; N, 5.64. Found C, 55.82; H, 6.08; N, 5.68.

3.5.21. Synthesis of 1-benzyloxycarbonyl-3-(1-(*S*)-benzyloxyethyl)-4-diethoxyphosphoryl-1,2-diaza-1,3-butadiene (26). To a room temperature solution of chlorohydrazone **25** (2.48 g, 5 mmol) in dry CH_2Cl_2 (25 mL), was added dropwise triethylamine (0.85 mL, 6 mmol) under nitrogen atmosphere. The mixture was stirred at this temperature for 45 min. The crude mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (2×20 mL) and the aqueous phase was extracted twice with CH_2Cl_2 (10 mL). The solvent was dried over $MgSO_4$ and evaporated under

vacuum. Diaza-alkene **26** proved to be unstable to chromatography and was then used in the next steps without further purification. > 98% conversion calculated by ^{31}P NMR on the crude reaction mixture. (R_f=0.62, AcOEt/hexanes 50:50). ^1H NMR ($CDCl_3$) δ 7.48–7.24 (m, 10H), 6.95 (d, $^2J_{PH}$=12.1 Hz, 1H, *E*), 5.72 (d, $^2J_{PH}$=10.6 Hz, 1H, *Z*), 5.42 (s, 2H), 5.32 (s, 2H), 4.57–4.38 (m, 3H), 4.17–4.08 (m, 4H), 1.70 (s, 3H, *E*), 1.68 (s, 3H, *Z*), 1.41–1.22 (m, 6H); ^{13}C NMR ($CDCl_3$) δ (*E*-isomer) 169.5 (d, $^2J_{PC}$=13.9 Hz), 161.3, 137.8–127.6 (m), 109.1 (d, $^1J_{PC}$=191.2 Hz), 71.3, 71.2, 70.5 (d, $^3J_{PC}$=12.8 Hz), 70.2, 70.1, 62.4 (d, $^2J_{PC}$=6.0 Hz), 62.3 (d, $^2J_{PC}$=5.9 Hz), 21.0, 16.4, 16.3; ^{31}P NMR ($CDCl_3$) δ 14.0 (*Z*), 13.7 (*E*); IR (NaCl) 2985, 1765, 1454, 1235, 1023.

3.5.22. Synthesis of *syn*-diethyl [1-benzylamino-3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)butyl] phosphonate (27a). (0.43 g, 75%) obtained as an oil from cholorohydrazone **25** (0.49 g, 1 mmol) and benzyl amine (0.13 mL, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A) and addition of benzyl amine at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.20, AcOEt/hexanes 50:50): ^1H NMR ($CDCl_3$) δ 10.62 (bs, 1H), 7.38–7.26 (m, 15H), 5.31–5.22 (m, 2H), 4.62–4.58 (m, 3H), 4.25–4.13 (m, 4H), 3.92–3.70 (m, 2H), 3.47 (d, $^2J_{PH}$=24.0 Hz, 1H), 2.48 (bs, 1H), 1.53 (d, $^3J_{HH}$=6.9 Hz, 3H), 1.38–1.28 (m, 6H);^{13}C NMR ($CDCl_3$) δ 153.4, 148.6, 139.1–127.0 (m), 77.1 (*mayor*), 75.5 (*minor*), 72.2 (*mayor*), 71.7 (*minor*), 67.2 (*minor*), 67.1 (*mayor*), 63.8 (d, $^2J_{PC}$=7.0 Hz, *mayor*), 63.3 (d, $^2J_{PC}$=6.8 Hz, *minor*), 63.0 (d, $^2J_{PC}$=7.2 Hz, *mayor*), 62.8 (d, $^2J_{PC}$=7.2 Hz, *minor*), 60.7 (d, $^1J_{PC}$=150.3 Hz, *minor*), 58.1 (d, $^1J_{PC}$=156.3 Hz, *mayor*), 52.5 (d, $^3J_{PC}$=12.7 Hz, *minor*), 51.1 (d, $^3J_{PC}$=14.8 Hz, *mayor*), 16.6–16.3 (m); ^{31}P NMR ($CDCl_3$) δ 22.0 (*minor*), 20.5 (*mayor*); IR (NaCl) 3310, 2985, 2919, 1752, 1493, 1215; MS (CI) *m/z* 568 (M^++1, 100). Anal. Calcd. for $C_{30}H_{38}N_3O_6P$: C, 63.48; H, 6.75; N, 7.40. Found C, 63.22; H, 6.83; N, 7.44.

3.5.23. Synthesis of *syn*-ethyl 3-[3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl)butylamino] propionate (27b). (0.37 g, 64%) obtained as a pale yellow oil from chlorohydrazone **25** (0.49 g, 1 mmol) and β-alanine ethyl ester hydrochloride (0.19 g, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of β-alanine ethyl ester hydrochloride at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.40, AcOEt): ^1H NMR ($CDCl_3$) δ 10.51 (bs, 1H), 7.36–7.28 (m, 10H), 5.28–5.15 (m, 2H), 4.61–4.52 (m, 3H), 4.24–4.10 (m, 6H), 3.79 (d, $^3J_{PH}$=23.4 Hz, 1H, *minor*), 3.56 (d, $^3J_{PH}$=23.6 Hz, 1H, *mayor*), 2.96–2.76 (m, 2H), 2.50–2.46 (m, 2H), 2.30 (bs, 1H), 1.51 (d, $^3J_{HH}$=6.8 Hz, 3H, *minor*), 1.47 (d, $^3J_{HH}$=6.9 Hz, 3H, *mayor*), 1.37–1.24 (m, 9H),; ^{13}C NMR ($CDCl_3$) δ 172.2, 153.3 (*minor*), 153.2 (*mayor*), 148.9, 137.0–127.9 (m), 76.4, 75.2, 72.0 (*mayor*), 71.7 (*minor*), 67.0, 63.7 (d, $^2J_{PC}$=6.9 Hz, *mayor*), 63.2 (d, $^2J_{PC}$=6.9 Hz, *minor*), 62.9 (d, $^2J_{PC}$=7.3 Hz, *mayor*), 62.7 (d, $^2J_{PC}$=7.2 Hz, *minor*), 61.9 (d, $^1J_{PC}$=149.7 Hz, *minor*), 60.4, 60.3, 60.2 (d, $^1J_{PC}$=154.2 Hz, *mayor*), 44.3 (d, $^3J_{PC}$=15.2 Hz, *mayor*), 44.2 (d, $^3J_{PC}$=13.3 Hz, *minor*), 35.1, 34.7, 16.4, 16.3, 14.1; ^{31}P NMR ($CDCl_3$) δ 21.7 (*minor*), 20.0 (*mayor*); IR (NaCl) 3310, 2979, 1725, 1500, 1228, 1023; MS (CI) *m/z* 578 (M^++1, 100). Anal. Calcd. for $C_{28}H_{40}N_3O_8P$: C, 58.22; H, 6.98; N, 7.27. Found C, 58.39; H, 7.01; N, 7.28.

3.5.24. Synthesis of *syn*-diethyl [3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-(4-methoxyphenylamino) butyl] phosphonate (27c). (0.48 g, 83%) obtained as a pale yellow oil from cholorohydrazone **25** (0.49 g, 1 mmol) and *p*-anisidine (0.15 g, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A), and addition of *p*-anisidine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.15, AcOEt/hexanes 50:50): ^1H NMR ($CDCl_3$) δ 10.40 (bs, 1H), 7.44–6.70 (m, 14H), 5.28 (d, $^2J_{HH}$=12.2 Hz, 1H), 5.18 (d, $^2J_{HH}$=12.1 Hz, 1H), 4.57–4.20 (m, 8H), 3.75 (s, 3H), 1.92 (bs, 1H), 1.42 (d, $^3J_{HH}$=6.9 Hz, 3H), 1.35–1.26 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 153.1, 149.1, 140.4–114.7 (m), 75.0, 71.7, 67.1, 64.0 (d, $^2J_{PC}$=7.0 Hz), 63.3 (d, $^2J_{PC}$=7.5 Hz), 57.1 (d, $^1J_{PC}$=154.1 Hz), 55.6, 16.6–16.2 (m); ^{31}P NMR ($CDCl_3$) δ 20.5 (*minor*), 19.8 (*mayor*); IR (NaCl) 3303, 2979, 1759, 1513, 1228; MS (CI) *m/z* 584 (M^++1, 100). Anal. Calcd. for $C_{30}H_{38}N_3O_7P$: C, 61.74; H, 6.56; N, 7.20. Found C, 61.92; H, 6.59; N, 7.18.

3.5.25. Synthesis of *syn*-ethyl 4-[3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl)butylamino] benzoate (27d). (0.48 g, 77%) obtained as a pale yellow oil from cholorohydrazone **25** (0.49 g, 1 mmol) and ethyl 4-aminobenzoate (0.20 g, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A) and addition of ethyl 4-aminobenzoate at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.30, AcOEt/hexanes 50:50): ^1H NMR ($CDCl_3$) δ 10.41 (bs, 1H), 7.92–6.74 (m, 14 H), 5.34–5.21 (m, 2H), 4.58–4.54 (m, 4H), 4.37–4.17 (m, 6H), 2.02 (bs, 1H), 1.48 (d, $^3J_{HH}$=6.9 Hz, 3H), 1.40–1.28 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 166.5, 153.2, 150.0, 136.5–112.8 (m), 75.0, 71.8, 67.3, 64.1 (d, $^2J_{PC}$=7.0 Hz), 63.4 (d, $^2J_{PC}$=7.6 Hz), 60.3, 55.7 (d, $^1J_{PC}$=154.0 Hz), 16.7–14.3 (m); ^{31}P NMR ($CDCl_3$) δ 19.6 (*minor*), 18.9 (*mayor*); IR (NaCl) 3270, 1706, 1606, 1487, 1281; MS (CI) *m/z* 626 (M^++1, 100). Anal. Calcd. for $C_{32}H_{40}N_3O_8P$: C, 61.43; H, 6.44; N, 6.72. Found C, 61.31; H, 6.42; N, 6.75.

3.5.26. Synthesis of *syn*-methyl 2-(*S*)-[3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl) butylamino]-3-methyl butyrate (28a). (0.51 g, 87%) obtained as a pale yellow oil from cholorohydrazone **25** (0.49 g, 1 mmol) and L-valine methyl ester hydrochloride (0.20 g, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of L-valine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.41, AcOEt/hexanes 50:50): ^1H NMR ($CDCl_3$) δ 10.5 (bs, 1H, *mayor*), 10.3 (bs, 1H, *minor*), 7.37–7.28 (m, 10H), 5.28–5.14 (m, 2H), 4.62–4.50 (m, 3H), 4.28–4.17 (m, 4H), 3.71 (s, 3H, *mayor*), 3.67 (s, 3H, *minor*), 3.59 (d, $^2J_{PH}$=20.4 Hz, 1H, *mayor*), 3.50 (d, $^2J_{PH}$=20.4 Hz, 1H, *minor*), 3.12 (d, $^3J_{HH}$=

5.1 Hz, 1H, *mayor*), 2.52 (bs, 1H), 2.10–2.00 (m, 1H), 1.51 (d, $^3J_{HH}$=6.8 Hz, 3H, *minor*), 1.46 (d, $^3J_{HH}$=6.9 Hz, 3H, *mayor*), 1.38–1.27 (m, 6H), 1.00–0.96 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 174.3 (*minor*), 173.9 (*mayor*), 154.8 (*mayor*), 153.3 (*minor*), 148.1 (*mayor*), 147.7 (*minor*), 137.1–128.1 (m), 76.8 (*mayor*), 75.7 (*minor*), 72.1 (*mayor*), 71.6 (*minor*), 71.4 (*minor*), 67.1(*mayor*), 65.8 (d, $^3J_{PC}$=13.7 Hz, *mayor*), 65.7 (d, $^3J_{PC}$=7.4 Hz, *minor*), 63.9–63.0 (m), 60.3 (d, $^1J_{PC}$=155.7 Hz, *minor*), 58.4 (d, $^1J_{PC}$=151.6 Hz, *mayor*), 51.5, 31.5 (*minor*), 31.4 (*mayor*), 19.2–13.7 (m); ^{31}P NMR ($CDCl_3$) δ 21.3 (*minor*), 19.6 (*mayor*); IR (NaCl) 3303, 2972, 1752, 1507, 1215; MS (CI) *m/z* 592 (M^++1, 100). Anal. Calcd. for $C_{29}H_{42}N_3O_8P$: C, 58.87; H, 7.16; N, 7.10. Found C, 58.71; H, 7.13; N, 7.10.

3.5.27. Synthesis of *syn*-methyl 2-(*S*)-[3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl) butylamino]-3-phenyl propionate (28b). (0.52 g, 81%) obtained as an oil from cholorohydrazone **25** (0.49 g, 1 mmol) and L-phenylalanine methyl ester hydrochloride (0.26 g, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of L-phenylalanine methyl ester hydrochloride at −40 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.37, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ 10.49 (bs, 1H), 7.37–7.16 (m, 15H), 5.29–5.19 (m, 2H), 4.54–4.45 (m, 3H), 4.24–3.76 (m, 5H), 3.73 (s, 3H, *mayor*), 3.63 (s, 3H, *minor*), 3.14–2.86 (m, 3H), 1.81 (bs, 1H), 1.49 (d, $^3J_{HH}$=6.8 Hz, 3H, *minor*), 1.39 (d, $^3J_{HH}$=6.9 Hz, 3H, *mayor*), 1.36–1.22 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 173.5, 153.3, 148.3, 137.2–126.7 (m), 76.5 (*mayor*), 75.7 (*minor*), 72.0 (*mayor*), 71.6 (*minor*), 67.2 (*minor*), 67.1 (*mayor*), 63.8 (d, $^2J_{PC}$=7.3 Hz, *mayor*), 63.3 (d, $^2J_{PC}$=7.2 Hz, *mayor*), 63.1 (d, $^2J_{PC}$=7.0 Hz, *minor*), 63.0 (d, $^2J_{PC}$=7.2 Hz, *minor*), 61.3 (d, $^3J_{PC}$=13.8 Hz, *mayor*), 61.2 (d, $^3J_{PC}$=6.7 Hz, *minor*), 59.6 (d, $^1J_{PC}$=153.3 Hz, *minor*), 58.9 (d, $^1J_{PC}$=151.2 Hz, *mayor*), 55.8, 41.1 (*mayor*), 39.6 (*minor*), 16.5–16.3 (m); ^{31}P NMR ($CDCl_3$) δ 21.1 (*minor*), 19.5 (*mayor*); IR (NaCl) 3297, 2932, 1739, 1493, 1215; MS (CI) *m/z* 640 (M^++1, 100). Anal. Calcd. for $C_{33}H_{42}N_3O_8P$: C, 61.96; H, 6.62; N, 6.57. Found C, 62.12; H, 6.61; N, 6.56.

3.5.28. Synthesis of *syn*-methyl 2-(*R*)-[3-(*S*)-benzyloxy-2-(benzyloxycarbonyl hydrazono)-1-(diethoxyphosphoryl) butylamino]-3-methyl butyrate (28c). (0.34 g, 57%) obtained as a yellow oil from cholorohydrazone **25** (0.49 g, 1 mmol) and D-valine methyl ester hydrochloride (0.20 g, 1.2 mmol) as described in the general procedure for the addition of primary amines to azo-alkenes **5** (method A) by using Et_3N (0.42 mL, 3 mmol) and addition of D-valine at 0 °C. The crude product was purified by flash-chromatography (silica gel, AcOEt/hexanes 50:50, R_f=0.24, AcOEt/hexanes 50:50): ^{1}H NMR ($CDCl_3$) δ (*mayor* diastereoisomer) 10.5 (bs, 1H), 7.73–7.28 (m, 10H), 5.27–5.15 (m, 2H), 4.59–4.54 (m, 3H), 4.27–4.21 (m, 4H), 3.62 (s, 3H), 3.49 (d, $^2J_{PH}$=19.7 Hz, 1H), 3.26 (bs, 1H), 2.84 (bs, 1H), 2.06–1.82 (m, 1H), 1.45 (d, $^3J_{HH}$=6.9 Hz, 3H), 1.38–1.27 (m, 6H), 0.99–0.98 (m, 6H); ^{13}C NMR ($CDCl_3$) δ (*mayor* diastereoisomer) 174.7, 154.8, 148.7, 137.1–128.0 (m), 77.0, 72.0, 67.2 (d, $^3J_{PC}$=8.7 Hz), 67.0, 63.8 (d, $^2J_{PC}$=7.4 Hz), 63.1 (d, $^2J_{PC}$=7.3 Hz), 59.7 (d, $^1J_{PC}$=159.7 Hz), 51.5, 31.7. 19.3–14.0 (m); ^{31}P NMR ($CDCl_3$) δ (*mayor* diastereoisomer) 19.7; IR (NaCl) 3317, 2959, 1739, 1500, 1222; MS (CI) *m/z* 592 (M^++1, 100). Anal. Calcd. for $C_{29}H_{42}N_3O_8P$: C, 58.87; H, 7.16; N, 7.10. Found C, 59.03; H, 7.18; N, 7.08. $[\alpha]_D^{20}$ −28.7 (*c* 0.60, CH_2Cl_2).

Acknowledgements

The present work has been supported by the Dirección General de Investigación del Ministerio de Ciencia y Tecnología (MCYT, Madrid DGI, PPQ2003-00910) and by the Universidad del País Vasco (UPV-GC/2002). Y. L. thanks the Consejería de Educación, Universidades e Investigación del Gobierno Vasco (Vitoria) for a predoctoral fellowship, and J. M. de los S. thanks the Ministerio de Ciencia y Tecnología (Madrid) for Ramón y Cajal Program financial support.

References and notes

1. For reviews see: (a) Murray, B. A. *Org. React. Mech.* **2003**, 1–33. (b) Enders, D.; Bettaray, W. *Pure Appl. Chem.* **1996**, *68*, 564–580. (c) Bergbreiter, D. E.; Momongan, M. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 2, p 503. (d) Buckingham, J. *Chem. Soc. Rev.* **1969**, *23*, 37–56.
2. (a) Melendez, R. E.; Lubell, W. D. *J. Am. Chem. Soc.* **2004**, *126*, 6759–6764. (b) Fox, D. L.; Ruxer, J. T.; Oliver, J. M.; Alford, K. L.; Salvatore, R. N. *Tetrahedron Lett.* **2004**, *45*, 401–405. (c) Malachowski, W. P.; Tie, C.; Wang, K.; Broadrup, R. L. *J. Org. Chem.* **2002**, *67*, 8962–8969. (d) Lee, H.; Song, J. W.; Choi, Y. S.; Park, H. M.; Lee, K. B. *J. Am. Chem. Soc.* **2002**, *124*, 11881–11893.
3. (a) Maehr, H.; Liu, C. M.; Pelleroni, N. J.; Smallheer, T.; Todaso, L.; Williams, T. H.; Blount, J. F. *J. Antibiotics* **1986**, *39*, 17–20. (b) Horimoto, H.; Shimada, N.; Naganawa, H.; Takita, T.; Umezawa, H. *J. Antibiotics* **1982**, *35*, 378–381. (c) Easmon, J.; Heinisch, G.; Pürstinger, G.; Langer, T.; Österreicher, J. K. *J. Med. Chem.* **1997**, *40*, 4420–4425. (d) Avery, M. A.; Mehrotra, S.; Bonk, J. D.; Vroman, J. A.; Goins, D. K.; Miller, R. *J. Med. Chem.* **1996**, *39*, 2900–2906.
4. For recent contributions see: (a) Enders, D.; Backes, M. *Tetrahedron: Asymmetry* **2004**, *15*, 1813–1817. (b) Frieestad, G. K.; Shen, Y.; Ruggles, E. L. *Angew. Chem., Int. Ed.* **2003**, *42*, 5061–5063. (c) El Kaim, L.; Grimaud, L.; Perroux, Y.; Tirla, C. *J. Org. Chem.* **2003**, *68*, 8733–8735.
5. For recent contributions see: (a) Reza, D. H.; Aghapoor, K.; Tajbakhsh, M. *Tetrahedron Lett.* **2004**, *45*, 4167–4169. (b) Patient, L.; Berry, M. B.; Coles, S. J.; Hursthouse, M. B.; Kilburn, J. D. *Chem. Commun.* **2003**, 2552–2553. (c) Wang, Y. G.; Zhang, J.; Lin, X. F.; Ding, H. F. *Synlett* **2003**, 1467–1468. (d) Abadi, A. H.; Abdel, H. E.; Hassa, G. S. *Chem. Pharm. Bull.* **2003**, *51*, 838–844.
6. For reviews see: (a) Attanasi, O. A.; De Crescentini, L.; Filippone, P.; Mantinelli, F.; Santeusanio, S. *Arkivoc* **2002**, 274–292. (b) Attanasi, O. A.; Filippone, P. *Synlett* **1997**, 1128–1140. (c) Schantl, J. G. In Kropf, H., Schaumann, E., Eds.; Houben-Weyl. Methoden der Organische Chemie; Thieme: Stuttgart, 1990; Vol. E15, p 909.

7. For recent contributions see: (a) Attanasi, O. A.; De Crescentini, L.; Favi, G.; Filippone, P.; Mantenilli, F.; Santeusanio, S. *J. Org. Chem.* **2004**, *69*, 2686–2692. (b) Attanasi, O. A.; De Crescentini, L.; Favi, G.; Filippone, P.; Giorgi, G.; Mantenilli, F.; Santeusanio, S. *J. Org. Chem.* **2003**, *68*, 1947–1953. (c) Attanasi, O. A.; Favi, G.; Filippone, P.; Giorgi, G.; Stanovnik, B.; Svete, J. *Synlett* **2003**, 995–996. (d) Avalos, M.; Babiano, R.; Cintas, P.; Clemente, F. R.; Gordillo, R.; Jiménez, J. L.; Palacios, J. C. *J. Org. Chem.* **2002**, *67*, 2241–2251.
8. (a) Attanasi, O. A.; De Crescentini, L.; Favi, G.; Filippone, P.; Mantenilli, F.; Santeusanio, S. *Synlett* **2004**, 549–551 and references therein cited. (b) Avalos, M.; Babiano, R.; Cintas, P.; Clemente, F. R.; Gordillo, R.; Jiménez, J. L.; Palacios, J. C. *J. Org. Chem.* **2001**, 5139–5145 and references therein cited.
9. (a) Ferguson, G.; Lough, A. J.; Mackay, D.; Weeratunga, G. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3361–3369. (b) Boeckman, R. K.; Reed, J. E.; Ge, P. *Org. Lett.* **2001**, *3*, 3651–3653. (c) Clarke, S. J.; Gilchrist, T. L.; Lemos, A.; Roberts, T. G. *Tetrahedron* **1991**, *47*, 5615–5624. (d) Schantl, J. G.; Nadenik, P. *Synlett* **1998**, 786–788. (e) South, M. S.; Jakuboski, T. L.; Westmeyer, M. D.; Dukesherer, D. R. *J. Org. Chem.* **1996**, *61*, 8921–8934. (f) Sommer, S. *Tetrahedron Lett.* **1977**, *1*, 117–120.
10. Palacios, F.; Aparicio, D.; de los Santos, J. M. *Tetrahedron* **1999**, *55*, 13767–13778.
11. For reviews see: (a) Engel, R. Handbook of Organophosphorus Chemistry; Dekker: New York, 1992. (b) Kafarski, P.; Lejezak, B. *Phosphorus Sulfur* **1991**, *63*, 193–215. (c) Hoagland, R. E. In *Biologically Active Natural Products*; Culter, H. G., Ed.; ACS Symposium Series 380; American Chemical Society: Washington, DC, 1988; p 182. (d) Toy, A. D. F.; Walsh, E. N. *Phosphorus Chemistry in Everyday Living*; American Chemical Society: Washington, DC, 1987; p 333.
12. (a) Palacios, F.; Alonso, C.; Rubiales, G.; Villegas, M. *Tetrahedron Lett.* **2004**, *45*, 4031–4034. (b) Palacios, F.; Herrán, E.; Rubiales, G. *J. Org. Chem.* **2002**, *67*, 2131–2135. (c) Palacios, F.; Alonso, C.; Amezua, P.; Rubiales, G. *J. Org. Chem.* **2002**, *67*, 1941–1946.
13. (a) Palacios, F.; Ochoa de Retana, A. M.; Martínez de Marigorta, E.; Rodríguez, M.; Pagalday, J. *Tetrahedron* **2003**, *59*, 2617–2623. (b) Palacios, F.; Oyarzabal, J.; Pascual, S.; Ochoa de Retana, A. M. *Org. Lett.* **2002**, *4*, 769–772. (c) Palacios, F.; Ochoa de Retana, A. M.; Pascual, S.; Oyarzabal, J. *Tetrahedron Lett.* **2002**, *43*, 5917–5919.
14. (a) Palacios, F.; Aparicio, D.; Ochoa de Retana, A. M.; de los Santos, J. M.; Gil, J. I.; López de Munain, R. *Tetrahedron: Asymmetry* **2003**, *14*, 689–708. (b) Palacios, F.; Ochoa de Retana, A. M.; Gil, J. I.; López de Munain, R. *Org. Lett.* **2002**, *4*, 2405–2408. (c) Palacios, F.; Aparicio, D.; Ochoa de Retana, A. M.; de los Santos, J. M.; Gil, J. I.; Alonso, J. M. *J. Org. Chem.* **2002**, *67*, 7283–7288.
15. (a) Palacios, F.; Aparicio, D.; de los Santos, J. M.; Vicario, J. *Tetrahedron* **2001**, *57*, 1961–1972. (b) Palacios, F.; Aparicio, D.; de los Santos, J. M.; Baceiredo, A.; Bertrand, G. *Tetrahedron* **2000**, *56*, 663–669. (c) Palacios, F.; Aparicio, D.; Ochoa de Retana, A. M.; de los Santos, J. M.; García, J.; Oyarzabal, J. *Tetrahedron* **1999**, *55*, 3105–3116. (d) Palacios, F.; Aparicio, D.; de los Santos, J. M. *Tetrahedron* **1996**, *52*, 4123–4132. (e) Palacios, F.; Aparicio, D.; de los Santos, J. M. *Tetrahedron* **1994**, *50*, 12727–12742.
16. For excellent reviews see: (a) Andreas, J.; Janek, C. F.; Bettray, W.; Peters, R.; Enders, D. *Tetrahedron* **2002**, *58*, 2253–2329. (b) Enders, D.; Thiebes, T. *Pure Appl. Chem.* **2001**, *73*, 573–578. (c) Enders, D.; Wortymann, L. K.; Peters, R. *Acc. Chem. Res.* **2000**, *33*, 157–169. (d) Enders, D. In Morrison, J. D., Ed.; Asymmetric Synthesis; Academic: New York, 1984; Vol. 3, p 275.
17. For a preliminary communication see: Palacios, F.; Aparicio, D.; López, Y.; de los Santos, J.M *Tetrahedron Lett.* **2004**, *45*, 4345–4348.
18. For reviews see: (a) Kafarski, P.; Lejczak, B. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 301–312. (b) *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*; Kukhar, V. P., Hudson, H. R., Eds.; Wiley: Chichester, 2000. (c) Fields, S. F. *Tetrahedron* **1999**, *55*, 12237–12273. (d) Hiratake, J.; Oda, J. *Biosci. Biotech. Biochem.* **1997**, *61*, 211–218.
19. For recent contributions see: (a) Chen, X.; Wiemer, D. F. *J. Org. Chem.* **2003**, *68*, 6108–6114. (b) Piotrowska, D. G.; Wroblewski, A. E. *Tetrahedron* **2003**, *59*, 8405–8410. (c) Palacios, F.; Aparicio, D.; Ochoa de Retana, A. M.; de los Santos, J. M.; Gil, J. I.; López de Munain, R. *Tetrahedron: Asymmetry* **2003**, *14*, 689–700. (d) Skropeta, D.; Schworer, R.; Schmidt, R. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3351–3354. (e) Davis, F. A.; Wu, Y.; Yan, H.; McCoul, W.; Prasad, K. R. *J. Org. Chem.* **2003**, *68*, 2410–2419.
20. (a) Smith, A. B.; Yager, K. M.; Taylor, C. M. *J. Am. Chem. Soc.* **1995**, *117*, 10879–10888. (b) Smithrud, D. B.; Benkovic, P. A.; Benkovic, S. J.; Taylor, C. M.; Yager, K. M.; Witherington, J.; Phillips, B. W.; Sprengler, P. A.; Smith, A. B.; Hirschmann, R. *J. Am. Chem. Soc.* **1997**, *119*, 278–282. (c) Hirschmann, R.; Smith, A. B.; Taylor, C. M.; Benkovic, P. A.; Taylor, S. D.; Yager, K. M.; Spengler, P. A.; Benkovic, S. J. *Science* **1994**, *265*, 234–237. (d) Meyer, J. H.; Bartlett, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 4600–4609. (e) Chackalamannil, S.; Chung, S.; Atamford, A. W.; McKittrick, B. A.; Wang, Y.; Tsai, H.; Cleven, R.; Fawzi, A.; Czarniecki, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1257–1260. (f) Steere, J. A.; Sampson, P. B.; Honek, J. F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 457–460. (g) Cristau, H. J.; Cooulombeau, A.; Genevis-Borella, A.; Pirat, J. L. *Tetrahedron Lett.* **2001**, *42*, 4491–4494. (h) Georgiadis, D.; Dive, V.; Yiotakis, A. *J. Org. Chem.* **2001**, *66*, 6604–6610. (i) Wallace, E. M.; Moliterni, J. A.; Moskal, M. A.; Neubart, A. D.; Marcopulos, N.; Stamford, L. B.; Trapani, A. J.; Savage, P.; Chou, M.; Jeng, A. Y. *J. Med. Chem.* **1998**, *41*, 1513–1523. (j) Chen, S.; Coward, J. K. *J. Org. Chem.* **1998**, *63*, 502–509. (k) Hirschmann, R.; Yager, K. M.; Taylor, C. M.; Witherington, J.; Sprengler, P. A.; Phillips, B. W.; Moore, W.; Smith, A. B. *J. Am. Chem. Soc.* **1997**, *119*, 8177–8190.
21. Only the preparation and some reactions of chiral aza-alkene containing a carbohydrate template at C-4 have been described[7d,8b]
22. Bozzini, S.; Feluga, G.; Nardin, A.; Pizzolini, G.; Pitacco, E.; Valentin, E. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1961–1969.
23. (a) Attanasi, O. A.; De Crescentini, L.; Filippone, P.; Mantenilli, F.; Santeusanio, S. *Synlett* **2003**, 1183–1185. (b) Abbiati, G.; Arcadi, A.; Attanasi, O. A.; De Crescentini, L.; Rossi, E. *Tetrahedron* **2001**, *57*, 2031–2038.
24. Diastereoisomers ratio were determined by ^{31}P NMR on the crude reaction mixture.
25. Shapiro, G.; Buechler, D.; Hennet, S. *Tetrahedron Lett.* **1990**, *31*, 5733–5736.
26. Lawrence, N. J. The Wittig reaction and related methods. In *Preparation of Alkenes. A Practical Approach*; Williams, J. M. J., Ed.; Oxford University Press: Oxford, 1996; p 35.
27. Dillen, J. L. M.; Meth-Cohn, O.; Moore, C.; Van Rooyen, P. H. *Tetrahedron* **1988**, *44*, 3127–3138.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2831–2838

Asymmetric synthesis of aryloxypropanolamines via OsO_4-catalyzed asymmetric dihydroxylation

Iliyas A. Sayyed, Vinay V. Thakur, Milind D. Nikalje, Gajanan K. Dewkar, S. P. Kotkar and A. Sudalai*

Chemical Engineering and Process Development Division, National Chemical Laboratory, Pashan Road, Pune 411 008, India

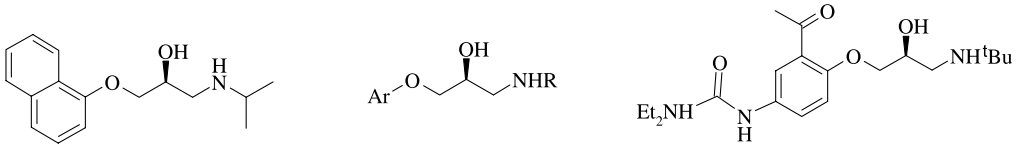
Received 18 October 2004; revised 4 January 2005; accepted 20 January 2005

Abstract—A simple and effective procedure for the enantioselective synthesis of several β-adrenergic blocking agents incorporating the first asymmetric synthesis of celiprolol, is described. The key steps are (i) sharpless asymmetric dihydroxylation of aryl allyl ethers to introduce chirality into the molecules and (ii) conversion of cyclic sulfates into the corresponding epoxides using a three-step procedure.

1. Introduction

β-Adrenergic blocking agents (β-blockers) are important drugs widely used for the treatment of hypertension, angina pectoris, glaucoma, anxiety and obesity. The discovery of propranolol (**1a**), the first successful drug having antianginal and antihypertensive effects, prompted the synthesis of many thousands of compounds containing an aryloxypropanolamine moiety.[1] The three fundamental goals of cardiovascular drugs are: lowering of blood pressure (antihypertensive), return of the heart to rhythmic beating (antiarrhythmics) and the general improvement of the heart muscle tone (cardiotonics).[2] Biochemically, the mechanism of action involves the adrenergic system in which the hormonal system provides the communication link between the sympathetic nervous system and involuntary muscle.[3] Blocking of the β-receptor system reduces the overall activity of the sympathetic nervous system. β-Blockers are thus used to increase life expectancy after heart attack. Biological systems, in most cases, recognize the members of a pair of enantiomers as different substances, and the two enantiomers will exhibit different responses. It has been shown for many pharmaceuticals that only one enantiomer contains all the desired activity, and the other is either totally inactive or highly toxic. Although (*S*)-isomers are known to be much more effective (100–500-fold) than the (*R*)-isomer,[4] these antihypertensive drugs are presently sold as racemic mixtures. To avoid unnecessary stress or in some cases toxicity to an organism caused by the (*R*)-isomers, the administration of optically pure (*S*)-isomer is desirable.

In the literature, there are several reports available for the synthesis of β-blockers (**1a–g**)[5] (Fig. 1) which include classical resolution via diastereomers, chromatographic



1a : (S)-Propranolol

1b: Ar = 2-methoxyphenyl, R=^{i}Pr, (S)-Moprolol
1c: Ar = 3-methylphenyl, R=^{i}Pr, (S)-Toliprolol
1d: Ar = 2-cyanophenyl, R=^{t}Bu, (S)-Bunitrolol
1e: Ar = 4-acetamidophenyl, R=^{i}Pr, (S)-Practolol
1f: Ar = 2,3-dimethylphenyl, R=^{t}Bu,(S)-Xibenolol

1g : (S)-Celiprolol

Figure 1.

Keywords: Antihypertensive; Asymmetric dihydroxylation; Epoxides; Cyclic sulfates.
* Corresponding author. Tel.: +91 20 25893300; fax: +91 20 25893359; e-mail: sudalai@dalton.ncl.res.in

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.074

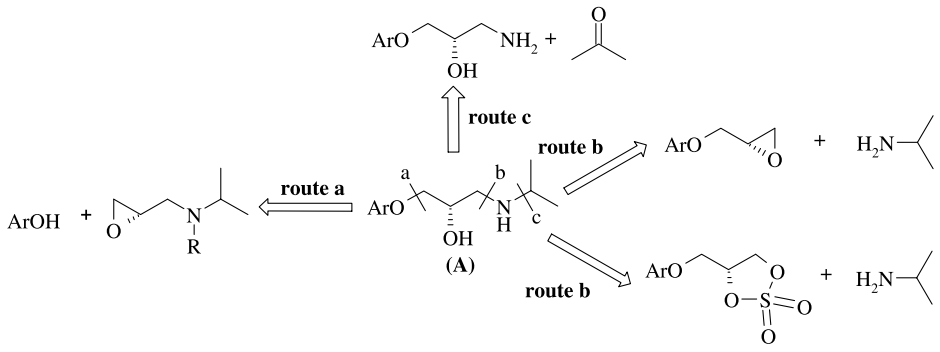


Figure 2. Retrosynthetic analysis of β-adrenergic blocking agents (**A**).

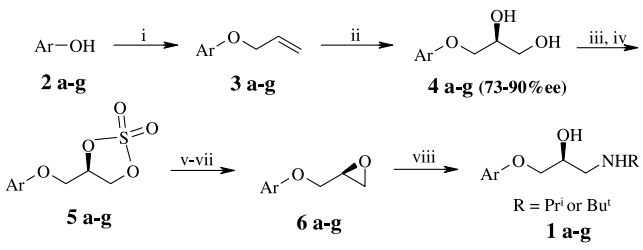
separation of enantiomers, enzymatic resolution, kinetic resolution and asymmetric synthesis via chiral pool strategy. Furthermore, many of these methods suffer from disadvantages such as low overall yields, use of expensive enzymes and resolving agents, low optical purity, the need for separation of diastereomers and the use of expensive chiral catalysts. In order to develop a new general route for the asymmetric synthesis of β-adrenergic blockers with good optical purity and yield, we decided to make use of sharpless asymmetric dihydroxylation (AD) and chemistry of chiral 1,2- cyclic sulfates.[6] Herein, we report catalytic enantioselective synthesis of seven such β-blockers (**1a–g**) from readily available starting materials (Fig. 1).

2. Results and discussion

Retrosynthetic analysis of these β-adrenergic blocking agents (**A**) is shown in Figure 2. There are three possible disconnections at the a, b and c bonds. Most of the previous synthetic routes are based on the disconnection of bonds at either a or b.

The general synthetic scheme we have employed for the synthesis of (*S*)-propranolol (**1a**), (*S*)-moprolol (**1b**), (*S*)-toliprolol (**1c**), (*S*)-bunitrolol (**1d**), (*S*)-practolol (**1e**), (*S*)-xibenolol (**1f**) and (*S*)-celiprolol (**1g**) is presented in Scheme 1.

Allylation of phenols **2a–g** (**2a**=α-naphthol, **2b**=2-methoxyphenol, **2c**=3-methylphenol, **2d**=2-cyanophenol, **2e**=4-acetamidophenol, **2f**=2,3-dimethylphenol, **2g**=2-hydroxy, 4-nitro- acetophenone) with allyl bromide gave allyl ethers **3a–g** in >97% yield.



Scheme 1. (i) K_2CO_3, $CH_2{=}CHCH_2Br$, acetone, reflux, 12 h, 97–99%; (ii) cat. OsO_4, $(DHQD)_2$-PHAL, $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/H_2O, 0 °C, 12 h, 94–98%, 73–90% ee; (iii) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C, 40 min. 96–99%; (iv) cat. $RuCl_3$ $3H_2O$, $NaIO_4$, CH_3CN:H_2O, 0 °C, 30 min. 94–98%; (v) LiBr, THF 25 °C, 2–3 h; (vi) 20% H_2SO_4, Et_2O, 25 °C, 10 h; (vii) K_2CO_3, MeOH, 0 °C, 2 h, 80–85% overall in three steps; (viii) R-NH_2, H_2O (cat.), reflux, 2 h, 99%.

These allylic ethers **3a–g** were then subjected for the Os-catalyzed sharpless asymmetric dihydroxylation (AD) using $(DHQD)_2$-PHAL (hydroquinidine 1,4-phthalazinediyl diether) as chiral ligand in the presence of $K_3Fe(CN)_6$/K_2CO_3 as co-oxidant to give the enantiomerically enriched diols **4a–g**. The diols **4a–g** were then treated with freshly distilled $SOCl_2$, Et_3N in CH_2Cl_2 at 0 °C to afford cyclic sulfites in 96–99% yield as 1:1 diastereomeric mixture. The formation of cyclic sulfite was clearly evident from the appearance of multiplets at δ 4.00–5.50 in its ^1H NMR spectrum. The cyclic sulfites of the corresponding diols were then converted into cyclic sulfates **5a–g** in 94–98% yield using $RuCl_3$-catalyzed oxidation. The ^1H NMR spectrum of cyclic sulfates **5a–g** showed the disappearance of several multiplets at δ 4.25–4.32, 4.72–4.86 and at 5.22–5.26 due to diastereomeric mixtures of cyclic sulfites. Finally, the cyclic sulfates **5a–g** were subjected to nucleophilic displacement with appropriate amine nucleophiles followed by hydrolysis of the resulting salts to afford the corresponding β-blockers **1**(**a–g**), respectively. However, these reactions resulted in very low yields of the final β-blockers (yields were often less than 30%). Hydrolysis of the salts of cyclic sulfates using various reaction conditions such as 20% H_2SO_4 in ether, 50% H_2SO_4 in ether, concd HCl, 20% aq NaOH and 50% aq NaOH was conducted but all of them failed to improve the yields of the final products. Hence, we decided to convert these cyclic sulfates **5a–g** into the corresponding epoxides **6a–g** using a three-step procedure. Thus, cyclic sulfates **5a–g** were first treated with anhydrous LiBr, followed by treatment with 20% aq H_2SO_4 in ether to give the corresponding bromoalcohols. These were then treated with anhydrous K_2CO_3 in MeOH at 0 °C to afford the corresponding epoxides **6a–g** in high overall yields (80–85% in three steps).[7]

Finally the epoxides **6a–g** were then subjected to regiospecific nucleophilic opening with the respective amines to furnish the corresponding β-blockers **1**(**a–g**) in excellent yields and enantiomeric excess (up to 99%). In case of celiprolol, the nitro group was hydrogenated at 20 psi H_2 pressure with 10% Pd/C as catalyst at room temperature to get the amine which was condensed with diethyl carbomyl chloride (DECC) to afford the corrosponding (*S*)-celiprolol.

3. Conclusion

In conclusion, we have developed a simple and efficient protocol for the asymmetric synthesis of seven β-blockers namely (*S*)-propranolol (**1a**) (67% overall yield, 90% ee), (*S*)-moprolol (**1b**) (74% overall yield, 68% ee), (*S*)-toliprolol (**1c**) (77% overall yield, 78% ee), (*S*)-bunitrolol (**1d**) (35% overall yield, 60% ee), (*S*)-practolol (**1e**) (31% overall yield, 82% ee), (*S*)-xibenolol (**1f**) (35% overall yield, 67% ee), and (*S*)-celiprolol (**1g**) (33% overall yield, 97% ee) in eight steps starting from the corresponding phenols **2a–g**. The asymmetric synthesis of celiprolol has been achieved for the first time.

4. Experimental

4.1. General information

Solvents were purified and dried by standard procedures before use; petroleum ether of boiling range 60–80 °C was used. Melting points are uncorrected. Optical rotations were measured using sodium D line on a JASCO-181 digital polarimeter. Infrared spectra were recorded on Shimadzu FTIR-8400 spectrometer. ^{1}H NMR and ^{13}C NMR were recorded on Bruker AC-200 and MSL-300 NMR spectrometers, respectively. Mass spectra were obtained with a Finnigan MAT-1020 B-70 eV mass spectrometer. Elemental analysis was carried out on a Carlo Erba CHNS-O analyzer. Enantiomeric excess was determined by chiral HPLC or by using chiral shift reagent Eu-(hfc)$_3$.

4.2. Preparation of allyl phenyl ethers 3a–g

A mixture of one of the phenols **2a–g** (10 mmol), allylbromide (12 mmol) and anhydrous K_2CO_3 (15 mmol) in dry acetone (20 mL) was refluxed under N_2 for 20 h (reactions monitored by TLC). The reaction mixture then cooled to room temperature, filtered through sintered funnel to remove solid residue and the filtrate was evaporated to dryness. The residue was purified by column chromatography using pet. ether/EtOAc (9:1) as eluent to get pure allyl phenyl ethers **3a–g** in 85–99% yield.

4.2.1. Allyl 1-naphthyl ether (3a). Yield: 1.78 g, 97%; gum; IR (neat, cm^{-1}): 744, 927, 999, 1012, 1028, 1125, 1200, 1260, 1458, 1520, 2829, 2930; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=4.68$ (2H, d, $J=4.0$ Hz), 5.29–5.54 (2H, m), 6.08–6.24 (1H, m), 6.78 (1H, d, $J=8.1$ Hz), 7.34–7.49 (4H, m), 7.76–7.81 (1H, m), 8.29–8.34 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=68.6$, 104.9, 117.0, 120.2, 122.0, 125.0, 125.7, 126.2, 127.3, 133.2, 134.4, 154.2; Analysis: $C_{13}H_{12}O$ requires C, 84.75; H, 6.57; found C, 84.69; H, 6.51%.

4.2.2. Allyl 2-methoxyphenyl ether (3b). Yield: 1.62 g, 99%; gum; IR (neat, cm^{-1}): 742, 927, 997, 1026, 1124, 1224, 1251, 1454, 1504, 1593, 2835, 2935; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=3.85$ (3H, s), 4.57–4.67 (2H, m), 5.24–5.45 (2H, m), 6.00–6.24 (1H, m), 6.87–6.90 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=55.5$, 69.5, 111.5, 113.4, 117.5, 120.4, 121.0, 133.2, 147.8, 149.2; MS *m/z* (% rel intensity): 164 (M^+, 80), 149 (10), 123 (94), 109 (25), 95 (100), 80 (30), 77 (95), 65 (25); Analysis: $C_{10}H_{12}O_2$ requires C, 73.15; H, 7.37; found C, 73.28; H, 7.34%.

4.2.3. Allyl 3-methylphenyl ether (3c). Yield: 1.43 g, 97%; gum; IR (neat, cm^{-1}): 670, 738, 780, 927, 1020, 1127, 1224, 1260, 1458, 1490, 1510, 1594, 2859, 2945; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=2.36$ (3H, s), 4.49–4.55 (2H, m), 5.25–5.48 (2H, m), 5.97–6.21 (1H, m), 6.75–6.79 (3H, m), 7.16 (1H, s); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=21.3$, 68.5, 111.4, 115.4, 117.2, 121.5, 129.0, 133.4, 139.2, 158.5; MS *m/z* (% rel intensity): 148 (M^+, 50), 133 (60), 119 (65), 105 (70), 91 (100), 77 (50); Analysis: $C_{10}H_{12}O$ requires C, 81.04; H, 8.16; found C, 81.12; H, 8.21%.

4.2.4. Allyl 2-cyanophenyl ether (3d). Yield: 1.54 g, 97%; gum; IR ($CHCl_3$, cm^{-1}): 3082, 2925, 2227, 1598, 1579, 1490, 1450, 1425, 1290, 1259, 1234, 1166, 1110, 995, 933, 788, 756, 732; ^{1}H NMR (200 MHz, $CDCl_3$)): $\delta=4.66$ (2H, d, $J=2.0$ Hz), 5.31–5.36 (1H, m), 5.44–5.53 (1H, m), 5.90–6.20 (1H, m), 6.90–7.10 (2H, m), 7.45–7.65 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=69.1$, 101.8, 112.5, 116.0, 117.7, 120.6, 131.6, 133.3, 134.0, 159.9; MS (*m/z*, RI): 159 (M^+, 100), 158 (98), 143 (5), 130 (14), 119 (20), 118 (18), 104 (7), 92 (21), 90 (22), 82 (6), 76 (12), 69 (7), 64 (16), 58 (8); Analysis: $C_{10}H_9NO$ requires C, 75.45; H, 5.69; N, 8.79; found C, 75.41; H, 5.79; N, 8.78%.

4.2.5. Allyl 4-acetamidophenoxy ether (3e). Yield: 1.81 g, 95%; crystalline solid; mp: 100–102 °C (EtOAc and hexane); IR ($CHCl_3$, cm^{-1}): 3298, 3020, 2962, 1685, 1605, 1589, 1514, 1435, 1280, 1217, 1118, 850; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=2.14$ (3H, s), 4.51 (2H, d, $J=4.0$ Hz), 5.20 (1H, d, $J=12.0$ Hz), 5.35 (1H, d, $J=16.0$ Hz), 5.95–6.20 (1H, m), 6.80 (2H, d, $J=8.0$ Hz), 7.30 (2H, d, $J=8.0$ Hz), 7.50 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=33.0$, 72.3, 79.1, 79.4, 123.7, 130.2, 141.8, 164.1, 177.2; Mass (*m/z*, RI): 191 (M^+, 18), 190 (6), 150 (12), 149 (5), 109 (11), 108 (100), 95 (3), 80 (12), 65 (4), 57 (5); Analysis: $C_{11}H_{13}NO_2$ requires C, 69.09; H, 6.85; N, 7.34; found 69.13; H, 6.87; N, 7.32%.

4.2.6. Allyl 2,3-dimethylphenyl ether (3f). Yield: 1.37 g, 85%; gum; IR ($CHCl_3$, cm^{-1}): 2935, 1593, 1503, 1454, 1251, 1224, 1178, 1026, 997, 927, 1593, 1503, 1454, 1251, 1224, 1178, 1026, 997, 927, 742; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=2.17$ (3H, s), 2.26 (3H, s), 4.49–4.51 (2H, m), 5.22 (1H, d, $J=10.0$ Hz), 5.40 (1H, d, $J=16.0$ Hz), 5.99–6.13 (1H, m), 6.66 (1H, d, $J=8.0$ Hz), 6.7(1H, d, $J=16.0$ Hz), 6.98–7.02 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=11.6$, 19.9, 68.9, 109.2, 116.6, 122.3, 125.3, 125.6, 133.8, 137.8, 156.5; Mass (*m/z*, RI): 162 (M^+, 32), 147 (35), 119 (54), 103 (22), 91 (100), 77 (88), 65 (15); Analysis: $C_{11}H_{14}O$ requires C, 81.44; H, 8.70%; found: C, 81.31; H, 8.48%.

4.2.7. Allyl-2-acetyl-4-nitrophenyl ether (3g). Yield: 2 g, 95%; white solid; mp: 78–80 °C; IR ($CHCl_3$, cm^{-1}): 3020, 2405, 1690, 1523, 1345, 1275, 1215, 1117, 756, 667; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=2.66$ (3H, s), 4.78 (2H, d, $J=4.0$ Hz), 5.38–5.52 (2H, m), 6.00–6.19 (1H, m), 8.31–8.42 (2H, m), 8.61 (1H, d, $J=2.0$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=31.4$, 70.1, 112.9, 119.2, 126.0, 128.3, 131.0, 141.0, 159.5, 161.9, 196.9; Analysis: $C_{11}H_{11}NO_4$ requires

C, 59.72; H, 5.011, N, 6.33; found: C, 59.79; H, 5.43; N, 6.48%.

4.3. Preparation of 1-(aryloxy)-2,3-dihydroxypropane 4a–g

A 100 mL RB flask was charged with $K_3Fe(CN)_6$ (18.0 mmol), K_2CO_3 (18.0 mmol), $(DHQD)_2$-PHAL (0.24 mmol) and *t*-BuOH/H_2O (1:1, 60 mL) and the resulting mixture was stirred for 10 min at 25 °C. It was then cooled to 0 °C and a solution of OsO_4 (256 μL, 0.124 mmol, 0.5 M solution in toluene) was added. The resulting reaction mixture was stirred at 0 °C for 5 min and then one of the olefins **3a–g** (6 mmol) was added. The reaction mixture was stirred at 0 °C for 20–22 h (monitored by TLC). It was quenched with sodium sulfite (4.0 g) and extracted with ethyl acetate (4×25 mL). Combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography using 50% EtOAc in pet. ether as eluent to yield pure diols **4a–g** as white solids in 84–98% yield.

4.3.1. (2*S*)-1-(1-Naphthoxy)-2,3-propanediol (4a). Yield: 1.25 g, 96%; white solid; mp: 113–114 °C; $[\alpha]^{25}_D$: +6.10 (*c* 1.1, MeOH), (lit.[8] +4.01 (*c* 1.1, MeOH), 60% ee); HPLC: 91% ee, Chiralcel OD-H, 5% EtOH/hexane, 1 mL/min. Retention time: (*R*): 13.23 min, (*S*): 16.55 min; IR ($CHCl_3$, cm^{-1}): 740, 780, 845, 993, 1020, 1130, 1257, 1379, 1390, 1458, 1515, 1598, 2845, 2910, 3280; ^1H NMR (200 MHz, $CDCl_3$): δ=3.55 (1H, br s), 3.80–3.95 (3H, m), 4.10–4.25 (3H, m), 6.83 (1H, d, *J*=6.1 Hz), 7.32–7.49 (4H, m), 7.77–7.81 (1H, m), 8.24–8.29 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=63.7, 69.1, 70.4, 104.8, 120.3, 121.7, 124.9, 125.4, 125.6, 126.2, 127.2, 134.3, 154.2; MS *m/z* (% rel intensity): 218 (M^+, 70), 144 (100), 127 (10), 115 (43), 89 (7), 77 (5); Analysis: $C_{13}H_{14}O_3$ requires C, 71.54; H, 6.47; found C, 71.52; H, 6.49%.

4.3.2. (2*S*)-1-(2-Methoxyphenyl)-2,3-propanediol (4b). Yield: 1.1 g, 94%; white solid; mp: 101–102 °C; $[\alpha]^{25}_D$: +6.70 (*c* 1.1, MeOH), 73% ee (lit.[9] +5.8 (*c* 1.1, MeOH), 63% ee); HPLC: 73% ee, Chiralcel OD-H, 5% EtOH/hexane, 1 mL/min. Retention time: (*R*): 11.18 min, (*S*): 15.21 min; IR ($CHCl_3$, cm^{-1}): 744, 837, 993, 1022, 1128, 1257, 1377, 1456, 1510, 1593, 2854, 2953, 3234; ^1H NMR (200 MHz, $CDCl_3$): δ=3.75–3.84 (2H, m), 3.86 (3H, s), 4.04–4.17 (3H, m), 6.90–6.97 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=55.9, 63.2, 69.8, 70.6, 111.5, 113.6, 120.6, 121.1, 147.7, 148.9, 159.4; MS *m/z* (% rel intensity): 198 (M^+, 28), 149 (10), 124 (100), 109 (80), 77 (13); Analysis: $C_{10}H_{14}O_4$ requires C, 60.60; H, 7.12; found C, 60.56; H, 7.14%.

4.3.3. (2*S*)-1-(3-Methylphenyl)-2,3-propanediol (4c). Yield: 1.06 g, 98%; white solid; mp: 61–62 °C; $[\alpha]^{25}_D$: +7.86 (*c* 1, EtOH) 80% ee (lit.[10] +9.5 (*c* 1, EtOH) 97% ee); HPLC: 80% ee, Chiralcel OD-H, 5% EtOH/hexane, 1 mL/min. Retention time: (*R*): 19.18 min, (*S*): 24.15 min; IR ($CHCl_3$, cm^{-1}): 690, 775, 933, 1055, 1159, 1259, 1290, 1453, 1490, 1585, 1602, 2877, 2927, 3390; ^1H NMR (200 MHz, $CDCl_3$): δ=2.30 (3H, s), 3.65–3.80 (2H, m), 4.00–4.25 (3H, m), 6.66–6.85 (3H, m), 7.20–7.30 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=21.2, 63.4, 68.8, 70.4, 111.2, 115.1, 121.7, 129.0, 139.2, 158.2; MS *m/z* (% rel intensity): 182 (M^+, 30), 133 (12), 121 (18), 109 (100), 92 (23), 77 (20); Analysis: $C_{10}H_{14}O_3$ requires C, 65.92; H, 7.74; found C, 65.86; H, 7.79%.

4.3.4. (2*S*)-1-(2-Cyanophenoxy)-2,3-propanediol (4d). Yield: 960 mg, 84%; white solid; mp: 140–142 °C (hexane and EtOAc); $[\alpha]^{25}_D$: +21.8 (*c* 0.5, EtOH); 65% ee, (lit.[11] $[\alpha]^{25}_D$ +9.4 (*c* 0.49, EtOH) for 28% ee); IR ($CHCl_3$, cm^{-1}): 3421, 3018, 2229, 1598, 1492, 1450, 1290, 1215; ^1H NMR (200 MHz, $CDCl_3$)): δ=3.50–3.90 (4H, m), 4.10–4.25 (3H, m), 7.90–7.05 (2H, m), 7.45 (1H, d, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=63.2, 69.8, 70.0, 101.4, 112.4, 116.5, 120.9, 133.3, 134.5, 160.2; MS (*m/z*, RI): 193 (M^+, 10), 162 (12), 149 (4), 133 (38), 119 (100), 104 (42), 91 (80), 85 (4), 75 (16), 64 (22), 57 (12); Analysis: $C_{10}H_{11}NO_3$ requires C, 62.17; H, 5.73; N, 7.25; found C, 62.10; H, 5.75; N, 7.25%.

4.3.5. (2*S*)-1-(4-Acetamidophenoxy)-2,3-propanediol (4e). Yield: 1.14 g, 85%; white solid; mp: 142–144 °C (EtOAc); $[\alpha]^{25}_D$ +7.0 (*c* 1.0, EtOH); HPLC; 80% ee, Chiralcel OD-H, λ=254 nm, 10% 2-propanol/hexane, 1 mL/min. Retention time: (*S*) 11.464. (*R*) 17.358 min; IR ($CHCl_3$, cm^{-1}): 3321, 3240, 3138, 3077, 2941, 2882, 1663, 1604, 1554, 1514, 1414, 1284, 1254, 1113, 1050; ^1H NMR (200 MHz, DMSO-d_6): δ=2.10 (3H, s), 3.60–3.75 (2H, m), 3.90–4.05 (3H, m), 4.24 (1H, br s), 4.45 (1H, br s), 6.84 (2H, d, *J*=8.0 Hz), 7.46 (2H, d, *J*=8.0 Hz), 9.35 (1H, br s); ^{13}C NMR (50 MHz): δ=23.4, 62.7, 69.8, 69.8, 114.1, 120.6, 132.1, 154.5; Mass (*m/z*, RI): 225 (M^+, 10), 183 (4), 151 (16), 135 (4), 117 (5), 110 (8), 109 (100), 108 (15), 93 (7), 74 (4), 65 (8), 60 (6), 57 (15); Analysis: $C_{11}H_{15}NO_4$ requires C, 58.63; H, 6.71; N, 6.22; found C, 58.63; H, 6.79; N, 6.26%.

4.3.6. (2*S*)-1-(2,3-Dimethylphenoxy)-2,3-dihydroxypropane (4f). Yield: 1.1 g, 94%; colourless solid; mp: 104–105 °C (EtOH); $[\alpha]^{25}_D$ +4.25 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 3431–3414, 3019, 2400, 1639, 1215, 751; ^1H NMR (200 MHz, $CDCl_3$): δ=2.14 (3H, s), 2.27 (3H, s), 2.55 (OH, br s), 3.0 (OH, br s), 3.77–3.90 (2H, m), 3.99 (2H, d, *J*=6.0 Hz), 4.05–4.2 (1H, m), 6.68 (1H, d, *J*=8.0 Hz), 6.78 (1H, d, *J*=8.0 Hz), 7.00 (1H, t, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=11.5, 19.9, 63.8, 69.2, 70.6, 109.1, 122.7, 125.0, 125.8, 137.9, 156.2; Mass (*m/z*, RI):196 (M^+, 18), 165 (0.2), 147 (8), 123 (100), 107 (40), 91 (12), 77 (8), 65 (0.2); Analysis: $C_{11}H_{16}O_3$ requires C, 67.32; H, 8.22%; found: C, 67.21; H, 8.16%.

4.3.7. (2*S*)-1-(2-Acetyl-4-nitrophenyl)-2,3-propanediol (4g). Yield: 1.46 g, 96%; yellow gum; $[\alpha]^{25}_D$: −5.31 (*c* 1.1, EtOH); HPLC: 97% ee, Chiracel-OD (25 cm) λ_{max}: 254 nm, 70:30 pet. ether/isopropanol, 1 mL/min. Retention time: (*S*): 7.08 min, (*R*): 8.26 min; IR: ($CHCl_3$, cm^{-1}): 740, 780, 845, 993, 1020, 1130, 1257, 1379, 1390, 1458, 1515, 1598, 2845, 2910, 3280; ^1H NMR (200 MHz, acetone-d_6): δ=2.66 (3H, s), 3.83–3.87 (2H, m), 4.16–4.34 (3H, m), 7.10 (1H, d, *J*=9.3 Hz), 8.33 (1H, dd, *J*=2.9, 9.0 Hz), 8.58 (1H, d, *J*=4.0 Hz); ^{13}C NMR (50 MHz, acetone-d_6): δ=31.0, 62.8, 69.8, 70.4, 112.7, 125.6, 127.6, 128.2, 140.5, 162.1,

196.9; Analysis: $C_{11}H_{13}NO_6$ requires C, 51.76; H, 5.13; N, 5.48; found C, 51.52; H, 5.08; N, 5.45%

4.4. Preparation of cyclic sulfates 5a–g

A. To a solution of one of the diols **4a–g** (4 mmol) and triethylamine (2.21 mL, 16 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added freshly distilled thionyl chloride (0.44 mL, 6 mmol) drop-wise under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 30–45 min (monitored by TLC). The reaction mixture was quenched by the addition of cold water (10 mL). The organic layer was separated and the aqueous layer extracted with EtOAc (3×15 mL). The combined organic extracts were washed with water, brine and dried over anhydrous Na_2SO_4. Evaporation of solvent under reduced pressure yielded pale yellow colored liquid, which was purified by the column chromatography using 10% EtOAc in pet. ether as a eluent to afford the corresponding cyclic sulfite as viscous yellow liquid in 96–99% yield.

B. To a solution of one of the cyclic sulfites (3 mmol) in CH_3CN: H_2O mixture (9:1, 8 mL) at 0 °C was added solid $NaIO_4$ (0.963 g, 4.5 mmol) and $RuCl_3 \cdot 3H_2O$ (0.012 g, 0.06 mmol). The reaction mixture was stirred for 30–40 min at 0 °C (monitored by TLC). After the reaction was completed, it was filtered through a pad of celite. Solvent evaporated under reduced pressure to give the crude product, which was purified by column chromatography using pet. ether/EtOAc (8:2) as eluent to afford cyclic sulfates **5a–g** in 86–98% yield.

4.4.1. (4*S*)-4-(1-Naphthoxymethyl)-1,3,2-dioxathiolane-2,2-dioxide (5a). Yield: 789 mg, 94%; gum; $[\alpha]^{25}_D$: +17.4 (*c* 0.5, EtOH); IR ($CHCl_3$, cm^{-1}): 651, 753, 984, 1024, 1130, 1190, 2113, 1255, 1398, 1460, 1510, 1600, 2854, 2940; ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.25–4.29 (2H, m), 4.60–4.67 (1H, m), 4.88–4.96 (1H, m), 5.36–5.41 (1H, m), 6.79 (1H, d, *J*=8.1 Hz), 7.26–7.53 (4H, m), 7.70–7.84 (1H, m), 8.16–8.21 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=66.5, 68.3, 77.9, 104.7, 121.1, 121.4, 125.0, 125.4, 126.5, 127.3, 134.3, 153.3; MS *m/z* (% rel intensity): 280 (M^+, 100), 157 (55), 144 (56), 137 (25), 123 (28), 118 (10), 91 (8); Analysis: $C_{13}H_{12}SO_5$ requires C, 55.71; H, 4.32; S, 11.44; found C, 55.56; H, 4.29; S, 11.42%.

4.4.2. (4*S*)-4-[(2-Methoxyphenyl)methyl]-1,3,2-dioxathiolane-2,2-dioxide (5b). Yield: 756 mg, 97%; gum; $[\alpha]^{25}_D$: +20.12 (*c* 1, EtOH); IR ($CHCl_3$, cm^{-1}): 651, 754, 819, 981, 1026, 1126, 1178, 1213, 1255, 1392, 1456, 1506, 1595, 2839, 2935, 3018; ^{1}H NMR (200 MHz, $CDCl_3$): δ=3.85 (3H, m), 4.31 (2H, t, *J*=6.2 Hz), 4.81 (1H, d, *J*=6.2 Hz), 4.85 (1H, d, *J*=2.1 Hz), 5.22–5.28 (1H, m), 6.94–7.10 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=55.6, 68.0, 69.7, 79.2, 112.4, 116.9, 120.9, 123.6, 146.9, 150.2; MS *m/z* (% rel intensity): 260 (M^+, 100), 216 (5), 137 (45), 123 (65), 109 (58), 95 (46), 77 (50); Analysis: $C_{10}H_{12}SO_6$ requires C, 46.15; H, 4.65; S, 12.32; found C, 46.21; H, 4.63; S, 12.26%.

4.4.3. (4*S*)-4-[(3-Methylphenyl)methyl]-1,3,2-dioxathiolane-2,2-dioxide (5c). Yield: 717 mg, 98%; gum; $[\alpha]^{25}_D$: +21.39 (*c* 1, EtOH); IR ($CHCl_3$, cm^{-1}): 652, 750, 819, 944, 1097, 1208, 1291, 1347, 1444, 1584, 2431, 2926, 3020; ^{1}H NMR (200 MHz, $CDCl_3$): δ=2.33 (3H, s), 4.01–4.14 (2H, m), 4.45–4.51 (1H, m), 4.78–4.86 (1H, m), 5.23–5.28 (1H, m), 6.68–6.84 (3H, m), 7.14–7.26 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=21.2, 65.5, 69.5, 79.1, 111.3, 115.4, 122.8, 129.3, 139.8, 157.4; MS *m/z* (% rel intensity): 244 (M^+, 23), 228 (16), 147 (10), 121 (96), 108 (92), 91 (100), 77 (15); Analysis: $C_{10}H_{12}SO_5$ requires C, 49.17; H, 4.95; S, 13.13; found C, 49.21; H, 4.86; S, 13.06%.

4.4.4. (2*R*)-1-(2-Cyanophenoxy)-1,3,2-dioxathiolane-2,2-dioxide (5d). Yield: 657 mg, 86%; gum; $[\alpha]^{25}_D$ +8.6 (*c* 2.0, EtOH); IR ($CHCl_3$, cm^{-1}): 3082, 2873, 2227, 1649, 1598, 1579, 1492, 1450, 1425, 1411, 1365, 1290, 1259, 1234, 1166, 1110, 1043, 995, 933, 839, 756, 590, 497; ^{1}H NMR (200 MHz, $CDCl_3$): δ=4.35–4.55 (1H, m), 4.90 (1H, d, *J*=2.0 Hz) 5.05 (1H, d, *J*=6.0 Hz), 5.25–5.45 (1H, m), 6.95–7.20 (2H, m), 7.50–7.70 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=96.9, 70.0, 78.8, 102.0, 112.6, 115.6, 122.1, 133.6, 134.5, 158.7; MS (*m/z*, RI): 255 (M^+, 6), 232 (3), 218 (4), 204 (4), 193 (11), 176 (4), 162 (15), 146 (6), 134 (15), 133 (56), 119 (100), 104 (45), 102 (15), 91 (72), 80 (27), 75 (24), 64 (45), 57 (26); Analysis: $C_{10}H_9NSO_5$ requires C, 47.05; H, 3.55; N, 5.48; found C, 47.01; H, 3.66; N, 5.49%.

4.4.5. (4*R*)-4-(4-Acetamidophenoxy)-1,3,2-dioxathiolane-2,2-dioxide (5e). Yield: 740 mg, 86%; gum; IR ($CHCl_3$, cm^{-1}): 3409, 3018, 1652, 1627, 1419, 1215, 1053, 1029, 757; ^{1}H NMR (200 MHz, DMSO-d_6): δ=2.13 (3H, s), 4.28 (2H, d, *J*=4.0 Hz), 4.70 (1H, dd, *J*=2.0, 6.0 Hz), 4.86 (1H, dd, *J*=2.0, 6.0 Hz), 5.20–5.40 (1H, m), 6.84 (1H, d, *J*=8.0 Hz), 7.50 (2H, d, *J*=8.0 Hz), 9.10 (1H, s); ^{13}C NMR (50 MHz): δ=23.6, 67.8, 70.9, 81.9, 116.1, 123.0, 156.1, 158.7, 171.5; Analysis: $C_{11}H_{13}NSO_6$ requires C, 45.79; H, 4.57; N, 4.89; S, 11.16; found C, 51.93; H, 4.47; N, 4.82; S, 11.13%.

4.4.6. (4*S*)-4-[(2,3-Dimethyl) methyl]-1,2,3-dioxathiolane-2,2-dioxide (5f). Yield: 665 mg, 86%; colourless solid; mp: 230–231 °C; $[\alpha]^{25}_D$ −8.8 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3020, 2926, 2431, 1584, 1444, 1347, 1291, 1208, 1097, 944, 819, 750, 652; ^{1}H NMR (200 MHz, $CDCl_3$): δ=2.15 (3H, s), 2.28 (3H, s), 4.26 (2H, d, *J*=4.0 Hz), 4.78–4.88 (2H, m), 5.2–5.35 (1H, m), 6.65 (1H, d, *J*=6.0 Hz), 6.84 (1H, d, *J*=6.0 Hz), 7.0 (1H, t, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 11.5, 19.9, 63.8, 68.6, 78.1, 108.9, 123.3, 125.3, 125.8, 138.3, 155.7; Mass (*m/z*, RI): 258 (M^+, 32), 162 (12), 159 (20), 145 (30), 135 (78), 122 (72), 105 (65), 91 (60), 77 (100), 65 (0.5); Analysis: $C_{11}H_{14}O_5S$ requires: C, 51.15; H, 5.46, S, 12.41%; found: C, 51.19; H, 5.39, S, 12.61%.

4.4.7. (4*S*)-4-[(2-Acetyl-4-nitrophenyl) methyl]-1,3,2-dioxathiolane-2,2-dioxide (5g). Yield: 932 mg, 98%; solid; mp: 138–140 °C; $[\alpha]^{25}_D$: −2.97 (*c* 0.4, EtOH); IR: ($CHCl_3$, cm^{-1}): 651, 753, 984, 1024, 1130, 1213, 1255, 1460, 1510, 1600, 1688, 2854, 2940; ^{1}H NMR (200 MHz, acetonitrile-d_3): δ=2.36 (3H, s), 4.39–4.54 (2H, m), 4.59 (1H, dd, *J*=4.1, 9.0 Hz), 4.74 (1H, dd, *J*=4.2, 9.0 Hz), 5.29–5.35 (1H, m), 7.00 (1H, d, *J*=9.3 Hz), 8.05 (1H, dd, *J*=2.9, 9.0 Hz), 8.26 (1H, d, *J*=4.1 Hz); ^{13}C NMR (50 MHz, acetonitrile-d_3): δ 32.5, 69.0, 71.5, 81.7, 115.4,

127.3, 130.2, 143.66, 162.7, 199.0; Analysis: $C_{11}H_{11}NSO_8$ requires C, 41.64; H, 3.49; N, 4.41; S, 10.10; found C, 41.62; H, 3.45; N, 4.46; S, 9.78%.

4.5. Preparation of epoxides 6a–g

A. To a solution of one of the cyclic sulfates **5a–g** (2.5 mmol) in dry THF (15 mL) was added anhydrous LiBr (1.04 g, 12 mmol) and the resulting reaction mixture was stirred for 40–50 min (monitored by TLC for the disappearance of cyclic sulfate) at 25 °C. After completion of the reaction the solvent was removed under reduced pressure. In the resulting residue diethyl ether (25 mL) and 20% H_2SO_4 (25 mL) were added and stirred at 25 °C for 4–5 h (monitored by TLC). After completion of the reaction the two layers were separated, the aqueous layer extracted with diethyl ether (3×15 mL), combined organic extracts were washed with saturated $NaHCO_3$, water and brine, dried over anhydrous sodium sulfate and evaporated under reduced pressure to give the corresponding bromoalcohols.

B. The above crude bromoalcohol (2 mmol) was dissolved in MeOH (20 mL) and treated with anhydrous K_2CO_3 (1.10 g, 8 mmol) at 0 °C. The resulting reaction mixture was stirred at 0 °C for 2 h (monitored by TLC). After completion the reaction was quenched by the addition of saturated NH_4Cl solution (10 mL) and extracted with CH_2Cl_2 (4× 15 mL), washed with water and brine, dried over anhydrous Na_2SO_4, evaporated under reduced pressure to give crude product. It was then purified by column chromatography using pet. ether/EtOAc (8:2) as eluents to give pure epoxides **6(a–g)** as oil in 80–85% yield.

4.5.1. (2*S*)-3-(1-Naphthyloxy)-1,2-epoxypropane (6a). Yield: 400 mg, 80% overall in two steps; gum; $[\alpha]^{25}_D$: +10.91 (*c* 1.3, EtOH); IR (neat, cm^{-1}): 748, 790, 916, 1021, 1123, 1190, 1240, 1260, 1454, 1510, 1590, 2890, 2990; ^1H NMR (200 MHz, $CDCl_3$): δ=2.75–2.79 (1H, m), 2.86–2.95 (1H, m), 3.39–3.44 (1H, m), 3.89 (1H, dd, *J*= 12.1, 6.2 Hz), 4.28 (1H, dd, *J*=12.1, 2.1 Hz), 6.73 (1H, d, *J*=8.14 Hz), 7.28–7.48 (4H, m), 7.74–7.79 (1H, m), 8.26–8.31 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=44.5, 50.0, 68.8, 104.9, 120.7, 121.9, 125.2, 125.6, 126.4, 127.3, 134.4, 154.1; MS *m/z* (% rel intensity): 200 (M^+, 100), 157 (28), 144 (65), 127 (18), 115 (53), 89 (10); Analysis: $C_{13}H_{12}O_2$ requires C, 77.98; H, 6.04; found C, 77.96; H, 6.04%.

4.5.2. (2*S*)-3-(2-Methoxyphenyl)-1,2-epoxypropane (6b). Yield: 382 mg, 85% overall in two steps; gum; $[\alpha]^{25}_D$: +9.83 (*c* 1.2, EtOH); IR (neat, cm^{-1}): 746, 779, 916, 1027, 1124, 1180, 1224, 1255, 1454, 1504, 1593, 2837, 2929, 3001; ^1H NMR (200 MHz, $CDCl_3$): δ=2.72 (1H, dd, *J*= 6.1, 4.0 Hz), 2.89 (1H, t, *J*=4.0 Hz), 3.35–3.44 (1H, m), 3.87 (3H, s), 3.99 (1H, dd, *J*=12.1, 6.1 Hz), 4.20 (1H, dd, *J*=12.1, 4.0 Hz), 6.90–6.93 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=44.7, 50.1, 55.9, 70.3, 112.5, 115.5, 120.9, 122.1, 148.3, 150.0; MS *m/z* (% rel intensity): 180 (M^+, 98), 150 (13), 137 (20), 124 (100), 109 (80), 95 (37), 81 (30), 77 (43), 65 (21); Analysis: $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.71; found C, 66.67; H, 6.81%.

4.5.3. (2*S*)-3-(3-Methylphenyl)-1,2-epoxypropane (6c). Yield: 344 mg, 84% overall in two steps; gum; $[\alpha]^{25}_D$: +13.43 (*c* 2.2, EtOH); IR (neat, cm^{-1}): 690, 775, 860, 900, 1041, 1053, 1161, 1261, 1290, 1454, 1488, 1585, 1602, 2871, 2923, 2999; ^1H NMR (200 MHz, $CDCl_3$): δ=2.33 (3H, s), 2.73–2.76 (1H, m), 2.87–2.92 (1H, m), 3.32–3.36 (1H, m), 3.91 (1H, dd, *J*=12.1, 3.1 Hz), 4.15 (1H, dd, *J*= 12.1, 4.1 Hz), 6.71–6.80 (3H, m), 7.13–7.25 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=21.4, 44.6, 50.1, 68.6, 111.5, 115.5, 112.0, 129.2, 139.5, 158.5; MS *m/z* (% rel intensity): 164 (M^+, 100), 134 (13), 119 (30), 108 (98), 91 (93), 77 (91), 65 (31), 57 (30); Analysis: $C_{10}H_{12}O_2$ requires C, 73.15; H, 7.37; found C, 73.21; H, 7.42%.

4.5.4. (2*S*)-1-(2-Cyanophenoxy)-1,2-epoxypropane (6d). Yield: 393 mg, 90%; gum; $[\alpha]^{25}_D$ +2.3 (*c* 2.3, $CHCl_3$); IR ($CHCl_3$, cm^{-1}): 4217, 3614, 3020, 2399, 2231, 1600, 1514, 1505, 1450, 1290, 1261, 1210, 1045, 1026, 908, 760, 669; ^1H NMR (200 MHz, $CDCl_3$)): δ=2.80–2.95 (2H, m), 3.35–3.45 (1H, m), 4.05 (1H, dd, *J*=6.0 Hz each), 4.30 (1H, dd, *J*=4.0, 8.0 Hz), 6.95 (2H, dd, *J*=8.0 Hz), 7.45–7. 65 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=44.2, 49.6, 69.2, 102.0, 112.5, 116.1, 121.2, 133.6, 134.3, 159.9; MS (*m/z*, RI): 175 (M^+, 8), 162 (10), 149 (10), 133 (28), 119 (45), 104 (36), 102 (32), 91 (80), 90 (32), 77 (20), 76 (18), 75 (28), 64 (52), 63 (45), 57 (100), 77 (72); Analysis: $C_{10}H_9NO_2$ requires C, 68.56; H, 5.17; N, 7.99; found C, 68.59; H, 5.17; N, 7.98%.

4.5.5. (2*S*)-3-(4-Acetamidophenoxy)-1,2-epoxypropane (6e). Yield: 414 mg, 80%; crystalline solid; mp: 104 °C (EtOAc and hexane); $[\alpha]^{25}_D$ +14.0 (*c* 2.0, EtOH); IR ($CHCl_3$, cm^{-1}): 3299, 2931, 1664, 1604, 1540, 1510, 1411, 1240, 1038, 828; ^1H NMR (200 MHz, $CDCl_3$)): δ=2.15 (3H, s), 2.76 (1H, dd, *J*=5.0, 3.0 Hz), 2.89 (1H, dd, *J*=5.0, 3.0 Hz), 3.25–3.45 (1H, m), 3.85 (1H, dd, *J*=8.0, 5.0 Hz), 4.15 (1H, dd, *J*=8.0, 5.0 Hz), 6.85 (2H, d, *J*=8.0 Hz), 7.40 (2H, d, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=24.0, 44.5, 50.1, 68.9, 114.8, 121.9, 131.5, 154.7, 168.7; Analysis: $C_{11}H_{13}NO_3$ requires C, 63.75; H, 6.32; N, 6.75; found C, 63.77; H, 6.35; N, 6.66%.

4.5.6. (2*S*)-3-(2,3-Dimethylphenyl)-1,2-epoxypropane (6f). Yield: 240 mg, 53.3%; gum; $[\alpha]^{25}_D$ −6.52 (*c* 2.3, $CHCl_3$); IR ($CHCl_3$, cm^{-1}) 3020, 2926, 2431, 1584, 1444, 1347, 1291, 1208, 1097, 944, 819, 750, 652; ^1H NMR (200 MHz, $CDCl_3$): δ=2.17 (3H, s), 2.27 (3H, s), 2.75 (1H, dd, *J*=2.0 Hz each), 2.87 (1H, t, *J*=6.0 Hz), 3.34–3.39 (1H, m), 3.91 (1H, dd, *J*=4.0, 6.0 Hz), 4.16 (1H, dd, *J*= 4.0 Hz each), 6.66 (1H, d, *J*=10.0 Hz), 6.78 (1H, d, *J*= 8.0 Hz), 7.0 (1H, t, *J*=8.0 Hz);^{13}C NMR (50 MHz, $CDCl_3$): δ=11.5, 19.9, 44.6, 50.3, 69.2, 109.6, 122.9, 125.8, 138.0, 156.5, 159.7; Analysis: $C_{11}H_{14}O_2$ requires: C, 74.13; H, 7.92%; found C, 74.41; H, 7.69%.

4.5.7. (2*S*)-3-(2-Acetyl-4-nitrophenyl)-1,2-epoxypropane (6g). Yield: 503 mg, 85%; solid; mp: 80–83 °C; $[\alpha]^{25}_D$: −10.7 (*c* 0.9, EtOH); IR: ($CHCl_3$, cm^{-1}): 413, 430, 440, 459, 471, 487, 756, 1017, 1116, 1216, 1277, 1345, 1485, 1523, 1586, 1610, 1685, 2930, 3020; ^1H NMR (200 MHz, $CDCl_3$): δ=2.70 (3H, s), 2.28 (1H, dd, *J*=2.1, 9.0 Hz), 3.00 (1H, dd, *J*=2.0, 9.0 Hz), 3.43–3.49 (1H, m), 4.57 (1H, dd, *J*=2.0, 10.0 Hz), 7.09 (1H, d, *J*=9.3 Hz), 8.34 (1H, dd, *J*= 2.9, 9.0 Hz), 8.63 (1H, d, *J*=4.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=31.5, 44.3, 49.3, 70.3, 112.9, 126.4, 128.5, 141.6, 159.6, 161.7, 196.9; MS *m/z* (% rel intensity): 237

(M^+, 20), 192 (8), 178 (40), 164 (18), 148 (10), 132 (60), 118 (30), 99 (35), 89 (10), 77 (20), 63 (35), 43 (100); Analysis: $C_{11}H_{11}NO_5$ requires C, 55.69; H, 4.67; N, 5.90; found C, 55.52; H, 4.61; N, 5.82%.

4.6. Preparation of (*S*)-propranolol (1a), (*S*)-moprolol (1b), (*S*)-toliprolol (1c), (*S*)-bunitrolol (1d), (*S*)-practolol (1e), (*S*)-xibenolol (1f) and (*S*)-celiprolol (1g) via opening of epoxides 6a–g

One of the epoxides **6a–g** (1.5 mmol) was dissolved in appropriate amines (10 mL) and refluxed in presence of water (0.5 mL) for 1 h. Excess of amine was removed under reduced pressure to afford **1(a–g)**.

In case of toliprolol, the resulting gum after evaporation of isopropylamine was dissolved in ether and dry HCl gas was passed through it for 15 min, the solvent was removed under reduced pressure and resulting solid recrystallized from MeOH+EtOAc afford (*S*)-toliprolol (**1c**) as its hydrochloride salt.

In case of celiprolol, the nitro group was hydrogenated at 20 psi H_2 pressure with 10% Pd/C as catalyst at room temperature to get the amine which was condensed with diethyl carbomyl chloride (DECC) to afford the corrosponding (*S*)-celiprolol.

4.6.1. (*S*)-(−)-Propranolol (1a). Yield: 384 mg, 99%; colourless solid; mp: 73–74 °C, (lit.[12] 72–73 °C); $[\alpha]^{25}_D$: −9.00 (*c* 0.5, EtOH), 90% ee (lit.[12] −9.9 (*c* 0.5, EtOH)); IR ($CHCl_3$, cm^{-1}): 570, 667, 750, 1029, 1120, 1175, 1210, 1240, 1450, 1500, 1594, 2930, 2960, 3300, 3432; ^1H NMR (200 MHz, $CDCl_3$): δ=1.09 (6H, d, *J*=6.1 Hz), 2.76–3.01 (5H, m), 4.08–4.19 (3H, m), 6.79 (1H, d, *J*=8.1 Hz), 7.34–7.51 (4H, m), 7.76–7.81 (1H, m), 8.22–8.27 (1H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=22.8, 48.7, 49.6, 68.4, 70.7, 104.8, 120.4, 121.7, 125.0, 125.4, 125.7, 126.2, 127.3, 134.4, 154.3; MS *m/z* (% rel intensity): 259 (M^+, 3), 144 (13), 115 (20), 84 (100), 72 (50), 69 (23), 56 (33); Analysis: $C_{16}H_{21}NO_2$ requires C, 74.10; H, 8.16; N, 5.40; found C, 74.25; H, 8.16; N, 5.30%.

4.6.2. (*S*)-(−)-Moprolol (1b). Yield: 351 mg, 98%; solid; mp: 84–85 °C, (lit.[12] 82–83 °C); $[\alpha]^{25}_D$: −3.90 (*c* 4.5, EtOH), 68% ee (lit.[11] −5.6 (*c* 4.5, EtOH)]; IR ($CHCl_3$, cm^{-1}): 667, 757, 1029, 1124, 1178, 1217, 1253, 1454, 1506, 1593, 2933, 2966, 3313, 3400; ^1H NMR (200 MHz, $CDCl_3$): δ=1.07 (6H, d, *J*=6.0 Hz), 2.69–2.90 (5H, m), 3.85 (3H, s), 3.97–4.07 (3H, m), 6.86–6.97 (4H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ=23.0, 48.8, 49.3, 55.9, 68.6, 73.1, 112.4, 115.3, 121.1, 121.9, 148.6, 150.1; MS *m/z* (% rel intensity): 239 (M^+, 5), 224 (10), 195 (52), 124 (7), 109 (6), 77 (12), 72 (100), 56 (12); Analysis: $C_{13}H_{21}NO_3$ requires C, 65.25; H, 8.84; N, 5.85; found C, 65.11; H, 8.64; N, 5.75%.

4.6.3. (*S*)-(−)-Toliprolol (1c) hydrochloride. Yield: 383 mg, 99%; gum; mp: 117–118 °C, (lit.[5k] 119 °C); $[\alpha]^{25}_D$: −21.54 (*c* 1.01, EtOH) 78% ee; (lit.[5k] −27.4 (*c* 1.01, EtOH)); IR ($CHCl_3$, cm^{-1}): 694, 775, 968, 1062, 1110, 1257, 1294, 1379, 1461, 1488, 1585, 1612, 2711, 2852, 2933, 3257, 3303; ^1H NMR (200 MHz, $CDCl_3$): δ=1.48 (6H, s), 2.30 (3H, s), 3.19–3.48 (3H, m), 3.94–4.15 (2H, m), 4.55–4.63 (1H, m), 6.71–6.79 (3H, m), 7.10–7.18 (1H, m), 8.50 (1H, br s), 8.57 (1H, br s); ^{13}C NMR (50 MHz, $CDCl_3$): δ=18.6, 18.8, 21.2, 47.8, 51.2, 65.5, 69.3, 111.2, 115.1, 121.8, 129.0, 139.3, 158.0; MS *m/z* (% rel intensity): 259 (M^+, 2), 236 (30), 223 (9), 208 (13), 179 (19), 108 (7), 91 (10), 72 (100); Analysis: $C_{13}H_{22}ClNO_2$ requires C, 60.11; H, 8.54; Cl, 13.65; N, 5.39; found C, 60.20; H, 8.55; Cl, 13.75; N, 5.41%.

4.6.4. (*S*)-Bunitrolol (1d). Yield: 279 mg, 75%; white solid; mp: 162 °C (EtOH) (lit.[11] 163–165 °C); $[\alpha]^{25}_D$ −10.0 (*c* 1.4, H_2O); HPLC: 60% ee, ChiraSpher NT, λ=254 nm, (*n*-hexane/EtOH/MeOH (60:20:20)/0.05% NH_3 (25%)), 0.5 mL/min. Retention time: (*S*) 9.359 min, (*R*) 12.354 min; IR ($CHCl_3$, cm^{-1}): 3400, 3019, 2995, 1485, 1410, 2227, 1554, 1490, 1215, 756; ^1H NMR (200 MHz, $CDCl_3$): δ=1.14 (9H, s), 2.37 (1H, br s), 2.70 (1H, dd, *J*=4.0, 6.0 Hz), 2.86 (1H, dd, *J*=4.0, 6.0 Hz), 3.80–4.15 (3H, m), 6.90 (2H, d, *J*=8.0 Hz), 7.45 (2H, d, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=28.6, 44.4, 50.6, 67.7, 71.6, 101.8, 112.4, 116.2, 120.8, 133.4, 134.2, 160.3; Analysis: $C_{14}H_{20}N_2O_2$ requires C, 67.71; H, 8.11; N, 11.28; found C, 67.75; H, 8.16; N, 11.25%.

4.6.5. (*S*)-Practolol (1e). Yield: 280 mg, 70%; white solid; mp: 125 °C (dioxane); lit.[5n] 128–129 °C; $[\alpha]^{25}_D$ −2.6 (*c* 1.0, EtOH); 82%ee (lit.[5n] $[\alpha]^{25}_D$ +3.5 (*c* 1.0, EtOH) for (*R*)-Practolol]; IR ($CHCl_3$, cm^{-1}): 3314, 3284, 2975, 2359, 1715, 1665, 1511, 1398, 1220, 1040, 769; ^1H NMR (200 MHz, $CDCl_3$): δ=0.95 (6H, d, *J*=6.0 Hz), 1.93 (3H, s), 3.30–3.50 (2H, m), 3.55–3.70 (1H, m), 3.80–4.10 (4H, m), 6.75 (2H, d, *J*=8.0 Hz), 7.44 (2H, d, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=23.7, 45.0, 49.9, 52.1, 69.0, 73.5, 115.9, 123.0, 133.7, 156.3, 171.4; Mass (*m/z*, RI): 248 (4), 222)3), 178 (5), 151 (40), 136 (20), 109 (100), 98 (15), 91 (10), 80 (32), 64 (60); Analysis: $C_{14}H_{22}N_2O_3$ requires C, 63.14; H, 8.31; N, 10.51; found C, 63.10; H, 8.25; N, 11.02%.

4.6.6. (*S*)-Xibenolol (1f). Yield: 214 mg, 57%; gum; $[\alpha]^{25}_D$ −17.58 (*c* 1.0, $CHCl_3$); 67% ee (lit.[5o] −25.4 (*c* 1.0, $CHCl_3$)]; IR ($CHCl_3$, cm^{-1}): 3421–3501, 2926, 2928, 2856, 1649, 1580, 1458, 1375, 1263, 1194 ^1H NMR (200 MHz, $CDCl_3$): δ=1.49 (9H, s), 2.12 (3H, s), 2.23 (3H, s), 3.07–3.36 (3H, m), 3.90–4.11 (2H, m), 4.66 (1H, br s), 6.62 (1H, d, *J*=8.0 Hz), 6.75–6.78 (1H, d, *J*=6.0 Hz), 6.96–7.03 (1H, t, *J*=8.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=11.4, 19.6, 25.6, 45.5, 57.4, 65.7, 69.9, 109.1, 122.6, 124.9, 125.7, 137.6, 156.0; Mass (*m/z*, RI): 233 (M^+–H_2O, 40), 218 (65), 161 (100), 147 (20), 122 (55), 112 (70), 91 (10), 77 (5); Analysis: $C_{15}H_{25}NO_2$ requires: C, 71.67; H, 10.02; N, 5.57%; found: C, 71.51; H, 9.89%; N, 5.51%.

4.6.7. (*S*)-(−) Celiprolol (1g). Yield: 511 mg, 90%; solid; mp 116–118 °C; lit.[5p] mp 117–118 °C; $[\alpha]^{25}_D$: −12.5 (*c* 1.76, EtOH), HPLC: 97% ee, Chiracel-OD 10% diethylamine/2-propanol, 1 mL/min. Retention time: (*S*): 10.35 min, (*R*): 12.67 min; IR: ($CHCl_3$, cm^{-1}): 673, 759, 824, 1044, 1076, 1162, 1221, 1307, 1382, 1430, 1500, 1651, 1677, 2794, 2966, 3320; ^1H NMR (200 MHz, $CDCl_3$): δ=1.39 (6H, t, *J*=10.0 Hz), 1.48 (9H, s), 2.57 (3H, s), 2.99 (1H, br s) 3.11–3.13 (3H, m), 3.38 (8H, m), 4.01–4.05 (2H, m), 6.84 (1H, d, *J*=10.0 Hz), 7.53 (1H, d, *J*=10.0 Hz), 7.70

(1H, s); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 8.5$, 13.8, 25.6, 31.0, 41.4, 46.0, 57.6, 65.2, 71.0, 113.5, 123.4, 126.9, 127.4, 133.1, 153.2, 155.4, 159.6, 199.8; Analysis: $C_{20}H_{33}N_3O_4$ requires C, 63.30; H, 8.76; N, 11.07; found C, 63.29; H, 8.81; N, 11.10.

Acknowledgements

IAS, VVT, MDN, GKD and SPK thank CSIR, New Delhi for the award of research fellowship. We also thank Dr. B. D. Kulkarni, Head, CEPD, for his constant encouragement. Financial support from DST, New Delhi is gratefully acknowledged.

References and notes

1. Stinson, S. C. *Chem. Eng. News* **1998**, *21*(September 21), 83.
2. Hanson, R. M. *Chem. Rev.* **1991**, *91*, 437.
3. Taylor, S. H.; Grimm, R. H. J. *Am. Heart J.* **1990**, *119*, 655.
4. (a) Howe, S. *Nature* **1966**, *210*, 1336. (b) Leftheris, K.; Goodman, M. J. *J. Med. Chem.* **1990**, *33*, 216. (c) Shiratsuchi, M.; Kawamura, K.; Akashi, T.; Ishihama, H.; Nakamura, M.; Takenaka, F. *Chem. Pharm. Bull.* **1987**, *35*, 3691.
5. **Propranolol**: (a) Dukes, M.; Smith, L. H. *J. Med. Chem.* **1971**, *14*, 326. (b) Tsuda, Y.; Yoshimoto, K.; Nishikawa, T.; *Chem. Pharm. Bull.* **1981**, *29*, 3593. (c) Iriuchijima, S.; Kojima, N. *Agric. Biol. Chem.* **1982**, *46*, 1153. (d) Katsuki, T. *Tetrahedron Lett.* **1984**, *25*, 2821. (e) Matsuo, N.; Ohno, N. *Tetrahedron Lett.* **1985**, *26*, 5533. (f) Kiunder, J. M.; Onami, T.; Sharpless, K. B. *J. Org. Chem.* **1989**, *54*, 1295. (g) Sakabura, S.; Takahashi, H.; Takeda, H.; Achiwa, K. *Chem. Pharm. Bull.* **1995**, *43*, 738. (h) Sessai, H.; Suzuki, T.; Itoh, N.; Shibasaki, M. *Appl. Organomet. Chem.* **1995**, *9*, 421. **Moprolol**: (i) Kazunori, K.; Akimasa, M.; Shigeki, H.; Takehisa, O.; Kiyoshi, W. *Agri. Biol. Chem.* **1985**, *49*, 207. (j) Ferrari, G., Vecchietti, V. US. 4683245 **1980**. **Toliprolol**: (k) Howe, R.; Rao, B. S. *J. Med. Chem.* **1968**, *11*, 1118. **Bunitrolol**: (l) Zhenya, H. *Gongye, Y.* **1987** *18*, 339 (Chinese). **Practolol**: (m) Thakkar, N. V.; Banerji, A. A.; Berinakatti; Hanumanthsa, S. *Biotechnology Lett.* **1995**, *17*, 217 (n) Danilewicz, J. C.; Kemp, J. E. G. *J. Med. Chem.* **1973**, *16*, 168. **Xibenolol**: (o) Hunma, S.; Ito, T.; Kambekawa, A. *Chem. Pharm. Bull.* **1985**, *33*, 760. **Celiprolol**: (p) Zoelss, G. *Arzneim-forsch.* **1983**, *33(1A)*, 2*(German)*. (q) Joshi, R. A.; Gurjar, M. K.; Tripathy, N. K. *Org. Proc. Res. Dev.* **2001**, *5*, 176.
6. (a) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483. (b) Johnson, R. A.; Sharpless, K. B. In *Catalytic Asymmetric Synthesis Ojima I*; Ojima, I., Ed.; VCH Publishers: New York, 1993; Chapter 4, pp 227–270.
7. Linli, He; Hoe-Sup, Byun; Robert, Bittman *J. Org. Chem.* **1998**, *16*, 5696.
8. Rao, A. V. R.; Gurjar, M. K.; Joshi, S. V. *Tetrahedron: Asymmetry* **1990**, *1*, 697.
9. Wang, Z. M.; Zhang, X. L.; Sharpless, K. B. *Tetrahedron Lett.* **1993**, *34*, 2267.
10. Theil, F.; Weidner, J.; Ballaschuh, S.; Kunath, A.; Schick, H. *J. Org. Chem.* **1994**, *59*, 388.
11. The Merck Index, 13th Ed. Merck and Co., Inc. Whitehouse Station, NJ.
12. Hou, X. L.; Li, B. F.; Dai, L. X. *Tetrahedron: Asymmetry* **1999**, *10*, 2319.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2839–2847

Synthesis of functionalized *m*-bistrifluoromethylbenzenes via cyclocondensation of 1,1,1,5,5,5-hexafluoroacetylacetone with enamines

Dmitriy M. Volochnyuk, Alexander N. Kostyuk,* Dmitriy A. Sibgatulin and Alexander N. Chernega

Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Murmanskaya 5, 02094 Kyiv-94, Ukraine

Received 15 October 2004; revised 4 January 2005; accepted 20 January 2005

Abstract—The reaction of 1,1,1,5,5,5-hexafluoroacetylacetone with push–pull enamines having a methyl group at the α-position was investigated. It was found that the reaction is sensitive both to the structure of enamines and to reaction conditions. As a result, a set of bistrifluoromethyldialkylanilines and ethyl bistrifluoromethylsalicylate was prepared. Plausible mechanisms and factors influencing the course of the reaction are discussed.

1. Introduction

Introduction of the trifluoromethyl group, an important functional group in organic chemistry allows chemists to considerably change physico-chemical properties of organic molecules.[1] Thus, derivatives of *m*-bistrifluoromethylbenzene are actively used in the design and synthesis of various ligands[2] and pharmaceutical substances.[3,4] Further development of methods for synthesis of functionalized *m*-bistrifluoromethylbenzenes is investigated. Although this fragment is actively used in many pharmaceutical research projects, in almost all of them 3,5-trifluoromethylaniline is used due to a known synthetic procedure described years ago.[5]

A few works such as the palladium catalyzed arylation of amines with 1-bromo-3,5-bis(trifluoromethyl)benzene[6] and the use of 2-methoxy-4,6-bis(trifluoromethyl) phenyllithium[7] give alternative approaches to the synthesis of bistrifluoromethylbenzene derivatives.

At the same time the commercially available symmetrical 1,3-biselectrophilic building block—1,1,1,5,5,5-hexafluoroacetylacetone has been used for synthesis of bistrifluoromethylated heterocyclic compounds. This approach has proved to be one of the most convenient methods for the synthesis of bistrifluoromethylated pyrazoles, isoxazoles,[8] pyridines,[9] pyrimidines,[10] pyrroles,[11] and diazepines.[12] To the best of our knowledge, the approach has not been applied to the synthesis of bistrifluoromethylated benzenes yet. In our previous work we have demonstrated the possibility of using tertiary push–pull enamines as 1,3-*CCC*-bisnucleophiles in the reactions with β-trifluoroacetylvinyl ethers for synthesis of monotrifluoromethylated functionalized dialkylanilines.[13] In this work we report our results on the reaction of tertiary push–pull enamines having a methyl group at the α-position with 1,1,1,5,5,5-hexafluoroacetylacetone **1**.

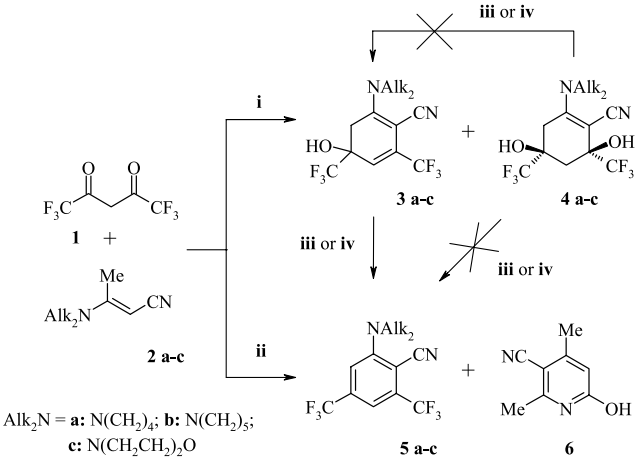
2. Results and discussion

2.1. Interaction of 1,1,1,5,5,5-hexafluoroacetylacetone with β-dialkylaminocrotonitriles

Enamines **2** derivatives of β-aminocrotonitrile react with 1,1,1,5,5,5-hexafluoroacetylacetone **1** in benzene at room temperature for 2–3 days affording a mixture of arene hydrate **3** and diol **4** in combined yield 35–40%, precipitated from the reaction mixture. Analysis of the reaction mixture by ^{19}F NMR reveals presence of the starting β-diketone **1** and traces of benzenes **5**. Numerous attempts to optimize the reaction conditions failed. Increasing the reaction temperature or time lead to formation of benzenes **5** and pyridone **6** products of the self-condensation of enamines **2**. Carrying

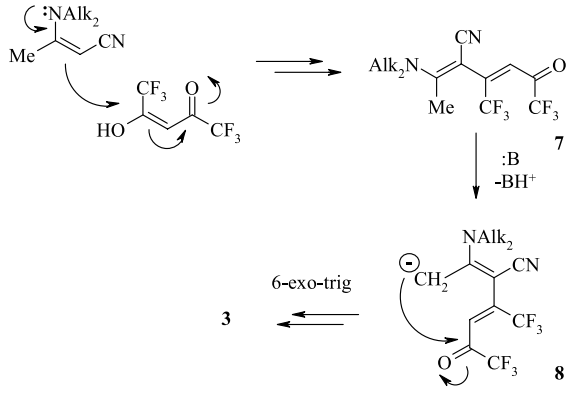
Keywords: 1,1,1,5,5,5-Hexafluoroacetylacetone; Push–pull enamines; Trifluoromethylated benzenes; Cyclocondensation.
* Corresponding author. Tel.: +380 44 5373218; fax: +380 44 5373253; e-mail: a.kostyuk@enamine.net

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.075

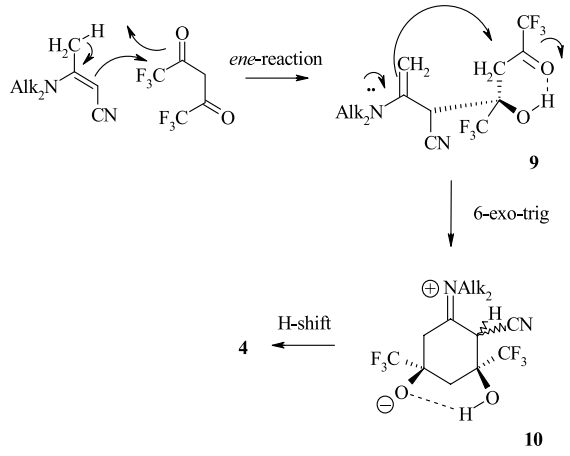


Scheme 1. Reagents and conditions: (i) benzene, rt, 2–3 days; (ii) benzene, reflux, 2 h; (iii) acetone, rt, 7–10 days; (iv) toluene, PTSA, reflux, 30 min.

out the reaction in boiling benzene or methanol results in conversion of 60–70% of the starting enamine, but the ratio of cyclization product **4** to pyridone **6** is ca. ~7:1 for enamine **2a**, and ~4:6 for enamine **2c** (Scheme 1). The ratio of reaction products **3** and **4** depends on the structure of the dialkylamino residue and reaction conditions (Table 1). Thus, for pyrrolidine enamine **2a**, the diol was registered in trace amount, while in the case of morpholine enamine **2c**, the ratio of diol/'hydrate' was 1:1. At the same time for enamine **2c**, diol **4c** was not registered at all when the reaction was run at 40–50 °C. It is worth noting that under the conditions in which hydrates **3** are transformed into benzenes **5**, diol **4** remains intact. Thus, we can draw the conclusion that diols **4** are not intermediate products in the chain of transformations **2**→**3**→**5**. We suppose that formation of **3** and **4** proceeds by different mechanisms. In the case of **3**, hexafluoroacetylacetone **1** reacts as an enol affording dienamine **7**, followed by 6-*exo-trig* cyclization to give 'hydrate' **3** (Scheme 2). At the same time the key step in the formation of diol **4** is the *ene*-reaction of enamines **2** with hexafluoroacetylacetone **1** which react in the ketone form like MeTFP,[14] affording terminal enamines **9** followed by spontaneous cyclization into diol **4**. Formation of only one possible diastereomer of **4** whose stereochemistry was solved by the single X-ray diffraction study could be rationalized by intramolecular hydrogen bonding (Scheme 3). Indirect proof for the proposed mechanisms is the growth of the 'hydrate' **3** produced upon increase of *C*-nucleophilicity of the enamines. Higher nucleophilicity would facilitate the reaction presented on Scheme 2,[13] and would not influence the reaction given on Scheme 3.[14]



Scheme 2.



Scheme 3.

The structures of compounds synthesized were confirmed by a set of physico-chemical methods, and for compounds **3a** and **4c** single X-ray diffraction studies were accomplished. (Figs. 1 and 2). It is worth noting that compounds of type **3** are allied to so-called arene hydrates whose simple representatives are highly unstable compounds under normal conditions.[15] In our previous work[13] two types of arene hydrates (type **A** and **B**, Fig. 3) which are stable under normal conditions have been described. In our viewpoint their stability is stipulated both by kinetic and thermodynamic factors. Substances **3** like arene hydrates of type **A** and **B** are stable compounds in the solid state, melting without decomposition. On heating in solution in the presence of catalytic amounts of acids such as PTSA 'arene hydrates' **3** eliminate water irreversibly turning into the corresponding benzenes **4**. It should be noted that arene

Table 1. Yields of products of the reaction of enamines **2** with hexafluoroacetylacetone **1**

Enamine	Conditions	Yield (%)			
		3	**4**	**5**	**6**
2a[a]	C_6H_6, rt, 3 days	31	Traces	6	—
2b[a]	C_6H_6, rt, 3 days	26	9	3	—
2c[a]	C_6H_6, rt, 3 days	21	23	5	—
2c[a]	C_6H_6, 40 °C, 3 h	14	Traces	15	—
2a[b]	C_6H_6, reflux, 4 h	—	—	43	6
2c[b]	C_6H_6, reflux, 4 h	—	—	28	24

[a] Refer according to ^{19}F NMR spectra of reaction mixture.
[b] Yields of **5** refer according to ^{19}F NMR spectra of reaction mixture and yield of **6** is for isolated product.

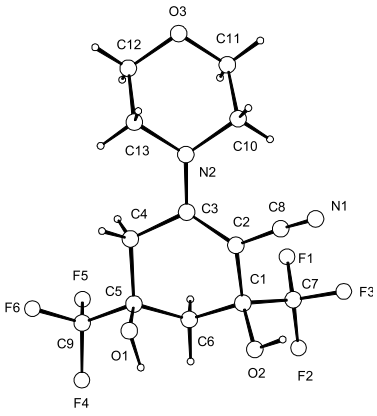


Figure 1. A perspective view and labeling scheme for the molecule **4a**.

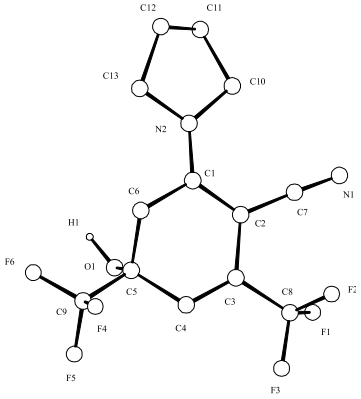


Figure 2. A perspective view and labeling scheme for the molecule **3a**.

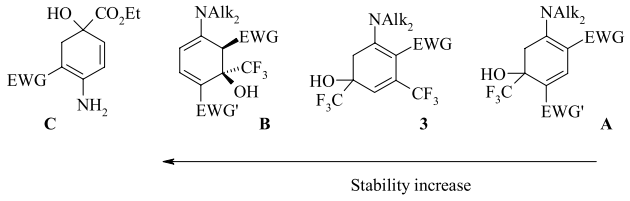


Figure 3.

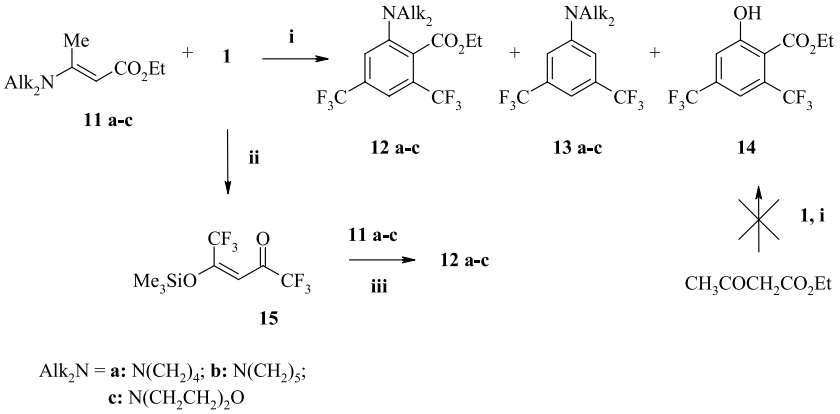
hydrates **3** hold an intermediate position in a stability series with respect to acidic catalysed elimination of water between previously described arene hydrates of types **A** and **B**. We suppose that unlike arene hydrates of types **A** and **B** the stability of **3** is stipulated only by kinetic factors, namely by strong destabilization of the forming carbcation on *E1*-elemination of water with two concurrently influencing trifluoromethyl groups. Besides, there are some similar stable arene hydrates of type **C** described in the literature where the ester group acts as an acceptor instead of the triflurometyl group.[16]

2.2. Interaction of 1,1,1,5,5,5-hexafluoroacetylacetone with β-dialkylaminocrotonoesters and α-methyl-β-enaminones

In going from enaminonitriles **2** to esters of β-dialkylaminocrotonic acids **11**, the reaction of the latter with hexafluoroacetylacetone **1** is complicated with side reactions. Besides the targeted dialkylaminoanthranilic acid esters **12**, symmetrical dialkylanilines **13**, and salicylic acid ester **14** formed in the reaction (Scheme 4). Increase of the basicity of dialkylamino residue leads to higher content of by-products (Table 2). We supposed that the cause of the side reactions which were not observed in the reactions with enones was the water forming in the reaction. To check the assumption the reaction was carried out in the presence of TMSCl as water scavenger affording the targeted esters of dialkylaminoanthranilic acids **12** as the sole products (Scheme 4).

It should be noted that salicylic acids **14** are not derived from hydrolysis of the starting enamine **11** so that under analogous conditions acetoacetic acid esters do not form salicylates **14**. In addition, under the reaction conditions the transformation of **12** into **13** does not occur.

Enaminones **16** derivatives of acetylacetone also react with hexafluoroacetylacetone **1** affording the product without EWG (Scheme 5). Thus, at room temperature in benzene both the expected acetophenones **17** and symmetrical anilines **13** are formed in the reaction. The higher basicity of dialkylamino group and the easier cleavage of the acetyl group mean that in the case of pyrrolidine enaminone, the

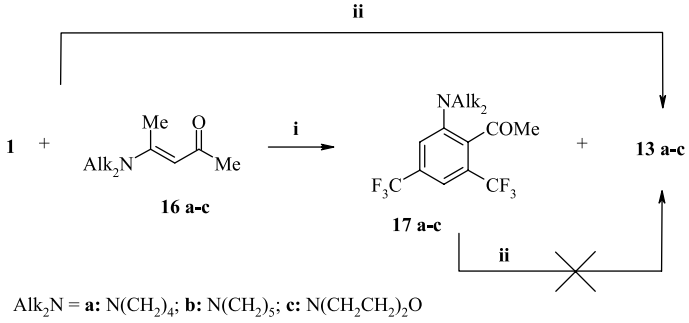


Scheme 4. Reagents and conditions: (i) benzene, rt, 3 days; (ii) 1 equiv Me_3SiCl, 1 equiv Et_3N, benzene, rt, 5 min; (iii) 1 equiv Me_3SiCl, 1 equiv Et_3N, benzene, rt, 1 day, 60 °C, 4 h.

Table 2. Yields of products of the reaction of enamines **11** and **16** with hexafluoroacetylacetone **1**[a]

Enamine	Conditions	Yield (%)		
		12 or **17**	**13**	**14**
11a	C_6H_6, rt, 3 days	15	5	32
11b	C_6H_6, rt, 3 days	17	Traces	29
11c	C_6H_6, rt, 3 days	39	Traces	14
11a	C_6H_6, TMSCl, Et_3N	93	—	—
11b	C_6H_6, TMSCl, Et_3N	71	—	—
11c	C_6H_6, TMSCl, Et_3N	69	—	—
16a	C_6H_6, rt, 3 days	Traces	45	—
16b	C_6H_6, rt, 3 days	26	10	—
16c	C_6H_6, rt, 3 days	27	6	—
16a	AcOH, 60 °C, 4 h	—	48	—
16b	AcOH, 60 °C, 4 h	—	45	—
16c	AcOH, 60 °C, 4 h	—	47	—

[a] Refer according to ^{19}F NMR spectra of reaction mixture.

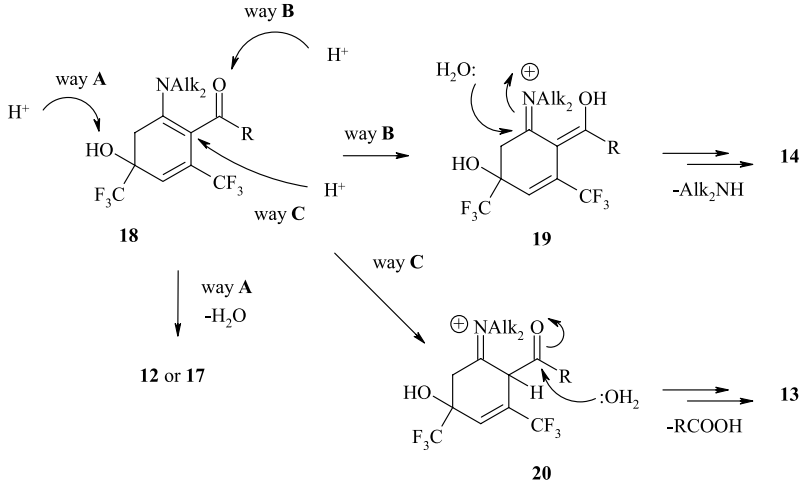


Scheme 5. Reagents and conditions: (i) benzene, rt, 3 days; (ii) AcOH, 60 °C, 4 h.

expected acetophenone **17a** is produced only in trace amounts. Under acidic conditions, influence of the dialkylamino residue basicity is reduced, so that the sole reaction products are anilines **13**.

Use of TMSCl in the reaction increases the conversion of hexafluoroacetylacetone **1** and decreases the yield of symmetrical anilines **13**. At the same time the reaction is accompanied by many side reactions thus complicating separation of the targeted products.

We suppose that enaminoesters **11** and enaminoketones **16** like enaminonitriles **2** react with hexafluoroacetylacetone **1** forming the corresponding arene hydrates **18** which have few protonation sites; three of them are noted on Scheme 6. Hexafluoroacetylacetone **1** as a strong CH-acid itself could act as a proton source in the reaction mixture. Protonation by pathway A followed by elimination of water affords targeted products **12** or **17**. Protonation at the oxygen atom of the enaminone fragment (pathway B) affords iminium salt **19** which upon hydrolysis gives the corresponding phenol **14**. In addition, protonation at the carbon atom of enamine fragment forming iminium salt **20** which could either hydrolize into the corresponding phenol **14** or enter into ketone cleavage giving symmetrical anilines **13**. Increase of the basicity of the dialkylamino residue



Scheme 6.

facilitates protonation by pathways B and C, so that the number of by-products increases. Actually, the increase of by-products was observed in series of enaminoesters **11** and enaminoketones **16**.

3. Conclusions

The reaction of 1,1,1,5,5,5-hexafluoroacetylacetone with push–pull enamines having a methyl group at the α-position was investigated. It has been found that the reaction is very sensitive both to structure of the starting enamines and to reaction conditions. As a result, a series of bistrifluoromethylated dialkylanilines bearing functional groups at the benzene ring, symmetrical bistrifluoromethylated dialkylanilines and ethyl ester of bistrifluoromethylated salicylic acid were obtained. Readily available starting materials and simple synthetic procedures make this method very attractive and convenient for the synthesis of various *m*-bistrifluoromethyl benzenes derivatives, useful building blocks for organic and medicinal chemistry.

4. Experimental

4.1. General

All solvents were purified and dried by standard methods. NMR spectra were recorded on a Varian VXR-300 spectrometer: ^{1}H and ^{13}C (300 and 75.4 MHz, respectively) with TMS as an internal standard; ^{19}F (282.2 MHz) with $CFCl_3$ as internal standard. IR spectra were recorded on a Nexus-470 spectrometer for samples in KBr discs. Mass spectra were obtained on a 'HEWLETT–PACKARD' HP GC/MS 5890/5972 instrument (EI, 70 eV) by GC inlet or on a MX-1321 instrument (EI, 70 eV) by direct inlet for the thermally labile arene hydrates. Microanalyses were performed in the Microanalytical Laboratory of the Institute of Organic Chemistry, National Academy of Sciences of Ukraine. Column chromatography was performed on silica gel (63–200 mesh, Merck). Silica gel Merck $60F_{254}$ plates were used for TLC. Starting enamines were prepared according to the literature.[17]

4.2. X-ray crystallography

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-235905 (**3a**) and CCDC-235904 (**4c**) and can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223/336 033; E-mail: deposit@ccdc.cam.ac.uk).

4.3. Interaction of 1 with β-dialkylaminocrotonitriles

4.3.1. 4-Hydroxy-2-(1-pyrrolidinyl)-4,6-bis(trifluoromethyl)-1,5-cyclohexadiene-1-carbonitrile (3a). Enamine **2a** (5 g, 36.8 mmol) was dissolved in benzene (25 mL) and to the solution formed **1** was added (7.65 g, 36.8 mmol). The reaction mixture was maintained at rt overnight. The precipitate formed was filtered and washed with cyclohexane affording **3a** (3.2 g, 27%) as a colourless solid. Mp 162 °C. ^{1}H NMR (acetone-d_6): $\delta=1.99$ (4H, br, m, CH_2), 2.99 and 3.07 (2H, AB-syst, $^{2}J_{HH}=16.8$ Hz), 3.85 (4H, br, s, NCH_2), 5.73 (1H, s, OH), 5.86 (1H, s, CH). ^{13}C NMR (DMSO-d_6): $\delta=24.7$, 34.5, 51.0, 65.1, 69.4 ($^{2}J_{CF}=28.5$ Hz), 110.3, 118.9, 123.6 ($^{1}J_{CF}=306.5$ Hz), 124.1 ($^{1}J_{CF}=272.1$ Hz), 131.6 ($^{2}J_{CF}=28.6$ Hz), 157.6. ^{19}F NMR (acetone): $\delta=-65.1$ (3F), −81.3 (3F). IR, ν_{max} (cm^{-1}): 3500–3200 (br), 2989, 2868, 2191, 1663, 1545, 1271. MS, *m/z* (%): 326 (M^+, 11), 308 (M^+-H_2O, 14), 307 (22), 280 (37), 257 (M^+-CF_3, 96), 148 (32), 119 (53), 69 (CF_3^+, 39), 55 (45), 42 (86), 41 (100). Anal. calcd for $C_{13}H_{12}F_6N_2O$: C 47.86; H 3.71; N 8.59. Found C 47.92; H 3.65; N 8.60.

4.3.2. 4-Hydroxy-2-(4-morpholinyl)-4,6-bis(trifluoromethyl)-1,5-cyclohexadiene-1-carbonitrile (3c). Enamine **2c** (1 g, 6.6 mmol) was dissolved in benzene (10 mL) and to the solution formed **1** was added (1.37 g, 6.6 mmol). The reaction mixture was maintained at 40 °C for 2 h and then it was left at rt overnight. The precipitate formed was filtered and washed with cyclohexane affording **3c** (316 mg, 14%) as a colourless solid. Mp 144–145 °C. ^{1}H NMR (acetone-d_6): $\delta=2.98$ and 3.05 (2H, AB-syst, $^{2}J_{HH}=16.8$ Hz), 3.67–3.92 (8H, m, CH_2), 5.85 (1H, s, OH), 5.96 (1H, s, CH). ^{13}C NMR (DMSO-d_6): $\delta=33.9$, 50.3, 63.8, 66.1, 69.7 ($^{2}J_{CF}=28.5$ Hz), 112.3, 116.4, 123.6 ($^{1}J_{CF}=306.5$ Hz), 124.1 ($^{1}J_{CF}=272.2$ Hz), 134.3 ($^{2}J_{CF}=33.0$ Hz), 161.7. ^{19}F NMR (acetone): $\delta=-67.0$ (3F), −82.9 (3F). IR, ν_{max} (cm^{-1}): 3510–3180 (br), 3078, 2919, 2868, 2193 1649, 1547, 1285. Mp 144–145 °C. MS, *m/z* (%): 342 (M^+, 78), 324 (M^+-H_2O, 56), 273 (M^+-CF_3, 48), 266 (60), 239 (100), 228 (32), 219 (29), 215 (35), 42 (34). Anal. calcd for $C_{13}H_{12}F_6N_2O_2$: C 45.62; H 3.53; N 8.19. Found C 45.65; H 3.56; N 8.22.

4.3.3. *cis*-4,6-Dihydroxy-2-(4-morpholinyl)-4,6-bis(trifluoromethyl)-1-cyclohexene-1-carbonitrile (4c) and 2-(4-morpholinyl)-4,6-bis(trifluoromethyl)-benzonitrile (5c). To a solution of enamine **2c** (5 g, 32.9 mmol) in benzene (25 mL) was added **1** (6.84 g, 32.9 mmol). The reaction mixture was maintained at rt for 3 days. The precipitate formed was filtered affording a mixture of **4c** and **3c** ~ 1:1. The mixture obtained was dissolved in acetone (25 mL) and maintained at rt for 7 days (the reaction mixture was monitored by ^{19}F NMR spectroscopy). After complete conversion of **3c** to **5c**, acetone was evaporated in vacuo and the residue was triturated with $CHCl_3$ (10 mL) affording **4c** (2.7 g, 23%). The $CHCl_3$ was evaporated in vacuo. The residue was extracted with boiling *n*-hexane (10 mL) and the *n*-hexane was evaporated in vacuo affording **5c** (2.66 g, 25%).

Compound **4c**. Colourless solid. Mp 175 °C (EtOAc). ^{1}H NMR (acetone-d_6): $\delta=2.10$ (1H, d, $^{2}J_{HH}=14.1$ Hz), 2.22 (1H, dd, $^{2}J_{HH}=14.1$ Hz, $^{4}J_{HH}=1.5$ Hz), 2.78 (1H, d, $^{2}J_{HH}=18.0$ Hz), 3.14 (1H, dd, $^{2}J_{HH}=18.0$ Hz, $^{4}J_{HH}=1.5$ Hz), 3.48–3.57 (2H, m, CH_2), 3.62–3.79 (6H, m, CH_2), 5.20 (1H, br. s, OH), 5.87 (1H, br. s, OH). ^{13}C NMR (acetone-d_6): $\delta=34.2$, 34.3, 51.1, 67.4, 72.4 ($^{2}J_{CF}=30.2$ Hz), 74.8 ($^{2}J_{CF}=30.4$ Hz), 79.2, 119.1, 126.0 ($^{1}J_{CF}=285.4$ Hz), 126.2 ($^{1}J_{CF}=284.4$ Hz), 161.3. ^{19}F NMR (acetone): $\delta=-78.9$ (3F), −82.6 (3F). IR, ν_{max} (cm^{-1}): 3384 (br), 3317 (br), 2983, 2933, 2868, 2184, 1555, 1453,

1261. MS, *m/z* (%): 360 (M^+, 16), 291 ($M^+ - CF_3$, 100). Anal. calcd for $C_{13}H_{14}F_6N_2O_3$: C 43.34; H 3.92; N 7.78. Found C 43.37; H 3.95; N 7.82.

Compound **5c**. Colourless solid. Mp 127–130 °C (cyclohexane) with sublimation. R_f (EtOAc)=0.68 ^{1}H NMR ($CDCl_3$): δ=3.33 (4H, t, $^3J_{HH}$=4.2 Hz, NCH_2), 3.93 (4H, t, $^3J_{HH}$=4.2 Hz, NCH_2), 7.42 (1H, s, CH), 7.56 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=51.0, 65.8, 104.1, 113.5, 114.8, 120.4, 121.6 ($^1J_{CF}$=275.2 Hz), 122.3 ($^1J_{CF}$=274.0 Hz), 134.0 ($^2J_{CF}$=34.2 Hz), 134.1 ($^2J_{CF}$=32.8 Hz), 157.5. ^{19}F NMR ($CHCl_3$): δ=−63.5 (3F), −64.9 (3F). IR, ν_{max} (cm^{-1}): 3083, 3052, 2868, 2843, 2228, 1619, 1445, 1395, 1281, 1142, 979. MS, *m/z* (%): 324 (M^+, 34), 266 (57), 239 (100), 219 (32). Anal. calcd for $C_{13}H_{10}F_6N_2O$: C 48.16; H 3.11; N 8.64. Found C 48.25; H 3.22; N 8.61

4.3.4. 2-(1-Pyrrolidinyl)-4,6-bis(trifluoromethyl)-benzonitrile (5a). To a solution of **3a** (100 mg) in toluene (25 mL) a few crystals of *p*-toluenesulfonic acid were added and the reaction mixture was refluxed for 0.5–1 h (The reaction was monitored by TLC using EtOAc as eluent). Toluene was evaporated in vacuo. The residue was extracted with boiling *n*-hexane (2 mL) and *n*-hexane was evaporated in vacuo affording **5a** (88 mg, 93%) as a colourless solid. Mp 96 °C. R_f (EtOAc)=0.81. ^{1}H NMR ($CDCl_3$): δ=2.06 (4H, t, $^3J_{HH}$=6.6 Hz, CH_2), 3.71 (4H, t, $^3J_{HH}$=6.6 Hz, NCH_2), 7.09 (1H, s, CH), 7.18 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=25.7, 50.9, 93.2, 109.9, 114.9, 116.0, 120.8 ($^1J_{CF}$=272.8 Hz), 124.1 ($^1J_{CF}$=271.0 Hz), 134.2 ($^2J_{CF}$=33.3 Hz), 136.6 ($^2J_{CF}$=31.0 Hz), 151.8. ^{19}F NMR ($CHCl_3$): δ=−62.2 (3F), −64.1 (3F). IR, ν_{max} (cm^{-1}): 2957, 2875, 2214, 1632, 1481, 1276, 1116, 1085, 1014, 869. MS, *m/z* (%): 308 (M+, 50), 307 (58), 289 (20), 280 (100), 245 (44), 219 (20), 188 (14), 69 (13), 42 (12), 41 (12). Anal. calcd for $C_{13}H_{10}F_6N_2$: C 50.66; H 3.27; N 9.09. Found C 50.61; H 3.22; N 9.07.

4.3.5. 2-(1-Piperidinyl)-4,6-bis(trifluoromethyl)-benzonitrile (5b). To a solution of enamine **2b** (1 g, 6.63 mmol) in benzene (25 mL) was added **1** (1.38 g, 6.63 mmol). The reaction mixture was maintained at rt for 3 days (the reaction mixture was monitored by ^{19}F NMR spectroscopy). The solvent was evaporated in vacuo. The residue was dissolved in acetone (10 mL) and maintained at rt for 5 days (the reaction mixture was monitored by ^{19}F NMR spectroscopy). Acetone was evaporated in vacuo, residue was extracted with boiling cyclohexane (15 mL) and maintained overnight. The precipitate formed was filtered affording **5b** (1.03 g, 48%) as a yellow solid. Mp 42 °C. R_f (EtOAc)=0.62. ^{1}H NMR ($CDCl_3$): δ=1.69 (2H, m, CH_2), 1.83 (4H, m, CH_2), 3.30 (4H, t, $^3J_{HH}$=5.7 Hz, NCH_2), 7.4 (1H, s, CH), 7.46 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=23.8, 25.9, 53.2, 105.3, 114.0, 114.5, 119.3, 121.6 ($^1J_{CF}$=272.8 Hz), 123.7 ($^1J_{CF}$=271.3 Hz), 135.2 ($^2J_{CF}$=34.2 Hz), 135.9 ($^2J_{CF}$=33.5 Hz), 159.1. ^{19}F NMR ($CHCl_3$): δ=−63.5 (3F), −64.9 (3F). IR, ν_{max} (cm^{-1}): 2950, 2858, 2802, 2227, 1620, 1445, 1395, 1280, 1141, 980. MS, *m/z* (%): 322 (M^+, 87), 321 (100), 293 (57), 281 (29), 245 (18), 239 (28), 84 (28), 69 (37), 55 (16), 43 (30), 42 (24), 41 (36). Anal. calcd for $C_{14}H_{12}F_6N_2$: C 52.18; H 3.75; N 8.69. Found C 52.25; H 3.72; N 8.65.

4.3.6. 2,4-Dimethyl-6-oxo-1,6-dihydropyridine-3-carbonitrile (6). To a solution of enamine **2c** (1 g, 6.6 mmol) in benzene (10 mL) was added **1** (1.37 g, 6.6 mmol). The reaction mixture was refluxed for 4 h. After cooling to rt the precipitate formed was filtered affording **6** (230 mg, 24%). The mother liquor was evaporated in vacuo. The residue was subjected to a column chromatography over silica gel using EtOAc as eluent affording **5c** (598 mg, 28%, R_f (EtOAc)=0.68).

Compound **6**. Colourless solid. Mp 293 °C (lit. 294–296 °C).[18] ^{1}H NMR (DMSO-d_6): δ=2.22 (3H, s, CH_3), 2.39 (3H, s, CH_3), 6.10 (1H, s, CH), 12.25 (1H, br. s. OH). MS, *m/z* (%): 148 (M^+, 67), 119 (100), 105 (21), 78 (11), 52 (11), 42 (13).

4.4. Interaction of 1 with β-dialkylaminocrotonoesters

4.4.1. General procedure for synthesis of compounds 12a–c in the presence of TMSCl and Et_3N. *Procedure A*. To a solution of **1** (1 g, 4.81 mmol) in dry benzene (10 mL) was added Me_3SiCl (4.81 mmol) and Et_3N (4.81 mmol). The reaction mixture was maintained at rt for 5 min. Enamine **11a–c** (4.81 mmol), Me_3SiCl (4.81 mmol) and Et_3N (4.81 mmol) was added consecutively. Reaction mixture was maintained at rt for 1 day and heated at 60 °C for 4 h. The precipitate formed was filtered off and the mother liquor was evaporated in vacuo. The residue was dried and recrystallised from *n*-hexane affording the corresponding ethyl ester of benzoic acid (**12a–c**).

4.4.2. Ethyl ester 2-(1-pyrrolidinyl)-4,6-bis(trifluoromethyl)-benzoic acid (12a). Pale yellow solid (1.59 g, 93%). Mp 48 °C. R_f (EtOAc)=0.68. ^{1}H NMR ($CDCl_3$): δ=1.39 (3H, t, $^3J_{HH}$=7.2 Hz, CH_3), 1.99 (4H, t, $^3J_{HH}$=6.6 Hz, CH_2), 3.35 (4H, t, $^3J_{HH}$=6.6 Hz, NCH_2), 4.37 (2H, q, $^3J_{HH}$=7.2 Hz, CH_2), 7.08 (1H, s, CH), 7.2 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=13.8, 25.9, 53.0, 62.6, 109.8, 114.2, 119.1, 121.9 ($^1J_{CF}$=272.2 Hz), 122.1 ($^1J_{CF}$=271 Hz), 130.2 ($^2J_{CF}$=31.2 Hz), 132.3 ($^2J_{CF}$=32.4 Hz), 146.9, 167.9. ^{19}F NMR ($CHCl_3$): δ=−60.8 (3F), −64.2 (3F). IR, ν_{max} (cm^{-1}): 2984, 2916, 2858, 1725, 1442, 1383, 1279, 1125, 978. MS, *m/z* (%): 355 (M^+, 25), 326 (100), 310 (31), 290 (38), 280 (18), 240 (14), 234 (15), 213 (18), 41 (11). Anal. calcd for $C_{15}H_{15}F_6NO_2$: C 50.71; H 4.26; N 3.94. Found C 50.75; H 4.31; N 4.01.

4.4.3. Ethyl ester 2-(1-piperidinyl)-4,6-bis(trifluoromethyl)-benzoic acid (12b). Yellow oil (1.26 g, 71%). R_f (EtOAc)=0.62. ^{1}H NMR ($CDCl_3$): δ=1.39 (3H, t, $^3J_{HH}$=7.2 Hz, CH_3), 1.56 (2H, m, CH_2), 1.66 (4H, m, CH_2), 2.97 (4H, t, $^3J_{HH}$=5.4 Hz, NCH_2), 4.42 (2H, q, $^3J_{HH}$=7.2 Hz, CH_2), 7.55 (1H, s, CH), 7.59 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=14.1, 24.0, 26.4, 54.4, 62.3, 117.7, 120.1, 121.8, 121.7 ($^1J_{CF}$=275.2 Hz), 122.3 ($^1J_{CF}$=274.0 Hz), 132.2 ($^2J_{CF}$=32.4 Hz), 132.3 ($^2J_{CF}$=32.4 Hz), 153.8, 166.4. ^{19}F NMR ($CHCl_3$): δ=−60.9 (3F), −64.1 (3F). IR, ν_{max} (cm^{-1}): 2938, 2883, 2853, 1730, 1440, 1390, 1282, 1130, 978. MS, *m/z* (%): 369 (M^+, 18), 340 (100), 324 (24), 294 (15), 97 (10), 69 (14), 57 (24), 55 (39), 43 (20), 41 (28). Anal. calcd for $C_{16}H_{17}F_6NO_2$: C 52.04; H 4.64; N 3.79. Found C 51.87; H 4.24; N 3.82.

4.4.4. Ethyl ester 2-(4-morpholinyl)-4,6-bis(trifluoromethyl)-benzoic acid (12c). Colourless solid (1.23 g, 69%). Mp 44 °C. R_f (EtOAc)=0.55. ^{1}H NMR ($CDCl_3$): δ=1.4 (3H, t, $^3J_{HH}$=7.2 Hz, CH_2), 3.04 (4H, t, $^3J_{HH}$=4.5 Hz, NCH_2), 3.8 (4H, t, $^3J_{HH}$=4.5 Hz, OCH_2), 4.44 (2H, q, $^3J_{HH}$=7.2 Hz, CH_2), 7.6 (1H, s, CH), 7.68 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=14.1, 53.2, 53.3, 67.1, 118.8, 121.6 ($^1J_{CF}$=275.2 Hz), 121.8, 122.3 ($^1J_{CF}$=274.0 Hz), 124.2, 132.9 ($^2J_{CF}$=32.8 Hz), 133.3 ($^2J_{CF}$=34.7 Hz), 151.9, 165.9. ^{19}F NMR ($CHCl_3$): δ=−61.0 (3F), −64.1 (3F). IR, ν_{max} (cm^{-1}): 3058, 2996, 2925, 2863, 1732, 1442, 1387, 1282, 1129, 978. MS, *m/z* (%): 371 (M^+, 27), 352 (17), 340 (18), 328 (52), 298 (100), 284 (73), 268 (70), 240 (55), 213 (44), 194 (23), 163 (24), 143 (20), 59 (37), 45 (25). Anal. calcd for $C_{15}H_{15}F_6NO_3$: C 48.53; H 4.07; N 3.77. Found C 48.47; H 4.02; N 3.82.

4.4.5. Ethyl ester 2-hydroxy-4,6-bis(trifluoromethyl)-benzoic acid (14). To a solution of enamine **11a** (5 g, 27.4 mmol) in benzene (75 mL) was added **1** (5.68 g, 27.4 mmol). The reaction mixture was maintained at rt for 3 days (the reaction mixture was monitored by ^{19}F NMR spectroscopy). The solvent was evaporated in vacuo. The residue obtained was placed in short silica gel column and washed with EtOAc (4×25 mL). EtOAc was evaporated in vacuo and the residue obtained was subjected to fractional distillation affording **14** (2.23 g, 27%) and **12a** (0.68 g, 7%). Colourless solid. Mp 24 °C. R_f (EtOAc)=0.56. ^{1}H NMR ($CDCl_3$): δ=1.44 (3H, t, $^3J_{HH}$=7.2 Hz, CH_3), 4.49 (2H, q, $^3J_{HH}$=7.2 Hz, CH_2), 7.48 (1H, s, CH), 7.53 (1H, s, CH), 10.8 (1H, br s, OH). ^{13}C NMR ($CDCl_3$): δ=13.5, 63.3, 114.6, 115.3, 119.4, 124.3 ($^1J_{CF}$=270.5 Hz), 124.5 ($^1J_{CF}$=273.2 Hz), 131.9 ($^2J_{CF}$=31.7 Hz), 135.6 ($^2J_{CF}$=32.9 Hz), 161.7, 168.1. ^{19}F NMR ($CHCl_3$): δ=59.6 (3F), −65.2 (3F). IR, ν_{max} (cm^{-1}): 3400–2800 (br), 2991, 2944, 1737 (sh), 1683, 1445, 1380, 1324, 1276, 1139, 1012, 962, 885, 730. MS, *m/z* (%): 302 (M^+, 23), 256 (100), 228 (40), 209 (27), 200 (17), 181 (14). Anal. calcd for $C_{11}H_8F_6O_3$: C 43.72; H 2.67; Found C 43.58; H 2.69.

4.4.6. General procedure for interaction of 1 with β-dialkylaminocrotonoesters. To a solution of enamines **11a–c** (9.71 mmol) in benzene (30 mL) was added **1** (2 g, 9.71 mmol). The reaction mixture was maintained at rt for 3 days (the reaction mixture was monitored by ^{19}F NMR spectroscopy). The solvent was evaporated in vacuo. The residue obtained was placed in a short silica gel column and washed with EtOAc (2×25 mL). EtOAc was evaporated in vacuo and the residue obtained was carefully dried in vacuo dissolved in anhyd *n*-hexane (30 mL). To the stirred solution obtained NaH (240 mg, 10 mmol) was carefully added and the solution was maintained until hydrogen evolution had stopped. The precipitate formed was filtered off under argon. The mother liquior was evaporated in vacuo and the residue obtained was subjected to a column chromatography over silica gel using EtOAc as eluent affording **12a–c**. The precipitate obtained was suspended in anhydrous CH_2Cl_2 (25 mL) and to the stirred suspension water (5 mL) was added dropwise and then aq HCl (30%, 1 mL) was added dropwise. The organic layer was separated and dried over Na_2SO_4. CH_2Cl_2 was evaporated in vacuo affording **14** (purity >95%).

4.5. Interaction of 1 with β-enaminones

4.5.1. 1-[3,5-Bis(trifluoromethyl)phenyl]-pyrrolidine (13a). To a solution of enamine **16a** (1 g, 6.54 mmol) in acetic acid (20 mL) was added **1** (1.36 g, 6.54 mmol). The reaction mixture was heated at 60 °C for 4 h. Solvent was evaporated in vacuo and residue was crystallized from mixture methanol/water −1:1 affording **13a** (370 mg, 20%) as a colourless solid. Mp 63 °C. R_f (EtOAc)=0.58. ^{1}H NMR ($CDCl_3$): δ=2.64 (4H, t, $^3J_{HH}$=6.3 Hz, CH_2), 3.34 (4H, t, $^3J_{HH}$=6.3 Hz, NCH_2), 6.85 (2H, s, CH), 7.08 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=25.4, 47.7, 107.9, 110.7, 125.2 ($^1J_{CF}$=270.3 Hz), 131.9 ($^2J_{CF}$=32.2 Hz), 147.9. ^{19}F NMR ($CHCl_3$): δ=−64.7. IR, ν_{max} (cm^{-1}): 2984, 2916, 2858, 1623, 1499, 1481, 1425, 1275, 1162, 1123, 1012, 853, 701, 682. MS, *m/z* (%): 283 (M^+, 61), 282 (100), 264 (21), 240 (32), 227 (71), 213 (40), 163 (13), 41 (10). Anal. calcd for $C_{12}H_{11}F_6N$: C 50.89; H 3.91; N 4.95. Found C 50.92; H 4.02; N 4.91.

4.5.2. 1-[3,5-Bis(trifluoromethyl)phenyl]-piperidine (13b). Enaminone **16b** (1 g, 5.98 mmol) was dissolved in acetic acid (20 mL) and to the solution formed **1** was added (1.24 g, 5.98 mmol). The reaction mixture was maintained at rt overnight and then heated at 60 °C for 4 h. The solvent was evaporated in vacuo. The residue was extracted with boiling *n*-hexane (15 mL). The hexane was evaporated in vacuo and the residue was crystallized from methanol affording **13b** (370 mg, 21%) as a colourless solid. Mp 60–62 °C. R_f (EtOAc)=0.64. ^{1}H NMR ($CDCl_3$): δ=3.25 (4H, t, $^3J_{HH}$=4.8 Hz), 3.87 (4H, t, $^3J_{HH}$=4.8 Hz), 7.24 (2H, s, CH), 7.32 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=24.0, 25.4, 49.5, 111.1, 114.8, 125.6 ($^1J_{CF}$=271.5 Hz), 132.4 ($^2J_{CF}$=31.8 Hz), 152.2. ^{19}F NMR ($CHCl_3$): δ=−64.2. IR, ν_{max} (cm^{-1}): 3077, 3062, 3011, 2938, 2883, 2853, 1619, 1480, 1413, 1283, 1141, 1132, 1024, 953, 859, 699, 682. MS, *m/z* (%): 297 (M^+, 61), 296 (100), 278 (19), 256 (28), 240 (49), 213 (32), 163 (11). Anal. calcd for $C_{13}H_{13}F_6N$: C 52.53; H 4.41; N 4.71. Found C 52.25; H 4.36; N 4.52.

4.5.3. 4-[3,5-Bis(trifluoromethyl)phenyl]-morpholine (13c). To a solution of enaminone **16c** (0.81 g, 4.81 mmol) in acetic acid (20 mL) was added **1** (1 g, 4.81 mmol). The reaction mixture was heated at 60 °C for 4 h. The solvent was evaporated in vacuo and residue was crystallized from methanol affording **13c** (0.268 g, 38%) as colourless solid. Mp 123 °C. R_f (*i*-PrOH)=0.39. ^{1}H NMR ($CDCl_3$): δ=1.63–1.72 (6H, m, $3CH_2$), 3.27 (4H, t, $^3J_{HH}$=4.8 Hz), 7.23 (3H, s, CH). ^{13}C NMR ($CDCl_3$): δ=48.3, 66.5, 112.4, 114.4, 125.3 ($^1J_{CF}$=270.9 Hz), 132.2 ($^2J_{CF}$=32.1 Hz), 151.7.^{19}F NMR ($CHCl_3$): δ=−64.2. IR, ν_{max} (cm^{-1}): 3082, 3060, 2983, 2918, 2847, 1617, 1487, 1405, 1286, 1170, 1114, 970, 857, 691, 683. MS, *m/z* (%): 299 (M^+, 41), 280 (14) 241 (100), 213 (27). Anal. calcd for $C_{12}H_{11}F_6NO$: C 48.17; H 3.71; N 4.68. Found C 48.21; H 3.73; N 4.56.

4.5.4. 1-[2-(1-Piperidinyl)-4,6-bis(trifluoromethyl)-phenyl]-ethanone (17b). Enaminone **16b** (1 g, 5.99 mmol) was dissolved in anhyd benzene (10 mL) and to the solution formed **1** (1.24 g, 5.99 mmol) was added. The reaction mixture was maintained at rt overnight. Benzene

was evaporated in vacuo. The residue was dissolved in methanol. To the solution formed a few drops of water was added (~0.1 mL). The precipitate formed was filtered affording **17b** (406 mg, 20%) as a colourless solid mp 88 °C. R_f (EtOAc)=0.73. ^{1}H NMR ($CDCl_3$): δ=1.54–1.6 (2H, m, CH_2), 1.66–1.71 (4H, m, CH_2) 2.56 (3H, s, CH_3), 2,92 (4H, t, $^3J_{HH}$=5.4 Hz, NCH_2), 7.57 (1H, s, CH), 7.63 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=23.7, 26.2, 31.1, 54.6, 118.1, 121.1, 124.1 ($^1J_{CF}$=271.9 Hz), 124.4 ($^1J_{CF}$=270.8 Hz), 128.3 ($^2J_{CF}$=32.1 Hz), 131.2 ($^2J_{CF}$=33.0 Hz), 140.8, 152.7, 202.6. ^{19}F NMR ($CHCl_3$): δ=−60.1 (3F), −64.1 (3F). IR, ν_{max} (cm^{-1}): 2950, 2858, 2802, 1710, 1386, 1273, 1131, 963, 897. MS, *m/z* (%): 339 (M^+, 47), 324 (100), 306 (39), 268 (25), 248 (17), 238 (27), 213 (16), 43 (26). Anal. calcd for $C_{15}H_{15}F_6NO$: C 53.1; H 4.46; N 4.13. Found C 53.06; H 4.41; N 4.06.

Note. When the residue after evaporating off benzene from the reaction mixture was subjected to a column chromatography over silica gel using EtOAc as eluent we would obtain **13b** (110 mg, 6%) and **17b** (400 mg, 20%).

4.5.5. 1-[2-(4-Morpholinyl)-4,6-bis(trifluoromethyl)-phenyl]-ethanone (17c). Enaminone **16c** (1 g, 5.99 mmol) was dissolved in anhydrous benzene (25 mL) and to a solution formed **1** (1.24 g, 5.99 mmol) was added. The reaction mixture was maintained at rt overnight. The benzene was evaporated in vacuo. The residue was dissolved in methanol. To a solution formed a few drops of water was added (~0.1 mL). The precipitate formed was filtered affording **17c** (430 mg, 21%) as a colourless solid Mp 56 °C. R_f (*i*-PrOH)=0.74. ^{1}H NMR ($CDCl_3$): δ=2.58 (3H, s, CH_3), 2.99 (4H, t, $^3J_{HH}$=4.5 Hz, NCH_2), 3.81 (4H, t, $^3J_{HH}$=4.5 Hz OCH_2), 7.60 (1H, s, CH), 7.69 (1H, s, CH). ^{13}C NMR ($CDCl_3$): δ=31.3, 53.4, 66.9, 119.1, 121.5, 124.4 ($^1J_{CF}$=275.0 Hz), 124.7 ($^1J_{CF}$=271.3 Hz), 128.1 ($^2J_{CF}$=28.9 Hz), 131.7 ($^2J_{CF}$=33.5 Hz), 141.2, 151.0, 202.2. ^{19}F NMR ($CHCl_3$): δ=−59.4 (3F), −62.0 (3F). IR, ν_{max} (cm^{-1}): 3077, 2984, 2847, 1707, 1439, 1380, 1274, 1142, 971. MS, *m/z* (%): 341 (M^+, 48), 326 (39), 296 (19), 280 (24), 268 (100), 255 (27), 240 (19), 213 (17), 43 (44). Anal. calcd for $C_{14}H_{13}F_6NO_2$: C 49.27; H 3.84; N 4.10. Found C 49.19; H 3.91; N 4.12.

Note. When the residue after evaporating off benzene from the reaction mixture was subjected to a column chromatography over silica gel using *i*-PrOH as eluent we would obtain **17c** (490 mg, 24%) and then after washing of column with EtOAc we would obtain **13c** (72 mg, 4%).

Acknowledgements

The authors acknowledge A. V. Turov, S. A. Alekseev (Department of Chemistry of Kyiv National Taras Shevchenko University), A. V. Mazepa (Bogatsky Physico-Chemical Institute, National Academy of Sciences of Ukraine, Department of Molecular Structure) and Yu. V. Kuzmenko (Central Customs Laboratory of National Customs Service of Ukraine) for spectral measurement.

References and notes

1. (a) *Fluorine in Bioorganic Chemistry*; Filler, R., Kobayasi, Y., Yagupolskii, L. M., Eds.; Elseiver: Amsterdam, 1993. (b) Filler, R. Fluorine Containing Drugs in Organofluorine Chemicals and Their Industrial Application; Pergamon: New York, 1979; (Chapter 6). (c) Hudlicky, M. Chemistry of Organic Fluorine Compounds; Ellis Horwood: Chichester, 1992.
2. For recent examples, see: (a) Arndt-Rosenau, M.; Hoch, M.; Sundermeyer, J.; Kipke, J. EP-16983, **2002**; *Chem. Abstr.* **2003**, 138, 170656. (b) Liedtke, J.; Loss, S.; Widauer, C.; Grutzmacher, H. *Tetrahedron* **2000**, *56*, 143–156.
3. For recent examples of using derivatives of 3,5-bistrifluoromethylaniline, see: (a) Tagmose, T. M.; Zaragoza, F.; Boonen, H. C. M.; Worsaae, A.; Mogensen, J. P.; Nielsen, F. E.; Jensen, A. F.; Hansen, J. B. *Bioorg. Med. Chem.* **2003**, *11*, 931–940. (b) Bongartz, J.-P.; Stokbroekx, R.; der Aa, M. V.; Luyckx, M.; Willems, M.; Ceusters, M.; Meerpoel, L.; Smets, G.; Jansen, T.; Wouters, W.; Bowden, C.; Valetta, L.; Herb, M.; Tominovich, R.; Tuman, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 589–592. (c) Dolezal, M.; Miletin, M.; Kunes, J.; Kralova, K. *Molecules* **2002**, *7*, 363–373. (d) Tagmose, T. M.; Mogensen, J. P.; Agerholm, P. C.; Arkhammar, P. O. G.; Wahl, P.; Worsaae, A.; Hansen, J. B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1749–1752. (e) Wilson, L. J.; Morris, T. W.; Wu, Q.; Renick, P. J.; Parker, C. N.; Davis, M. C.; McKeever, H. D.; Hershberger, P. M.; Switzer, A. G.; Shrum, G.; Sunder, S.; et al *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1149–1152. (f) Palanki, M. S. S.; Erdman, P. E.; Gayo-Fung, L. M.; Shevlin, G. I.; Sullivan, R. W.; Suto, M. J.; Goldman, M. E.; Ransone, L. J.; Bennett, B. L.; Manning, A. M. *J. Med. Chem.* **2000**, *43*, 3995–4004.
4. For recent examples of using derivatives of 3,5-bistrifluoromethylphenol, see (a) Ryckmans, T.; Balancon, L.; Berton, O.; Genicot, C.; Lamberty, Y.; Lallemand, B.; Pasau, P.; Pirlot, N.; Quere, L.; Talaga, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 261–264. (b) Malamas, M. S.; Sredy, J.; Gunawan, I.; Mihan, B.; Sawicki, D. R.; Seestaller, L.; Sullivan, D.; Flam, B. R. *J. Med. Chem.* **2000**, *43*, 995–1010.
5. Ross, S. D.; Markarian, M.; Schwarz, M. *J. Am. Chem. Soc.* **1953**, *75*, 4967–4969.
6. (a) Harris, M. S.; Geis, O.; Buchwald, S. L. *J. Org. Chem.* **1999**, *64*, 6019–6022. (b) Antane, S. *Synth. Commun.* **2003**, *33*, 2145–2150.
7. Dmowski, W.; Piasecka-Maciejewska, K. *J. Fluorine Chem.* **1996**, *78*, 59–64.
8. (a) Sloop, J. C.; Bumgardner, C. L.; Loehle, W. D. *J. Fluorine Chem.* **2002**, *118*, 135–148. (b) Singh, S. P.; Kumar, D.; Batra, H.; Rozas, I.; Elguero, J. *Can. J. Chem.* **2000**, *78*, 1109–1120.
9. Volochnyuk, D. M.; Pushechnikov, A. O.; Krotko, D. G.; Sibgatulin, D. A.; Kovaleva, S. A.; Tolmachev, A. A. *Synthesis* **2003**, 1531–1540 and references cited therein.
10. (a) Burgart, Ya. V.; Kuzueva, O. G.; Pryadeina, M. V.; Kappe, C. O.; Saloutin, V. I. *Russ. J. Org. Chem.* **2001**, *37*, 869–880. (b) Chu-Moyer, M. Y.; Ballinger, W. E.; Beebe, D. A.; Berger, R.; Coutcher, J. B.; Day, W. W.; Li, J.; Mylari, B. L.; Oates, P. J.; Weekly, R. M. *J. Med. Chem.* **2002**, *45*, 511–528.
11. Ohkura, H.; Berbasov, D. O.; Soloshonok, V. A. *Tetrahedron Lett.* **2003**, *44*, 2417–2420.
12. Skryabina, Z. E.; Burgart, Ya. V.; Saloutin, V. I. *Bull. Acad. Sci. USSR Div. Chem. Sci.* **1991**, *40*, 788–794.
13. Volochnyuk, D. M.; Kostyuk, A. N.; Sibgatulin, D. A.;

Chernega, A. N.; Pinchuk, A. M.; Tolmachev, A. A. *Tetrahedron* **2004**, *60*, 2361–2371.
14. Volochnyuk, D. M.; Kostyuk, A. N.; Sibgatulin, D. A.; Petrenko, A. E. *Synthesis* **2004**, 2545–2549.
15. (a) Staroscik, J.; Rickborn, B. *J. Am. Chem. Soc.* **1971**, *93*, 3046–3047. (b) Brion, F. *Tetrahedron Lett.* **1982**, *23*, 5299–5302.
16. Padva, A.; Dimitroff, M.; Waterson, A. G.; Wu, T. *J. Org. Chem.* **1997**, *62*, 4088–4096.
17. (a) Hickmott, P. W.; Sheppard, G. *J. Chem. Soc. (C)* **1971**, 1358–1364. (b) Dedina, J.; Kuthan, J.; Palecek, J.; Schraml, J. *Collect. Czech. Chem. Commun.* **1975**, *40*, 3476–3490.
18. Yalisheva, N. Z.; Chistyakov, V. V.; Granik, V. G. *Khim. Heterocycl. Compd. (Engl. Transl.)* **1986**, *22*, 70–73.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2849–2856

A stereoselective synthesis of spiro-dioxolanes via the multicomponent reaction of dicarbomethoxycarbene, aldehydes and 1,2- or 1,4-diones

Vijay Nair,[a,*] Sindhu Mathai,[a] Smitha C. Mathew[a] and Nigam P. Rath[b]

[a]*Organic Chemistry Division, Regional Research Laboratory, Trivandrum 695 019, India*
[b]*Department of Chemistry, University of Missouri, St Louis, MO 63121-4499, USA*

Received 25 August 2004; revised 4 January 2005; accepted 20 January 2005

Abstract—The three component reaction of acyclic carbonyl ylides generated from dicarbomethoxycarbene and aldehydes with 1,2- and 1,4-diones is described. The reaction afforded the corresponding spiro-dioxolanes in good yields.

1. Introduction

The phenomenal success of the Huisgen 1,3-dipolar cycloaddition reaction in the construction of heterocycles paved the way for the use of unconventional dipoles such as ylides in this reaction. Much of the work in this area, however, has been confined to cyclic carbonyl ylides[1,2] often generated by the Rh(II) catalyzed decomposition of diazo compounds with suitably positioned carbonyl groups. Imaginative and important applications of cycloadditions involving cyclic carbonyl ylides, especially in the synthesis of natural products, were developed by Padwa and co-workers.[3] In contrast, the chemistry of acyclic carbonyl ylides remained largely undeveloped. Our interest in acyclic carbonyl ylides has its origin in our observation that zwitterions generated by the reaction of isocyanides and nucleophilic carbenes with DMAD can be efficiently trapped by carbon heteroatom π bonds leading to the synthesis of highly functionalized heterocycles.[4]

The chemistry of acyclic carbonyl ylides can be traced to the work of Büchner and Curtius who were the first to report the reaction of dicarbomethoxycarbene with carbonyl compounds.[5] The products of the reaction were characterized as dioxolanes by Dieckmann[6] in 1910. Later Huisgen and de March investigated the chemistry of carbonyl ylides generated from dicarbomethoxycarbene and carbonyl compounds in detail.[7] They were able to establish the intermediacy of carbonyl ylides by trapping it with DMAD. A successful attempt to trap the ylide by a carbon–carbon double bond was reported by Maas[8] who succeeded in obtaining tetrahydrofuran derivatives by a three component reaction of electrophilic carbene, aldehydes and maleate or fumarate. Recently, Jiang[9] et al. have reported a stereoselective synthesis of dioxolanes by the 1,3-dipolar cycloaddition reaction of acyclic carbonyl ylides to aldehydes. A stereospecific synthesis of epoxides involving the collapse of the ylides reported by Doyle is also noteworthy.[10]

Against this literature background, and in the context of our general interest in the chemistry of 1,2-diones,[11] we have explored the reaction of the carbonyl ylides generated from dicarbomethoxycarbene and aldehydes to *o*-quinones. Our preliminary results showing the formation of dioxolanes have already been published.[12] The details of this work along with the results of our extended investigations involving isatins and *p*-quinones are presented in this paper.

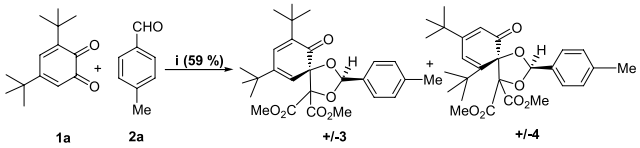
2. Results and discussion

2.1. Reaction with *o*-benzoquinones

Our studies commenced by the Rh(II) catalyzed decomposition of dimethyl diazomalonate in the presence of *p*-tolualdehyde and 3,5-di-*tert*-butyl-1,2-benzoquinone. A facile reaction occurred affording a regioisomeric mixture of dioxolanes **3** and **4** in the ratio 3:1 (Scheme 1).[12]

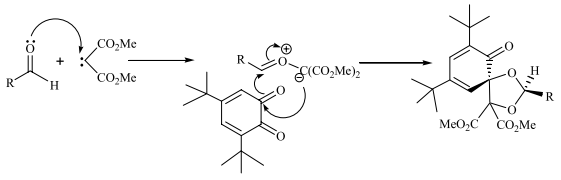
Keywords: Dicarbomethoxycarbene; Carbonyl ylide; Huisgen 1,3-dipolar cycloaddition; Spiro-dioxolane.
* Corresponding author. Tel.: +91 471 2490406; fax: +91 471 2491712; e-mail: vijaynair_2001@yahoo.com

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.076



Scheme 1. (i) $N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$, dry benzene, argon, 80 °C, 14 h.

Mechanistically the reaction may be considered to involve the formation of a carbonyl ylide by the reaction of the carbene and the aldehyde and its trapping by the quinone carbonyls (Scheme 2). The diastereoselectivity of the reaction may be rationalized by the concerted nature of the carbonyl ylide cycloaddition and the observed relative stereochemistry of the products may be attributed to the preferred *trans* geometry of the ylide.

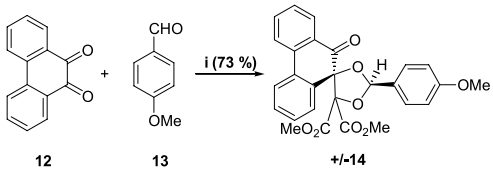


Scheme 2.

The reaction was found to be general with respect to a variety of aromatic aldehydes, especially those containing electron donating groups, and 1,2-benzoquinones. The dioxolane derivatives were obtained in moderate to good yields. The reaction with 4-*tert*-butyl-1,2-benzoquinone afforded an inseparable mixture of regioisomers in the ratio 1:1. In all cases the structure of the products was established by spectroscopic analysis; IR, ^{1}H NMR and ^{13}C NMR data were completely consistent with the assigned structure (Table 1).

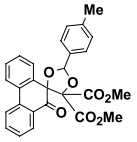
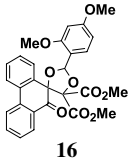
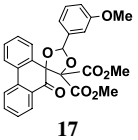
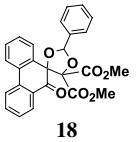
2.2. Reaction with phenanthrene quinone

Subsequent to the above investigations, we examined the addition of acyclic carbonyl ylides to phenanthrenequinone. In a prototype experiment, *p*-anisaldehyde and phenanthrenequinone were exposed to dimethyl diazomalonate in the presence of catalytic amount of Rh(II) acetate in refluxing benzene under an atmosphere of argon for 14 h. The reaction afforded the corresponding dioxolane derivative in 73% yield as a single diastereoisomer (Scheme 3).[12]



Scheme 3. (i) $N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$, dry benzene, argon, 14 h.

Table 2

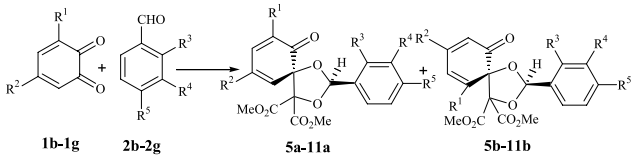
Entry	Aldehyde	Products	Yield (%)
1		 **15**	85
2		 **16**	78
3		 **17**	68
4		 **18**	49

The reaction was extended to a number of other aldehydes; in all cases good yields of the spiro-dioxolane derivatives were obtained and the results are summarized in Table 2.

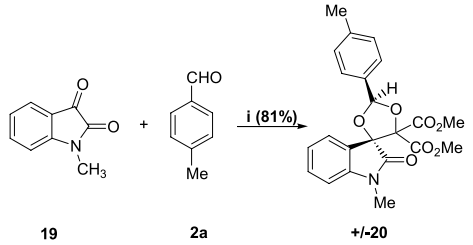
2.3. Reaction with isatins

In the next phase of our studies we extended the reaction to another class of 1,2-dicarbonyl compounds viz. isatins. When *N*-methyl isatin was allowed to react with acyclic carbonyl yilde generated by the reaction of *p*-tolualdehyde

Table 1.



o-Benzoquinone	Aldehyde	Substituents	Product	Yield (%)/(ratio)
1b	**2b**	$R^1=R^2=C(CH_3)_3$, $R^3=R^4=R^5=H$	**5a**, **5b**	62 (3:1)
1c	**2c**	$R^1=R^2=C(CH_3)_3$, $R^3=R^5=H$, $R^4=OCH_3$	**6a**, **6b**	40 (3:1)
1d	**2d**	$R^1=R^2=C(CH_3)_3$, $R^3=R^5=OCH_3$, $R^4=H$	**7a**, **7b**	34 (3:1)
1e	**2e**	$R^1=CHPh_2$, $R^2=C(CH_3)_3$, $R^3=R^4=H$, $R^5=CH_3$	**8a**, **8b**	74 (2:1)
1f	**2f**	$R^1=CHPh_2$, $R^2=C(CH_3)_3$, $R^3=R^4=R^5=H$	**9a**, **9b**	65 (1.4:1)
1g	**2g**	$R^1=H$, $R^2=C(CH_3)_3$, $R^3=R^4=H$, $R^5=CH_3$	**10a**, **10b**	41 (1:1)
1h	**2h**	$R^1=H$, $R^2=C(CH_3)_3$, $R^3=R^4=R^5=H$	**11a**, **11b**	45 (1:1)



Scheme 4. (i) $N_2(CO_2Me)_2$, $Rh_2(OAc)_4$, dry benzene, argon, 16 h.

and dicarbomethoxycarbene, a product was formed in high yield (Scheme 4).

The structure of the product was assigned initially by routine spectroscopic methods. The IR spectrum of **20** showed strong bands at 1757 cm^{-1} and 1728 cm^{-1} due to the ester and the lactam carbonyls, repectively. In the ^1H NMR spectrum, resonance signal due to the methyl group on the aromatic ring was seen as a singlet at δ 2.36, while the signal due to the *N*-methyl protons appeared at 3.19. Signals due to the two methoxy groups were discernible at δ 3.64 and δ 3.80. The sharp singlet observed at δ 6.94 can be assigned to the acetal proton. The ^{13}C NMR spectrum was also in agreement with the structure assigned, with the lactam carbonyl displaying a signal at δ 173.8. The two ester carbonyls gave signals at δ 166.9. The signal characteristic of spirocarbon was observed at δ 85.6. The signals δ 26.2 and δ 21.4 were assigned to the *N*-methyl and *C*-methyl carbons, respectively. Conclusive evidence for the relative stereochemistry was obtained by single crystal X-ray analysis (Fig. 1).[13]

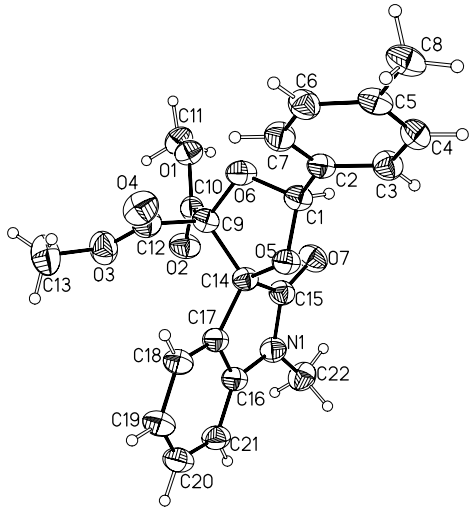
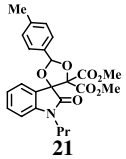
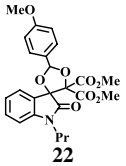
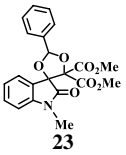
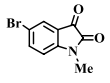
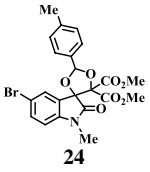
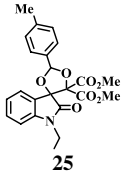
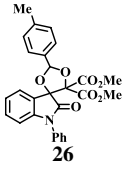


Figure 1. Single crystal X-ray structure of **20**.

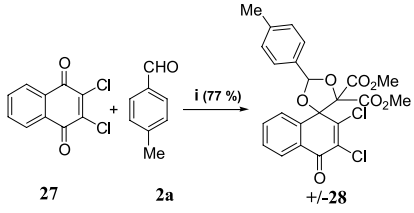
Evidently the regiospecificity observed in this reaction is attributable to the higher electrophilicity of the ketogroup vis a vis the amide carbonyl. The reaction was found to be general with various isatin derivatives and aromatic aldehydes; the spiro oxindoles were obtained as single diastereoisomers in high yields. The results are summarized in Table 3.

Table 3

Entry	Isatin	Aldehyde	Product	Yield (%)
1			 **21**	95
2			 **22**	93
3			 **23**	76
4			 **24**	82
5			 **25**	78
6			 **26**	98

2.4. Reaction with 1,4-quinones

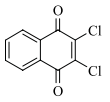
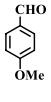
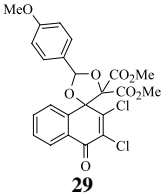
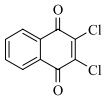
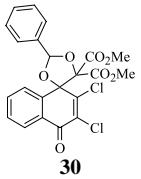
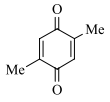
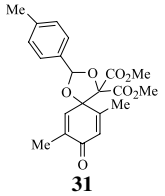
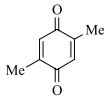
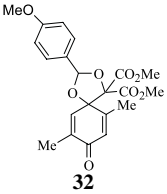
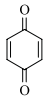
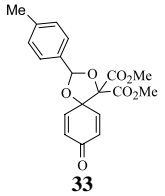
In view of the encouraging results obtained in the reaction of 1,2-diones with the acyclic carbonyl ylides generated by the reaction of aldehydes and dicarbomethoxycarbene, it was obligatory to extend the same to 1,4-diones. A limited investigation was conducted and the results are given in this section. The reaction of 2,3-dichloro-1,4-naphthoquinone with acyclic carbonyl ylide generated from *p*-tolualdehyde and dicarbomethoxycarbene constituted our initial experiment (Scheme 5).



Scheme 5. (i) $N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$, dry benzene, argon, 16 h.

The structure of the adduct **28** was established by spectroscopic methods. The IR spectrum displayed strong absorptions at 1762, 1688 cm^{-1} corresponding to the ester and enone carbonyls, respectively. The ^1H NMR spectrum was in consonance with the structure proposed. The *C*-methyl protons resonated at δ 2.45, while the peaks due to the carbomethoxy protons appeared at δ 3.13 and 3.78. Signal due to the acetal proton was discernible as a singlet at δ 6.76. The ^{13}C NMR spectrum was also in good agreement with the structure proposed. The peak at δ 176.2 corresponds to the quinone carbonyl group. Two ester carbonyls resonated at δ 164.5 and δ 163.3. The peak at δ 86.7 was typical of a spirocarbon. The carbomethoxy carbons resonated at δ 54.3 and δ 52.8 and the *C*-methyl carbon displayed its signal at δ 21.5. All the other signals were also in good agreement with the structure assigned. The reaction was extended to a few other quinones and the results are presented in Table 4.

Table 4

Entry	1,4-Quinone	Aldehyde	Product	Yield (%)
1			 **29**	54
2			 **30**	57
3			 **31**	56
4			 **32**	51
5			 **33**	20

3. Theoretical calculations

From the results presented in the previous sections, it is clear that acyclic carbonyl ylide adds across either one of the C=O bonds of the *o*-quinones. In order to explain the observed mode of cycloaddition, we have carried out some calculations using semi-emprical MNDO method with the aid of TITAN® software (version 1).

From Figure 2, it is clear that the predominant interaction is between the HOMO of the dipole and the LUMO of the dipolarophile, which leads to the pathway, favored both in terms of energetics and symmetry considerations.

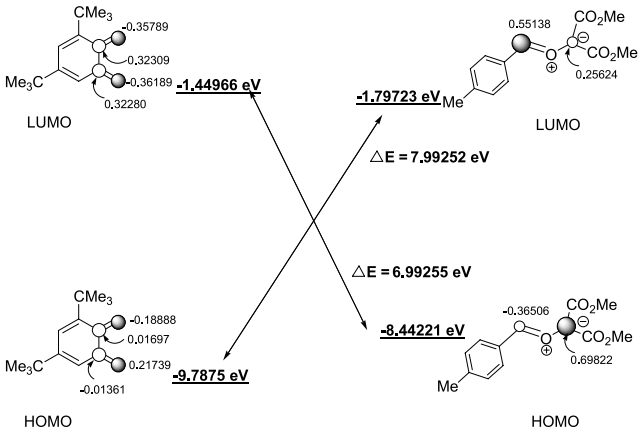


Figure 2. Molecular orbital correlation diagram of 3,5-di-*tert*-butyl-1,2-benzoquinone **1** and the acyclic carbonyl ylide.

4. Conclusion

In conclusion, we have demonstrated that carbonyl ylides generated from dicarbomethoxycarbene and aldehydes react efficiently with 1,2-quinones as well as 1,4-quinones leading to novel spiro-dioxolanes. In all cases the cycloaddition is regio- and stereoselective. With isatins the ylide preferentially adds to the more electron deficient carbonyl group making the reaction regiospecific. Here also the reaction is stereoselective and affords novel spiro-oxindole derivatives in high yields. Theoretical calculations strongly support the observed reactivity of the quinonoid compound toward carbonyl ylide. The novel three component reaction described herein may prove to be the method of choice for the synthesis of spirodioxolanes.

5. Experimental

5.1. General

All the reactions were carried out in oven dried glasswares under an atmosphere of argon unless otherwise mentioned. NMR spectra were recorded at 300 (^1H) and 75 (^{13}C) MHz, respectively on a Brüker DPX-300 MHz NMR spectrometer. Chemical shifts (δ) are reported relative to TMS (^1H) and $CDCl_3$ (^{13}C) as the internal standards. Coupling constant (*J*) is reported in Hertz (Hz). IR spectra were recorded on a Nicolet Impact 400D FT-IR spectrophotometer. Elemental analyses were performed on a Perkin Elmer-2400 Elemental Analyzer. Melting points were recorded on a Büchi melting point apparatus and are uncorrected. Dimethyl diazomalonate was prepared according to a literature procedure.[14] Commercial grade solvents were distilled prior to use.

5.1.1. Dimethyl-7,9-bis(1,1-dimethylethyl)-10-oxo-2-(4-methylphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 3 and dimethyl-6,8-bis(1,1-dimethylethyl)-10-oxo-2-(4-methylphenyl)-1,3-dioxaspiro[4.5]-deca6,8-diene-4,4-dicarboxylate 4: typical procedure and spectral data. A mixture of 3,5-di-*tert*-butyl-1,2-benzoquinone **1** (0.1 g, 0.45 mmol), *p*-tolualdehyde 2 (0.054 g, 0.45 mmol), dimethyl diazomalonate (0.079 g, 0.5 mmol) and 2 mol% of $Rh_2(OAc)_4$ was refluxed in 5 mL of dry benzene under atmosphere of argon for 14 h. The solvent was then removed in vacuo and the residue on chromatographic separation on silica gel using hexane–ethyl acetate (95:5) gave the spirodioxolanes **3** (0.094 g, 44%) and **4** (0.032 g, 15%) as yellow crystalline solids. The products were recrystallized from ethyl acetate–hexane solvent system.

5.1.2. Cycloadduct 3. Mp 160–161 °C, IR (KBr) ν_{max}: 2965, 2866, 1745, 1646, 1427, 1374, 1288, 1228, 1122, 1069, 1003, 963, 937, 817, 791, 738, 645 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.60 (d, 2H, $J=8.0$ Hz), 7.19 (d, 2H, $J=8.0$ Hz), 6.87 (d, 1H, $J=2.2$ Hz), 6.78 (s, 1H), 5.72 (d, 1H, $J=2.2$ Hz), 3.80 (s, 3H), 3.73 (s, 3H), 2.37 (s, 3H), 1.25 (s, 9H), 1.14 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 198.2, 167.0, 147.1, 143.5, 139.6, 134.4, 132.8, 128.9, 127.8, 123.4, 123.3, 107.1, 84.0, 53.0, 34.9, 34.6, 29.3, 28.5, 21.5. Elemental analysis calcd for $C_{27}H_{34}O_7$: C, 68.92, H, 7.28, Found: C, 69.00, H, 7.41.

5.1.3. Cycloadduct 4. Mp 102–104 °C, IR (KBr) ν_{max}: 2959, 2919, 2866, 1752, 1659, 1639, 1580, 1440, 1387, 1295, 1222, 1129, 1016, 963, 824, 658, 492 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.56 (d, 2H, $J=8.0$ Hz), 7.20 (d, 2H, $J=8.0$ Hz), 6.59 (s, 1H). 6.37 (d, 1H, $J=1.41$ Hz), 5.74 (d, 1H, $J=1.41$ Hz), 3.75 (s, 3H), 3.71 (s, 3H), 2.39 (s, 3H), 1.21 (s, 9H), 1.12 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 204.6, 167.6, 153.1, 139.1, 131.9, 128.9, 126.8, 123.4, 117.2, 107.1, 53.5, 52.8, 37.7, 35.5, 30.7, 28.1, 21.4. Elemental analysis calcd for $C_{27}H_{34}O_7$: C, 68.92, H, 7.28, Found: C, 68.80, H, 7.52.

5.1.4. Dimethyl-7,9-bis(1,1-dimethylethyl)-10-oxo-2-(phenyl)-1,3-dioxaspiro[4.5]-deca-6,8-diene-4,4-dicarboxylate 5a. Yellow viscous liquid, IR (neat) ν_{max}: 2960, 2872, 1750, 1677, 1512, 1460, 1258, 1134, 793, 705 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.73–7.72 (m, 2H), 7.40–7.38 (m, 3H), 6.88 (d, 1H, $J=2.1$ Hz), 6.80 (s, 1H), 5.72 (d, 1H, $J=2.1$ Hz), 3.81 (s, 3H), 3.74 (s, 3H), 1.25 (s, 9H), 1.15 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 198.1, 167.2, 166.9, 147.2, 143.5, 135.6, 134.0, 129.8, 128.2, 127.9, 123.2, 107.6, 106.9, 91.6, 84.1, 53.0, 52.9, 34.9, 34.6, 29.2, 28.4. HRMS (EI): *m/z* Calcd for $C_{26}H_{32}O_7$ [M+]: 456.2148. Found: 456.2143.

5.1.5. Dimethyl-6,8-bis(1,1-dimethylethyl)-10-oxo-2-(phenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene-4,4-dicarboxylate 5b. Yellow viscous liquid, IR (neat) ν_{max}: 2960, 2872, 1750, 1698, 1590, 1434, 1372, 1253, 1108, 1062, 1015, 824, 736 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.81–7.66 (m, 3H), 7.42–7.35 (m, 3H), 6.64 (s, 1H), 6.38 (d, 1H, $J=1.6$ Hz), 5.75 (d, 1H, $J=1.6$ Hz), 3.75 (s, 3H), 3.72 (s, 3H), 1.21 (s, 9H), 1.12 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 204.5, 164.5, 164.0, 146.3, 143.7, 135.5, 129.9, 127.9, 126.8, 123.4, 122.1, 117.2, 106.9, 92.0, 53.6, 52.8, 37.7, 35.5, 30.4, 28.0. HRMS (EI): *m/z* Calcd for $C_{26}H_{32}O_7$ [M+]: 456.2148. Found: 456.2144.

5.1.6. Dimethyl 7,9-bis(1,1-dimethylethyl)-10-oxo-2-(3-methoxyphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 6a. Yellow viscous liquid, IR (neat) ν_{max}: 2959, 2873, 1752, 1677, 1601, 1463, 1369, 1269, 1124, 793 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.31–7.23 (m, 2H), 6.93–6.87 (m, 2H), 6.78 (s, 1H), 6.67 (s, 1H), 5.72 (d, 1H, $J=2.1$ Hz), 3.84 (s, 3H), 3.80 (s, 3H), 3.74 (s, 3H), 1.25 (s, 9H), 1.14 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 196.4, 167.1, 166.9, 159.6, 147.2, 143.6, 137.9, 129.2, 129.1, 123.2, 120.5, 119.6, 116.5, 112.6, 107.5, 102.5, 83.9, 55.1, 53.0, 52.9, 34.7, 34.6, 29.2, 28.5. HRMS (EI): *m/z* Calcd for $C_{27}H_{38}O_8$ [M+]: 486.2254. Found: 486.2265.

5.1.7. Dimethyl-6,8-bis(1,1-dimethylethyl)-10-oxo-2-(3-methoxyphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 6b. Yellow viscous liquid, IR (neat) ν_{max}: 2960, 1756, 1672, 1602, 1463, 1367, 1268, 1113, 1052, 960, 874, 787, 726, 692, 499 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.33–7.22 (m, 2H), 6.93–6.87 (m, 2H), 6.60 (s, 1H), 6.39 (s, 1H), 5.74 (s, 1H), 3.82 (s, 3H), 3.75 (s, 3H), 3.71 (s, 3H), 1.21 (s, 9H), 1.13 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 204.4, 163.9, 163.4, 153.0, 136.2, 129.2, 123.4, 122.1, 120.3, 119.2, 117.1, 116.6, 106.8, 92.0, 91.0, 55.2, 53.5, 52.7, 35.5, 35.3, 30.6, 28.0. HRMS (EI): *m/z* Calcd for $C_{27}H_{34}O_8$ [M+]: 486.2254 Found: 486.2274.

5.1.8. Dimethyl-7,9-bis(1,1-dimethylethyl)-10-oxo-2-(2,4-dimethoxyphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 7a. Yellow crystalline solid, mp 68–69 °C, IR (KBr) ν_{max}: 2959, 1746, 1660, 1615, 1510, 1460, 1372, 1280, 1208, 1156, 1126, 1059, 939, 843, 796 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.88 (d, 1H, $J=8.7$ Hz), 7.08 (s, 1H), 6.85 (s, 1H), 6.54 (d, 1H, $J=8.7$ Hz), 6.40 (s, 1H), 5.72 (s, 1H), 3.81 (s, 6H), 3.79 (s, 3H), 3.68 (s, 3H), 1.22 (s, 9H), 1.14 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 198.1, 167.3, 167.1, 162.0, 159.3, 146.8, 143.6, 134.2, 129.7, 123.7, 116.3, 104.6, 102.0, 98.0, 83.9, 55.5, 55.2, 52.9, 34.8, 34.6, 29.2, 28.4. Anal. Calcd for $C_{28}H_{36}O_9$: C, 65.10; H, 7.02. Found: C, 64.83; H, 7.30.

5.1.9. Dimethyl-6,8-bis(1,1-dimethylethyl)-10-oxo-2-(2,4-dimethoxyphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 7b. Yellow viscous liquid, IR (neat) ν_{max}: 2959, 1762, 1677, 1603, 1508, 1463, 1372, 1267, 1158, 1126, 1032, 944, 837 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.98 (d, 1H, $J=8.6$ Hz), 7.11 (s, 1H), 6.83 (s, 1H), 6.55 (m, 1H), 6.34 (s, 1H), 5.88 (s, 1H), 3.83 (s, 6H), 3.79 (s, 3H), 3.78 (s, 3H), 1.22 (s, 9H), 1.16 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 197.7, 167.3, 166.9, 162.0, 159.1, 145.8, 143.7, 134.2, 130.7, 128.9, 123.7, 119.4, 104.9, 102.1, 97.9, 83.7, 55.3, 55.2, 53.0, 52.9, 34.8, 34.7, 30.0, 28.5. HRMS (EI): *m/z* Calcd for $C_{28}H_{36}O_9$ [M+]: 516.2359, Found: 516.2386.

5.1.10. Dimethyl-7-benzhydryl-9-*tert*-butyl-10-oxo-2-(4-methylphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 8a. Yellow viscous liquid, IR (neat) ν_{max}: 2965, 1755, 1682, 1439, 1284, 1232, 1124, 1067, 787, 705 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.56 (d, 2H, $J=7.9$ Hz), 7.33–7.06 (m, 12H), 6.61 (s, 1H), 6.49 (m, 1H), 5.75 (d, 1H, $J=1.8$ Hz), 5.47 (s, 1H), 3.81 (s, 3H), 3.57 (s, 3H), 2.36 (s,

3H), 1.10 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 197.2, 166.9, 166.8, 147.0, 141.6, 141.5, 139.7, 139.4, 138.9, 132.3, 129.3, 128.8, 128.7, 128.3, 127.7, 126.5, 123.3, 106.7, 91.7, 83.5, 52.9, 52.8, 49.2, 34.6, 28.2, 21.3. HRMS (EI): *m/z* Calcd for $C_{36}H_{36}O_7$ [M+]: 580.2461. Found: 580.2458.

5.1.11. Dimethyl 6-benzhydryl-8-*tert*-butyl-10-oxo-2-(4-methylphenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 8b. Yellow crystalline solid, mp 66–67 °C, IR (KBr) ν_{max}: 3027, 2960, 1760, 1677, 1501, 1439, 1232, 1124, 1072, 1015, 736, 705 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.27–7.00 (m, 12H), 6.79 (m, 2H), 6.38 (s, 1H), 6.18 (s, 1H), 6.00 (s, 1H), 4.96 (s, 1H), 3.75 (s, 3H), 3.55 (s, 3H), 2.34 (s, 3H), 1.11 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 202.3, 166.1, 164.3, 162.0, 149.2, 143.2, 142.5, 138.8, 131.7, 129.9, 128.6, 128.4, 128.2, 128.1, 127.9, 126.6, 125.7, 118.9, 107.2, 94.6, 88.8, 53.6, 53.4, 52.9, 35.5, 28.0, 21.4. HRMS (EI): *m/z* Calcd for $C_{36}H_{36}O_7$ [M+]: 580.2461. Found: 580.2447.

5.1.12. Dimethyl-7-benzhydryl-9-*tert*-butyl-10-oxo-2-(phenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 9a. Yellow crystalline solid, mp 124–125 °C, IR (KBr) ν_{max}: 2965, 2948, 1755, 1672, 1473, 1268, 1118, 1002, 700 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.68 (m, 2H), 7.39–7.11 (m, 13H), 6.65 (s, 1H), 6.51 (s, 1H), 5.75 (s, 1H), 5.48 (s, 1H), 3.83 (s, 3H), 3.58 (s, 3H), 1.20 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 197.1, 167.0, 166.7, 165.5, 147.3, 141.7, 139.0, 138.8, 129.9, 129.5, 129.4, 129.0, 128.9, 128.7, 128.5, 128.3, 127.9, 123.3, 107.8, 106.8, 96.3, 83.7, 53.1, 53.0, 49.4, 31.4, 28.2. Anal. Calcd for $C_{35}H_{34}O_7$: C, 74.19; H, 6.05; Found: C, 74.40; H, 6.15.

5.1.13. Dimethyl-6-benzhydryl-8-*tert*-butyl-10-oxo-2-(phenyl)-1,3-dioxaspiro[4.5]deca-6,8-diene 4,4-dicarboxylate 9b. Yellow crystalline solid, mp 136–138 °C, IR (KBr) ν_{max}: 2960, 1755, 1677, 1455, 1227, 1124, 1010, 705 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.27–7.02 (m, 13H), 6.77–6.76 (m, 2H), 6.41 (s, 1H), 6.18 (m, 1H), 6.01 (m, 1H), 4.94 (s, 1H), 3.75 (s, 3H), 3.57 (s, 3H), 1.11 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 202.0, 165.8, 164.1, 162.0, 149.1, 142.9, 142.2, 134.4, 129.6, 128.9, 128.2, 128.0, 127.8, 126.5, 126.3, 118.8, 106.9, 94.9, 88.6, 53.5, 53.3, 52.9, 35.4, 27.9. Anal. Calcd for $C_{35}H_{34}O_7$: C, 74.19; H, 6.05; Found: C, 74.24; H, 6.32.

5.1.14. Dimethyl-8-*tert*-butyl-2-(4-methylphenyl)-10-oxo-1,3-dioxaspiro[4.5]deca-6,8-diene-4,4-dicarboxylate 10a and dimethyl-7-*tert*-butyl-2-(4-methylphenyl)-10-oxo-1,3-dioxaspiro[4.5]deca-6,8-diene-4,4-dicarboxylate 10b. IR (neat) ν_{max}: 2968, 2875, 1748, 1674, 1586, 1497, 1438, 1431, 1393, 1272, 1129, 1058, 998, 921, 795, 729 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.61–7.55 (m, 4H), 7.21–7.19 (m, 4H), 7.08–7.05 (m, 1H), 6.75 (s, 1H), 6.73 (s, 1H), 6.55–6.51 (m, 1H), 6.15–6.08 (m, 2H), 5.95 (m, 1H), 5.80 (m, 1H), 3.82 (s, 6H), 3.75 (s, 6H), 2.36 (s, 6H), 1.20 (s, 9H), 1.14 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 203.8, 197.4, 168.1, 165.8, 161.7, 139.9, 139.8, 130.4, 129.2, 128.9, 128.1, 127.9, 127.5, 126.5, 126.4, 120.0, 107.6, 106.9, 90.0, 89.9, 53.6, 53.3, 53.1, 52.8, 34.7, 34.5, 28.4, 28.3. HRMS (EI): *m/z* Calcd for $C_{23}H_{26}O_7$ [M+]: 414.1678. Found: 414.1673.

5.1.15. Dimethyl-8-*tert*-butyl-2-(phenyl)-10-oxo-1,3-dioxaspiro[4.5]deca-6,8-diene-4,4-dicarboxylate 11a and dimethyl-7-*tert*-butyl-2-(phenyl)-10-oxo-1,3-dioxaspiro[4.5]deca-6,8-diene-4,4-dicarboxylate 11b. IR (neat) ν_{max}: 3039, 2968, 1755, 1673, 1579, 1497, 1459, 1431, 1393, 1272, 1129, 1058, 998, 921, 795, 729 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.73–7.67 (m, 4H), 7.39–7.38 (m, 6H), 7.08–7.02 (m, 1H), 6.77 (s, 1H), 6.76 (s, 1H), 6.56–6.52 (m, 1H), 6.15–6.08 (m, 2H), 5.95 (m, 1H), 5.79 (m, 1H), 3.80 (s, 6H), 3.74 (s, 6H), 1.18 (s, 9H), 1.13 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 198.6, 198.0, 166.9, 166.7, 166.6, 162.2, 139.9, 139.4, 135.2, 134.2, 132.4, 129.9, 129.6, 128.5, 128.4, 128.2, 128.0, 127.7, 125.6, 124.8, 118.9, 107.1, 106.5, 91.4, 83.4, 82.2, 53.1, 53.0, 52.9, 35.3, 34.5, 28.2, 27.8. HRMS (EI): *m/z* Calcd for $C_{22}H_{24}O_7$ [M+]: 400.1522. Found: 400.1533.

5.1.16. Dimethyl-2′-(4-methoxylphenyl)-10′-oxospiro-[1,3-dioxolane-4,9′-(10′H)-phenanthrene]-5′,5′-dicarboxylate 14. Phenanthrenequinone (0.104 g, 0.5 mmol), *p*-anisaldehyde (0.068 g, 0.5 mmol) and dimethyl diazomalonte (0.087 g, 0.55 mmol) was allowed to react with 2 mol% of $Rh_2(OAc)_4$ under an atmosphere of argon at reflux for 14 h. The solvent was then removed in vacuo and the residue purified by chromatographic separation on silica gel eluting with hexane–ethyl acetate (80:20) to give the spirodioxolane **14** (0.173 g, 73%) as a colorless crystalline solid, mp 177–178 °C. IR (KBr) ν_{max}: 2962, 1748, 1708, 1620, 1526, 1458, 1256, 1108, 1047, 845 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.01–7.86 (m, 3H), 7.78 (d, 2H, J=8.7 Hz), 7.70–7.62 (m, 2H), 7.48–7.28 (m, 3H), 7.00 (d, 2H, J=8.7 Hz), 6.71 (s, 1H), 3.85 (s, 3H), 3.60 (s, 3H), 3.18 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 197.2, 165.8, 164.8, 161.0, 136.6, 134.7, 132.2, 129.7, 129.0, 128.7, 128.6, 128.5, 128.1, 127.2, 123.5, 122.7, 113.9, 107.5, 94.1, 88.0, 55.2, 53.1, 52.5. Anal. Calcd for $C_{27}H_{22}O_8$: C, 68.35; H, 4.67. Found: C, 68.50; H, 4.84.

5.1.17. Dimethyl-2′-(4-methylphenyl)-10′-oxospiro[1,3-dioxolane-4,9′-(10′H)-phenanthrene]-5′,5′-dicarboxylate 15. Colorless crystalline solid, mp 128–129 °C. IR (KBr) ν_{max}: 2965, 2929, 1760, 1687, 1510, 1472, 1432, 1375, 1301, 1235, 1209, 1117, 1090, 1016, 953, 940, 748 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.00–7.83 (m, 3H), 7.73 (d, 2H), 7.67–7.60 (m, 2H), 7.46–7.37 (m, 2H), 7.29–7.21 (m, 3H), 6.71 (s, 1H), 3.59 (s, 3H), 3.16 (s, 3H), 2.39 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 196.9, 165.5, 164.6, 139.7, 136.5, 134.6, 132.1, 129.5, 129.1, 128.6, 128.5, 128.4, 127.8, 127.2, 123.4, 122.6, 107.4, 93.7, 88.1, 52.9, 52.4, 21.4. Anal. Calcd for $C_{27}H_{22}O_7$: C, 70.73; H, 4.84. Found: C, 70.97; H, 4.45.

5.1.18. Dimethyl-2′-(2,4-dimethoxylphenyl)-10′-oxospiro[1,3-dioxolane-4,9′-(10′H)-phenanthrene]-5′,5′-dicarboxylate 16. Colorless crystalline solid, mp 186–187 °C, IR (KBr) ν_{max}: 3056, 2962, 1753, 1688, 1615, 1451, 1436, 1281, 1240, 1128, 1115, 1074, 1033, 948, 901, 753 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 8.02–7.85 (m, 3H), 7.70– 7.62 (m, 3H), 7.47–7.26 (m, 3H), 7.00 (s, 1H), 6.97–6.87 (m, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.61 (s, 3H), 3.18 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 197.1, 165.9, 164.8, 153.8, 152.6, 134.7, 132.2, 130.6, 129.6, 128.8, 128.6, 128.4, 128.1, 124.1, 123.5, 122.7, 116.4, 113.3, 112.5, 102.9, 88.0, 56.6, 55.8, 53.1, 52.5 Calcd for $C_{28}H_{24}O_9$: C, 66.66; H, 4.80. Found: C, 66.26; H, 4.73.

5.1.19. Dimethyl-2′-(3-methoxylphenyl)-10′-oxospiro [1,3-dioxolane-4,9′-(10′H)-phenanthrene]-5′,5′-dicarboxylate 17. Colorless viscous liquid, IR (neat) ν_{max}: 3012, 2955, 2846, 1755, 1693, 1600, 1460, 1439, 1398, 1274, 1144, 1051, 943, 891, 731 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00–7.84 (m, 3H), 7.68–7.59 (m, 3H), 7.43–7.25 (m, 3H), 7.00–6.94 (m, 2H), 6.74 (s, 1H), 3.83 (s, 3H), 3.59 (s, 3H), 3.16 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 197.0, 165.7, 164.6, 159.7, 137.7, 136.5, 134.5, 132.1, 129.7, 129.6, 128.7, 123.5, 122.7, 120.1, 119.6, 115.9, 112.3, 107.2, 93.8, 88.0, 55.3, 53.1, 52.5 HRMS (EI): *m/z* Calcd for $C_{27}H_{22}O_8$ [M+]: 474.1315. Found: 474.1315.

5.1.20. Dimethyl-2′-(phenyl)-10′-oxospiro[1,3-dioxolane-4,9′-(10′H)-phenanthrene]-5′,5′-dicarboxylate 18. Colorless viscous liquid, IR (neat) ν_{max}: 3068, 2965, 1755, 1693, 1610, 1444, 1434, 1279, 1237, 1134, 1108, 1077, 1036, 953, 901, 756 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.00–7.83 (m, 5H), 7.60–7.32 (m, 8H), 6.77 (s, 1H), 3.54 (s, 3H), 3.12 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 196.9, 165.4, 164.4, 136.3, 134.7, 134.6, 131.9, 129.8, 129.5, 128.3, 128.3, 127.7, 127.1, 123.4, 122.6, 107.1, 93.6, 87.8, 52.8, 52.3. HRMS (EI): *m/z* Calcd for $C_{26}H_{20}O_7$ [M+]: 444.1209. Found: 444.1206.

5.1.21. Dimethyl-1′,2′-dihydro-1′-methyl-2-(4-methylphenyl)-2′-oxospiro[1,3-dioxolane-4,3′-[3H]indole]-5′,5′-dicarboxylate 20. A mixture of *N*-methyl isatin (0.081 g, 0.5 mmol), *p*-tolualdehyde (0.060 g, 0.5 mmol) and dimethyl diazomalonate (0.087 g, 0.55 mmol) in 5 mL of dry benzene was refluxed with 2 mol% of Rh(II) acetate under an atmosphere of argon for 16 h. The residue obtained after the removal of the solvent was subjected to chromatography on silica gel using hexane–ethyl acetate (80:20) as the solvent to afford the product **20** (0.166 g, 81%) as a colorless crystalline solid, mp 168–169 °C. IR (KBr) ν_{max}: 2962, 1757, 1728, 1616, 1457, 1440, 1384, 1371, 1299, 1243, 1032 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.69 (d, 2H, J=7.1 Hz), 7.38–7.19 (m, 4H), 7.04–6.99 (m, 1H), 6.94 (s, 1H), 6.83 (d, 1H, J=7.5 Hz), 3.80 (s, 3H), 3.64 (s, 3H), 3.19 (s, 3H), 2.36 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 173.8, 166.9, 145.3, 139.9, 132.4, 131.5, 128.9, 127.9, 125.2, 122.8, 122.7, 108.9, 106.3, 88.9, 85.6, 53.1, 52.9, 26.2, 21.4. Anal. Calcd for $C_{22}H_{21}NO_7$: C, 64.23; H, 5.14; N, 3.40. Found: C, 63.94; H, 5.03; N, 3.43.

5.1.22. Dimethyl-1′,2′-dihydro-1′-^{n}propyl-2-(4-methylphenyl)-2′-oxospiro[1,3-dioxolane-4,3′-[3H]indole]-5′,5′-dicarboxylate 21. Colorless crystalline solid, mp 123–124 °C, IR (KBr) ν_{max}: 2975, 1762, 1620, 1445, 1263, 1148, 1034 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.69 (d, 2H, J=7.7 Hz), 7.35–7.19 (m, 4H), 7.04–7.01 (m, 1H), 6.91 (s, 1H), 6.83 (d, 1H, J=7.8 Hz), 3.77 (s, 3H), 3.73–3.68 (m, 1H), 3.63 (s, 3H), 3.57–3.47 (m, 1H), 2.35 (s, 3H), 1.77–1.70 (m, 2H), 1.00 (t, 3H, J=7.3 Hz). ^{13}C NMR ($CDCl_3$) δ 173.4, 166.7, 144.8, 139.6, 132.5, 131.2, 128.8, 128.1, 127.7, 125.1, 122.3, 108.9, 106.0, 88.7, 85.3, 52.8, 41.7, 21.2, 20.4, 11.2. Anal. Calcd for $C_{24}H_{25}NO_7$: C, 65.59; H, 5.73; N, 3.19. Found: C, 65.43; H, 5.85; N, 3.19.

5.1.23. Dimethyl-1′,2′-dihydro-1′-^{n}propyl-2-(4-methoxylphenyl)-2′-oxospiro[1,3-dioxolane-4,3′-[3H]indole]-5′,5′-dicarboxylate 22. Colorless crystalline solid, mp 161–163 °C, IR (KBr) ν_{max}: 2967, 2876, 1757, 1723, 1620, 1469, 1374, 1289, 1244, 1130, 1038, 839 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.75 (d, 2H, J=8.6 Hz), 7.34–7.24 (m, 2H), 7.03–6.84 (m, 5H), 3.81 (s, 3H), 3.79 (s, 3H), 3.75–3.70 (m, 1H), 3.67 (s, 3H), 3.60–3.51 (m, 1H), 1.79– 1.72 (m, 2H), 1.02 (t, 3H, J=7.4 Hz). ^{13}C NMR ($CDCl_3$) δ 173.6, 167.1, 166.9, 161.1, 144.9, 131.4, 129.9, 129.6, 127.3, 125.2, 124.8, 122.6, 113.7, 109.1, 106.1, 88.8, 85.3, 55.2, 53.1, 53.0, 41.8, 20.5, 11.5. Anal. Calcd for $C_{24}H_{25}NO_8$: C, 63.29; H, 5.53; N, 3.08. Found: C, 63.43; H, 5.37; N, 3.41.

5.1.24. Dimethyl-1′,2′-dihydro-1′-methyl-2-(phenyl)-2′-oxospiro[1,3-dioxolane-4,3′-[3H]indole]-5′,5′-dicarboxylate 23. Colorless crystalline solid, mp 169–170 °C, IR (KBr) ν_{max}: 3022, 2948, 2840, 1755, 1735, 1620, 1472, 1378, 1290, 1256, 1128, 1054, 1013, 919 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.83–7.80 (m, 2H), 7.42–7.34 (m, 4H), 7.24 (d, 1H, J=7.5 Hz), 7.05–6.99 (m, 1H), 6.97 (s, 1H), 6.84 (d, 1H, J=7.8 Hz), 3.79 (s, 3H), 3.65 (s, 3H), 3.17 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 173.6, 166.8, 166.7, 145.1, 135.1, 131.6, 130.0, 128.2, 128.2, 127.9, 124.9, 122.8, 122.2, 108.9, 106.1, 88.6, 85.5, 53.1, 52.9, 26.1. Anal. Calcd for $C_{21}H_{19}NO_7$: C, 63.47; H, 4.82; N, 3.52. Found: C, 63.65; H, 4.97; N, 3.74.

5.1.25. Dimethyl-5′-bromo-1′-methyl 2′-oxo-2-(4-methylphenyl)-1′,2′-dihydrospiro[1,3-dioxolane-4,3′-indole]-5,5-dicarboxylate 24. Colorless crystalline solid, mp 175–176 °C, IR (KBr) ν_{max}: 2955, 2829, 1750, 1729, 1610, 1485, 1438, 1364, 1303, 1243, 1121, 1031, 994 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.69 (d, 2H, J=8.0 Hz), 7.53–7.49 (m, 1H), 7.36–7.21 (m, 3H), 6.93 (s, 1H), 6.74 (d, 1H, J=8.3 Hz), 3.80 (s, 3H), 3.73 (s, 3H), 3.20 (s, 3H), 2.39 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 173.4, 166.8, 144.4, 140.1, 134.3, 132.1, 129.1, 128.6, 127.9, 124.5, 115.4, 110.2, 106.7, 89.0, 85.2, 53.1, 53.1, 26.3, 21.4. HRMS (EI): *m/z* Calcd for $C_{22}H_{20}NO_7Br$ [M+]: 489.0423. Found: 489.0425.

5.1.26. Dimethyl-1′,2′-dihydro-1′-ethyl-2-(4-methylphenyl)-2′-oxospiro[1,3-dioxolane-4,3′-[3H]indole]-5′,5′-dicarboxylate 25. Colorless crystalline solid, mp 126–128 °C, IR (KBr) ν_{max}: 2982, 1752, 1723, 1615, 1479, 1378, 1297, 1249, 1121 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.69 (d, 2H, J=8.0 Hz), 7.36–7.31 (m, 1H), 7.26–7.19 (m, 3H), 7.02–6.70 (m, 1H), 6.94 (s, 1H), 6.84 (d, 1H, J=7.8 Hz), 3.77 (s, 3H), 3.74–3.67 (m, 1H), 3.64 (s, 3H), 3.56–3.49 (m, 1H), 2.36 (s, 3H), 1.02 (t, 3H, J=7.4 Hz). ^{13}C NMR ($CDCl_3$) δ 173.5, 166.8, 144.8, 139.8, 131.3, 128.9, 127.9, 125.1, 122.5, 109.0, 106.1, 88.4, 85.7, 53.0, 52.9, 41.7, 21.3, 20.5, 11.4. HRMS (EI): *m/z* Calcd for $C_{23}H_{23}N_2O_7$ [M+]: 439.1504. Found: 439.1505.

5.1.27. Dimethyl-1′,2′-dihydro-1′-phenyl-2-(4-methylphenyl)-2′-oxospiro[1,3-dioxolane-4,3′-[3H]indole]-5′,5′-dicarboxylate 26. Colorless viscous liquid. IR (neat) ν_{max}: 3052, 2955, 2927, 1754, 1729, 1615, 1506, 1472, 1432, 1375, 1301, 1244, 1210, 1124, 1090, 1016, 953, 942, 759, 708 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.73 (d, 2H, J=8.0 Hz), 7.54–7.39 (m, 4H), 7.33–7.19 (m, 5H), 7.00 (s, 1H), 6.77 (d, 2H, J=7.9 Hz), 3.78 (s, 3H), 3.67 (s, 3H), 2.36 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 172.9, 166.8, 145.2, 139.7, 133.5, 131.9, 129.5, 128.8, 127.8, 126.4, 125.1, 123.0, 109.9, 106.2, 88.8, 85.4, 53.2, 53.0, 21.2. HRMS (EI): *m/z* Calcd for $C_{27}H_{23}NO_7$ [M+]: 473.1471. Found: 473.1474.

5.1.28. Dimethyl-2-(4-methylphenyl)-4′-oxo-2′,3′-dichloro-4H-spiro[1,3-dioxolane-4,1′-naphthalene]-5,5-dicarboxylate 28. Colorless crystalline solid, mp 155–157 °C, IR (KBr) ν_{max}: 2962, 2921, 2861, 1762, 1688, 1600, 1431, 1276, 1222, 1135, 1081, 1020, 960, 926 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.15–8.12 (m, 1H), 7.66 (d, 2H, J= 8.0 Hz), 7.53–7.46 (m, 3H), 7.32 (d, 2H, J=7.9 Hz), 6.76 (s, 1H), 3.78 (s, 3H), 3.13 (s, 3H), 2.45 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.2, 164.5, 163.3, 149.8, 140.4, 140.0, 133.2, 131.4, 129.6, 129.4, 127.2, 126.9, 126.6, 107.4, 92.7, 86.7, 54.3, 52.8, 21.5. Anal. Calcd for $C_{23}H_{18}Cl_2O_7$: C, 57.88; H, 3.80. Found: C, 58.14; H, 3.43.

5.1.29. Dimethyl-2-(4-methoxyphenyl)-4′-oxo-2′,3′-dichloro-4H-spiro[1,3-dioxolane-4,1′-naphthalene]-5,5-dicarboxylate 29. Colorless crystalline solid, mp 142–143 °C, IR (KBr) ν_{max}: 2860, 2846, 1770, 1760, 1620, 1589, 1522, 1439, 1403, 1300, 1263, 1144, 1072, 1036, 963, 912, 834, 710 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.04 (d, 1H, J= 6.9 Hz), 7.64–7.45 (m, 5H), 6.98–6.88 (m, 2H), 6.66 (s, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 3.04 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 175.9, 164.4, 163.1, 160.9, 149.7, 140.2, 134.6, 133.0, 132.7, 130.0, 129.5, 128.5, 128.0, 127.9, 126.8, 125.9, 114.0, 107.2, 93.0, 86.5, 55.2, 54.2, 52.6. Anal. Calcd for $C_{23}H_{18}Cl_2O_8$: C, 56.00; H, 3.68. Found: C, 55.99; H, 3.71.

5.1.30. Dimethyl-2-phenyl-4′-oxo-2′,3′-dichloro-4H-spiro[1,3-dioxolane-4,1′-naphthalene]-5,5-dicarboxylate 30. Colorless crystalline solid, mp 175–176 °C, IR (KBr) ν_{max}: 2955, 1755, 1681, 1607, 1452, 1283, 1236, 1155, 1061, 1020, 953, 791, 697 cm^{-1}. ^1H NMR ($CDCl_3$) δ 8.2 (d, 1H, J=7.9 Hz), 7.79–7.76 (m, 2H), 7.51–7.49 (m, 6H), 6.78 (s, 1H), 3.88 (s, 3H), 3.12 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 176.1, 164.4, 163.1, 140.6 140.2, 134.2, 132.7, 130.1, 130.0, 129.6, 128.7, 128.4, 127.1, 126.9, 126.5, 107.2, 92.7, 86.7, 54.3, 52.8. Anal. Calcd for $C_{22}H_{16}Cl_2O_7$: C, 57.04; H, 3.48. Found: C, 56.92; H, 3.32.

5.1.31. Dimethyl-6,9-dimethyl-2-(4-methylphenyl)-8-oxo-1,3-dioxospiro[4.5]deca-6,9-diene-4,4-dicarboxylate 31. Colorless viscous liquid, IR (neat) ν_{max}: 2955, 1755, 1688, 1661, 1445, 1384, 1249, 1121, 1074, 663 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.47 (d, 2H, J=8.0 Hz), 7.21 (d, 2H, J= 8.0 Hz), 6.81 (s, 1H), 6.62 (s, 1H), 6.16 (s, 1H), 3.81 (s, 3H), 3.73 (s, 3H), 2.38 (s, 3H), 1.94 (s, 3H), 1.82 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 185.2, 165.9, 164.5, 140.2, 139.3, 138.5, 131.9, 128.9, 128.5, 126.2, 125.9, 107.2, 105.3, 90.6, 83.0, 53.3, 53.2, 21.2, 19.6, 15.4. HRMS (EI): m/z Calcd for $C_{21}H_{22}O_7$ [M+]: 386.1365. Found: 386.1399.

5.1.32. Dimethyl-6,9-dimethyl-2-(4-methoxylphenyl)-8-oxo-1,3-dioxospiro[4.5]deca-6,9-diene-4,4-dicarboxylate 32. Colorless viscous liquid, IR (neat) ν_{max}: 2948, 2840, 1755, 1688, 1654, 1526, 1438, 1391, 1256, 1189, 1135, 1081 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.52 (d, 2H, J=8.7 Hz), 6.92, (d, 2H, J=8.7 Hz), 6.79 (s, 1H), 6.59 (s, 1H), 6.10 (s, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.74 (s, 3H), 1.93 (s, 3H), 1.84 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 185.3, 166.1, 164.7, 155.4, 140.3, 138.6, 128.9, 128.0, 127.7, 113.8, 105.4, 90.4, 83.0, 55.2, 53.3, 53.2, 19.7, 15.6. HRMS (EI): m/z Calcd for $C_{21}H_{22}O_8$ [M+]: 402.1315. Found: 402.1320.

5.1.33. 2-(4-Methylphenyl)-8-oxo-1,3-dioxospiro[4.5]-deca-6,9-diene-4,4-dicarboxylate 33. Colorless viscous liquid, IR (neat) ν_{max}: 2068, 1755, 1667, 1438, 1294, 1243, 1108 cm^{-1}. ^1H NMR ($CDCl_3$) δ 7.44 (d, 2H, J= 7.9 Hz), 7.21 (d, 2H, J=7.9 Hz), 7.10–6.91 (m, 2H), 6.53 (s, 1H), 6.38–6.23 (m, 2H), 3.82 (s, 3H), 3.71 (s, 3H), 2.38 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 184.1, 165.3, 145.9, 143.4, 141.2, 133.1, 131.2, 130.1, 129.2, 126.5, 106.2, 89.4, 79.6, 53.4, 53.2, 21.4. HRMS (EI): m/z Calcd for $C_{19}H_{18}O_7$ [M+]: 357.0974. Found: 357.0935.

Acknowledgements

S. M. thanks the CSIR, New Delhi for a research fellowship.

References and notes

1. For reviews on the chemistry of carbonyl ylides, see (a) Padwa, A.; Hornbuckle, S. F. *Chem. Rev.* **1991**, *91*, 263. (b) Padwa, A. *J. Chin. Chem. Soc.* **1993**, *40*, 97. (c) Padwa, A.; Weingarten, M. D. *Chem. Rev.* **1996**, *96*, 223. (d) Hodgson, D. M.; Pierad, F. Y. T. M.; Stupple, P. A. *Chem. Soc. Rev.* **2001**, *30*, 50. (e) Muthusamy, S.; Mehta, G. *Tetrahedron* **2002**, *58*, 9477.
2. Mc Mills, M. C.; Wright, D. In *Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*; Padwa, A., Pearson, W. H., Eds.; Wiley: London, 2003; p 253.
3. (a) Padwa, A.; Sandanayaka, V. P.; Curtis, E. A. *J. Am. Chem. Soc.* **1994**, *116*, 2667. (b) Padwa, A.; Sandanayaka, V. P.; Curtis, E. A. *Tetrahedron Lett.* **1995**, *36*, 1989. (c) Padwa, A.; Curtis, E. A.; Sandanayaka, V. P. *J. Org. Chem.* **1997**, *62*, 1317.
4. Nair, V.; Rajesh, C.; Vinod, A. U.; Bindu, S.; Sreekanth, A. R.; Mathen, J. S.; Balagopal, L. *Acc. Chem. Res.* **2003**, *36*, 899.
5. Büchner, E.; Curtius, T. *Ber. Dtsch. Chem. Ges.* **1883**, *16*, 2230.
6. Dieckmann, W. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 1024.
7. (a) de March, P.; Huisgen, R. *J. Am. Chem. Soc.* **1982**, *104*, 4952. (b) Huisgen, R.; de March, P. *J. Am. Chem. Soc.* **1982**, *104*, 4953.
8. Alt, M.; Maas, G. *Tetrahedron* **1994**, *50*, 7435.
9. Jiang, B.; Zhang, X.; Luo, Z. *Org. Lett.* **2002**, *4*, 2453.
10. Doyle, M. P.; Hu, W.; Timmons, D. *Org. Lett.* **2001**, *3*, 3741.
11. Nair, V.; Sheela, K. C.; Sethumadhavan, D.; Dhanya, R.; Rath, N. P. *Tetrahedron* **2002**, *58*, 10341 and references cited therein.
12. Nair, V.; Mathai, S.; Nair, S. M.; Rath, N. P. *Tetrahedron Lett.* **2003**, *44*, 8407.
13. Crystallographic data for compound **20** has been deposited with Cambridge Crystallographic data Centre with deposition number CCDC 258280.
14. Regitz, M. *Synthesis* **1972**, 351.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2857–2869

Mechanistic approaches to asymmetric synthesis of aziridines from guanidinium ylides and aryl aldehydes

Toyokazu Haga[a,b] and Tsutomu Ishikawa[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan*
[b]*Production Department, Technology Group, Eisai Co., Ltd., 22-Sunayama, Hasaki-machi, Kashima-gun, Ibaraki 314-0255, Japan*

Received 20 December 2004; accepted 19 January 2005

Abstract—To approach more realistic mechanisms for asymmetric aziridine synthesis from guanidinium ylides and aryl aldehydes, reactions were systematically carried out by using a variety of *p*-substituted benzaldehydes under modified conditions. Two kinds of reaction mechanisms controlled by the nature of the *p*-substituents of aryl aldehydes is proposed for the two-steps aziridine synthesis composed of a C–C bond formation by nucleophilic addition of guanidinium ylides to aryl aldehydes (step 1) and the fragmentation of intermediate adducts to aziridine products by intramolecular nucleophilic substitution (step 2). A S_Ni-like mechanism via cationic-like transition state is proposed for step 2 in the asymmetric synthesis using EDG-substituted benzaldehydes, whereas with EWG-substituted benzaldehydes, a S_N2-like mechanism is proposed. Hammett analysis, based on the diastereomeric ratio in the aziridine products, is consistent with the proposed rate-determining steps in these two mechanisms. A second Hammett analysis, based on the enantiomeric ratio of the aziridine products, clearly reveals the difference in the susceptibilities to the electronic substituents effect between step 1 and step 2.

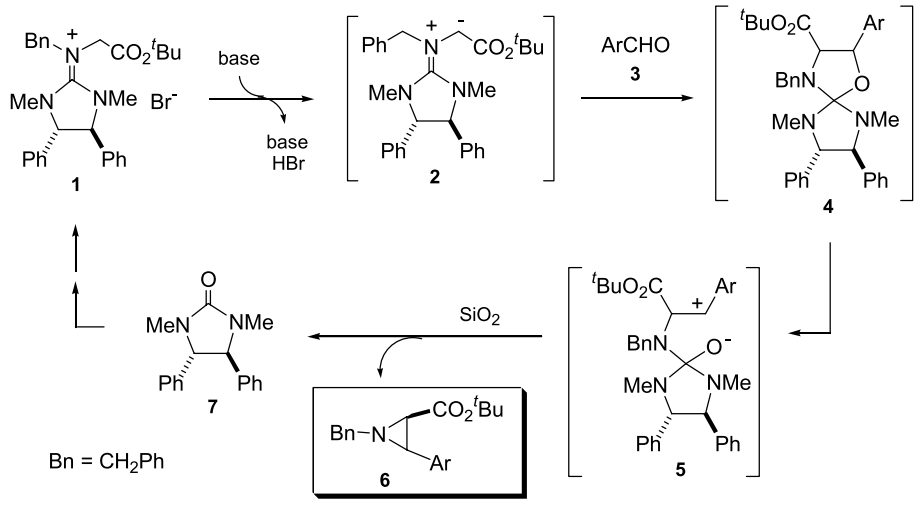
1. Introduction

Three-membered nitrogen heterocycles, aziridines, are very important molecules not only as key components of biologically active natural products such as mitomycins, but also as reactive synthetic precursors for a wide variety of nitrogen-containing compounds.[1] Among them 3-arylaziridine-2-carboxylates are versatile precursors for the synthesis of amino acid derivatives including unnatural type products.[2] Therefore, there have been many approaches to aziridine preparation involving asymmetric synthesis, which could be basically classified into three types of reactions:[1] (i) intramolecular substitution of β-aminoalcohols by nucleophilic nitrogens, (ii) addition of carbenes to imines, and (iii) addition of nitrenes to olefins. Recently, we reported a new synthetic method for the preparation of 3-arylaziridine-2-carboxylates **6** from guanidinium salts which carry a glycine unit (e.g., **1**) and aryl (including heterocyclic) aldehydes **3**.[3] In the proposed cyclic mechanism, guanidinium ylide **2** is formed from the guanidinium salt **1** under basic conditions and acts as a nucleophile. Finally, urea **7**, a synthetic precursor of **1**, is produced as a co-product of aziridines after treatment with silica gel (SiO_2). Introduction of chiral centers into the guanidinium template results in effective asymmetric induction in the aziridine formation. In this unique cycle, aziridines are generated with excellent to moderate stereoselectivity depending upon the choice of the aryl aldehydes **3**. In general *trans*-aziridines are efficiently obtained with satisfactory enantioselectivity when aryl aldehydes carrying an electron-donating group (EDG) such as piperonal (**3a**) are used as electrophiles. Based on stereochemical results we postulated a mechanism for the asymmetric induction through carbocation-alkoxide intermediates **5** and spiro intermediates **4** (Scheme 1).

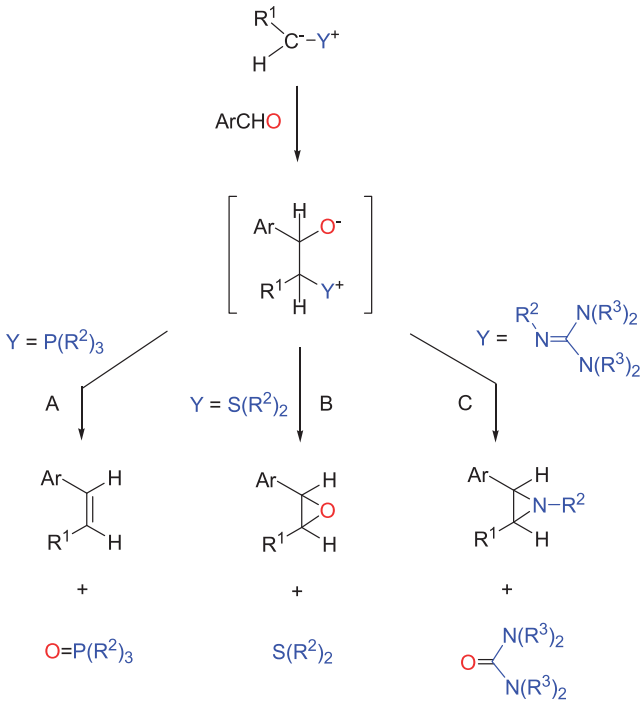
In ylide chemistry, the Wittig reaction is a well known process;[4] a phosphonium ylide reacts with a carbonyl compound such as aldehydes or ketones to give an alkene by C–C bond formation together with phosphine oxide, in which the phosphorus atom of the ylide acts as oxygen acceptor from the carbonyl substrate (see A, Scheme 2). In the reaction of sulfonium ylide with a carbonyl substrate, the product is oxirane in which the oxygen atom comes from the carbonyl substrate[5] (see B, Scheme 2). However, in the guanidinium ylides mechanism, the external nitrogen atom, among three ylide nitrogens, is incorporated into aziridines and the remaining amidine moiety acts as an oxygen acceptor to be converted to urea (see C, Scheme 2). Thus, these three types of ylide-participating reactions with aryl

Keywords: Asymmetric synthesis; Aziridine; Guanidinium ylide; Mechanism; Hammett analysis.
* Corresponding author. Tel./fax: +81 43 290 2910;
e-mail: benti@p.chiba-u.ac.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.088



Scheme 1. The postulated mechanism for asymmetric aziridine synthesis from guanidinium salt **1** and aryl aldehydes **3**.



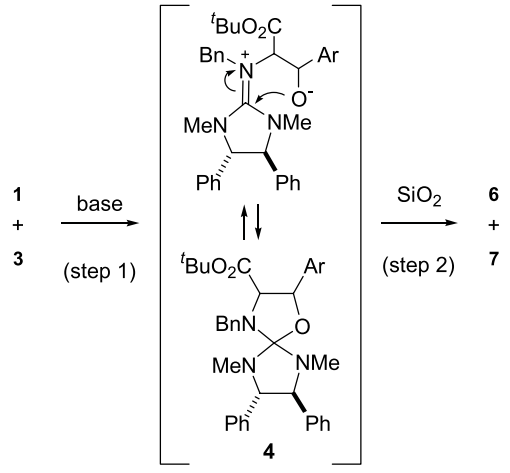
Scheme 2. Schematic reaction profiles of phosphonium, sulfonium, and guanidinium ylides with aryl aldehydes.

aldehydes afford quite comparable profiles in the product formation.

Despite the reasonable mechanisms proposed for the reactions of phosphonium[4] and sulfonium ylides[5] with carbonyl substrates, the reactivity and the overall stereoselectivity observed in the guanidinium ylide chemistry could not be rationally explained by the mechanism[3] proposed in Scheme 1. The presence in some cases of *cis*-aziridine as major isomer, forces us to reevaluate the role of carbocation-alkoxide intermediates **5**. Thus, reactions were systematically carried out by using a variety of *p*-substituted benzaldehydes under modified conditions and, as a result, we proposed more realistic mechanisms to cover the overall aziridine preparation from guanidinium ylides by application of Hammett relation. In this paper we present two different reaction pathways depending upon the presence of either EDG or electron-withdrawing group (EWG) on the aryl aldehyde components.

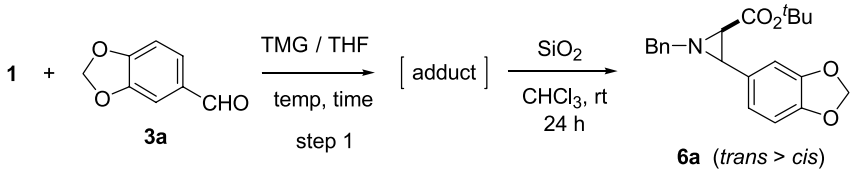
2. Results and discussion

Aziridine synthesis is basically composed of two steps (Scheme 3), as previously mentioned in a communication.[3] Step one is a C–C bond formation between a guanidinium salt **1** and an aryl aldehyde **3** under basic conditions in which an initially-formed zwitter ionic species is in equilibria with a non-ionic spiro compound **4**. The second step is the fragmentation of the intermediate adduct, triggered with SiO_2, to afford aziridine product **6** and urea **7**. This two steps reaction can be followed by a normal phase TLC: a first intermediate adduct is observed as a highly polar product then, after SiO_2 treatment, a less-polar component is generated.



Scheme 3. C–C bond formation between a guanidinium salt **1** and an aryl aldehyde **3** followed by fragmentation to aziridine **6** and urea **7**.

Table 1. Effect of temperature on the C–C bond formation (step 1) of **1** and **3a**



Run	Temperature (°C)	Time (h)	*trans*-**6a**		*cis*-**6a** yield (%)[a]
			Yield (%)[a]	ee (%)[b]	
1	−35	>48[c]	73	84	4
2	−20	49	86	83	5
3	−10	23	91	85	5
4	10	4.5	88	92	5
5	25	4.5	89	91	6
6	40	4.5	88	91	6
7	60	4.5	86	89	5
8	−20	4[d]	34	84	3

A 1.5 M equiv of TMG was used.
[a] Determined by quantitative HPLC.
[b] Determined by chiral HPLC.
[c] A trace of **3a** was detected at 48 h.
[d] Unreacted **3a** was observed on TLC.

In this mechanistic approach, (4*S*,5*S*)-4,5-diphenylimidazoline-derived guanidinium salt **1** and piperonal (**3a**) have been selected as an ylide and aryl aldehyde source because effective production of *trans*-aziridine *trans*-**6a** has been observed with high diastereomeric excess (de) and enantiomeric excess (ee). Although 1,1,3,3-tetramethylguanidine (TMG) has not been used only as a base but also as solvent in the previous communication,[3] the reaction conditions were modified to use a limited amount of tetrahydrofuran (THF) as solvent in order to obtain reproducible results.[†] Thus, *trans*-**6a** and *cis*-**6a** were obtained in 89 and 6% yields, respectively, in the reaction of **1** and **3a** at 25 °C for 4.5 h followed by fragmentation with SiO_2 in chloroform for 24 h, as reported previously[3] (see run 5, Table 1). The ee of the former *trans*-**6a** is 91%.

2.1. Examination of reaction conditions: effect of temperature

First we examined the effect of temperature on the C–C bond formation of step 1 to determine whether the reaction was controlled by either kinetic or thermodynamic mode (Table 1). The reaction was carried out at various temperature until the starting materials disappeared on TLC and, then, the formed adduct intermediate was treated with SiO_2 for 24 h (runs 1–7). Only small difference in product profiles was observed in all reactions examined and, in addition, identical enantioselectivity in aziridine products were obtained in shorter treatment (4 h, run 8) as well as in longer one (49 h, run 2). These facts indicate that step 1 is basically under kinetic control, although higher asymmetric induction at higher temperature (runs 1–3 vs 4–7) suggests the partial participation of thermodynamic mode to the C–C bond formation. Moreover, variable ee of *trans*-**6a** dependent upon the conditions used, even with a small difference, suggests that no equilibration between the intermediate and the starting piperonal occurs during the SiO_2 treatment (step 2). Thus, it is reasonable to deduce that the overall reaction of this aziridine formation is controlled by kinetic mode.

Next, we approached to the structure of the intermediate adduct by spectroscopic means; however, direct NMR study on the reaction mixture obtained in step 1 using a variety of solvents ($CDCl_3$, CD_3CN, benzene-d_6, and pyridine-d_5) resulted in a complex signal pattern in all cases examined. Although trials for the isolation of the adduct derived from piperonal (**3a**) were failed, the intermediate adduct in the reaction using *p*-chlorobenzaldehyde (**3g**) mentioned later was successfully isolated as a ca. 5:1 mixture of two isomeric polar compounds (see Section 4.2.11), which was smoothly converted into the corresponding aziridines by SiO_2 treatment [*trans*-**6g**: 22% yield (84% ee); *cis*-**6g**: 55% yield (86% ee)]. Unfortunately, in this case, a decisive structural information on the adduct could not be taken even using 2D NMR techniques under various conditions. These situations force us to attempt to chemically trap the zwitter ionic intermediate adduct (see Scheme 3).

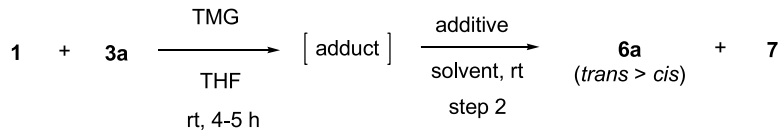
2.2. Chemical approaches to the adduct: fragmentation to aziridine with acid anhydrides

Trials for the derivatization of the intermediate adduct using *p*-nitrobenzenesulfonyl chloride, *m*-bromobenzoyl chloride, or *tert*-butyldimethylsilyl triflate were unsuccessful. On the other hand, it was found that treatment with acetic anhydride (Ac_2O) in chloroform immediately convert the intermediate adduct into *trans*-**6a** in 86% yield with 87% ee together with *cis*-**6a** (7% yield) and urea **7** (80% yield), but no trapped product (run 1, Table 2). The same asymmetric induction[‡] as with SiO_2 suggests that both reactions occurred under the same stereochemical controls,

[†] In the preliminary communication,[3] we reported the effectiveness of reaction under solvent-free conditions; however, inconstant results were sometimes obtained in scale-up reactions. After precise examination, homogeneous condition in limited amounts of THF solution led to reproducible results. Other organic bases examined such as DBU did not initiate the reaction.

[‡] It was confirmed by chiral HPLC analysis. See Section 4.

Table 2. Effect of additives on the fragmentation (step 2) of the intermediate adduct obtained from **1** and **3a**



Run	Additive/solvent	M equiv/mL	Time (h)	*trans*-**6a**		*cis*-**6a** yield (%)[a]	**7** yield (%)[b]
				Yield (%)[a]	ee (%)[c]		
1	$Ac_2O/CHCl_3$	2.2[d]/10	0.3	86	87	7	80
2	$(EtCO)_2O/CHCl_3$	2.2/10	1	87	85	7	–[e]
3	$(^iPrCO)_2O/CHCl_3$	2.2/10	2	83	90	7	–[e]
4	$(^tBuCO)_2O/CHCl_3$	2.2/10	14.5	84	91	8	–[e]
5	$(PhCO)_2O/CHCl_3$	2.2/0.8	2	68[b]	92	–[e]	–[e]
6	Ac_2O/THF	2.2/0.4	0.3	77	84	6	–[e]
7	$(CH_2CO)_2O$/THF	2.2/10	43	57	87	3	–[e]
8	$(^tBuCO)_2O$/THF	2.2/0.4	28	53	90	4	–[e]
9	$(Boc)_2O$/THF	2.2/0.4	89	71	86	6	–[e]
10	$Ac_2S/CHCl_3$	2.2/10	0.5	68	88	6	79

A 1.1 M equiv of TMG was used because excess amount had no effect on the reaction.
[a] Determined by quantitative HPLC.
[b] Isolated yield, but not optimized.
[c] Determined by chiral HPLC.
[d] Incomplete reaction was observed when treated with 1.1 M equiv of Ac_2O for 0.7 h. Further addition of Ac_2O (total 2.2 M equiv) led to the conversion to aziridines. Therefore, 2.2 M equiv of an acid anhydride was used at the beginning of all experiments.
[e] Not isolated.

prompting us to further examine the fragmentation reaction with various acid anhydrides for the scope and limitation.

Although alkyl acid anhydrides effectively act as initiators in the reaction, reaction rate is dependent upon the bulkiness of the acid anhydride used and, thus, the bulkier the alkyl group is, the longer it takes for the conversion to occur while the enantioselectivity is higher (runs 2–4, Table 2). We as well observed that this aziridine preparation method is sensitive to steric demands of not only nucleophiles but also electrophiles; either replacement of benzyl or alkoxycarbonylmethylene groups on the external nitrogen of guanidinium salts to bulkier ones or the use of ketone substrates in place of aldehydes led to nearly no production of aziridine products.[§] These facts suggest that the reaction site of the intermediate adduct is fairly crowded and that the acylation with an acid anhydride is a rate-determining step in this fragmentation sequence.

Aziridine could also be obtained when changing the solvent from chloroform to THF (runs 1 vs 6 and 4 vs 8, Table 2); however, the activity is slightly lowered in THF. In addition di-*t*-butyl dicarbonate was found to trigger the fragmentation reaction (run 9, Table 2).

The oxygen atom in the urea molecule should originate from the aldehyde oxygen of piperonal (**3a**) when SiO_2 is used as an initiator of fragmentation. However, the possibility of the urea oxygen to be delivered from the oxygen atom of Ac_2O could not be completely excluded in the treatment of the intermediate adduct with Ac_2O. Therefore we examined the reaction using diacetyl sulfide (Ac_2S) in place of Ac_2O, in which the alternative product will be thiourea. The fragmentation proceeds rapidly to give *trans*-**6a** in 68% yield with 88% ee together with urea **7** (79% yield) (run 10, Table 2), no thiourea could be detected at the end of the reaction.[¶] From this experiment, we can conclude that the urea oxygen comes from the aldehyde oxygen of piperonal (**3a**) even in the acid anhydride-triggered fragmentation.

The fragmentation reaction may be initiated by the corresponding acid possibly generated by the acylation of the remaining TMG with an acid anhydride. The excess TMG in a reaction mixture should be stoichiometrically calculated to be 0.1 M equiv because of the use of 1.1 M equiv of TMG in our experiments. Thus, the reaction mixture containing an adduct intermediate was treated with 10 M extra of acetic acid (1.1 M equiv) under the same conditions. However, incomplete fragmentation was observed even after 0.5 h,[‖] in contrast to smooth conversion with Ac_2O (0.3 h), strongly indicating that an acid anhydride is a real initiator for the fragmentation reaction. This deduction is supported by a comparable experiment using pivalic acid (2.2 M equiv), in place of pivalic anhydride (run 4 in Table 2), as an initiator, in which longer reaction time (24 h vs 14. 5 h) is needed for the completion of fragmentation.[**] Furthermore, low conversion to aziridine products[††] in the presence of BF_3 Et_2O (2.2 M equiv) even after 41 h suggests that a Lewis acid is not an effective initiator for the fragmentation reaction, too.

[§] For example, although *p*-nitroacetophenone produced an intermediate adduct, reverse reaction to the starting ketone was observed on TLC when treated with Ac_2O for 4 days at room temperature. Aziridine was obtained in only 4% yield and the ketone was recovered in 57% yield.

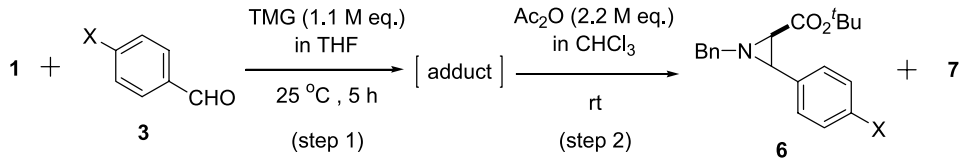
[¶] Urea was identified by spectral data [δ_C 161.8 ppm (CO); *m/z*: 267 ($M+H)^+$]. Precise examination of the reaction mixture obtained in run 10 in Table 2 using Ac_2S led to the isolation of *O*-acetyl-β-aminoalcohol **8**, but no sulfur-containing products, even in minute amount (2%). The same compound **8** was also isolated as a side-product (1%) in the case of the Ac_2O treatment (run 1, Table 2). The corresponding *N*-acetylated product was not obtained.

[‖] The reaction was completed when treated with an aditional amount of AcOH (1.1 M equiv; total 2.2 M equiv) for further 0.5 h, affording *trans*-**6a** [81% (83%ee)] and *cis*-**6a** (7%), respectively.

** *trans*-**6a** [88% (92% ee)] and *cis*-**6a** (8%) were obtained.

†† *trans*-**6a** [17% (84% ee)] and *cis*-**6a** [23% (89% ee)] were obtained.

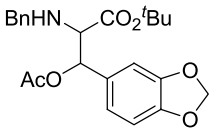
Table 3. Reactions of **1** and various *p*-substituted benzaldehydes (**3b–3j**) under the new conditions



Run	X in **3**	Time (h) in step 2	Total **6** (%)	*trans*-**6** (%)[a] (ee)[b]	*cis*-**6** (%)[a] (ee)[b]	Ratio of *trans*-**6** (%)[c]	**7** (%)[a]	**3** (%)[a]
1	NMe_2 (**3b**)	0.5	—[d]	—	—	—	—	95
2	O^{n}Bu (**3c**)	0.5	67	64 (92)	3 (–)[e]	96	81	29
3	OMe (**3d**)	0.5	81	77 (91)	4 (–)[e]	95	65	12
4	CH_3 (**3e**)	1.5	76	31 (93)	45 (90)	41	90	None
5	H (**3f**)	3	80	22 (88)	58 (86)	28	100	None
6	Cl (**3g**)	17	92	33 (84)	59 (86)	36	—[f]	None
7	CO_2Me (**3h**)	17.5	80	28 (72)	52 (79)	35	69	None
8	CN (**3i**)	17.5	53	35 (32)	18 (16)	66	50	None
9	NO_2 (**3j**)	49	70	41 (11)	29 (10)	59	67	None

[a] Isolated yield.
[b] Determined by chiral HPLC.
[c] (trans-**6**/total **6**)×100.
[d] No aziridine product is formed even with an excess of **3b** (3.5 M equiv). The same result is obtained when *p*-diethylaminobenzaldehyde is used.
[e] Not measured.
[f] Not purified.

Thus, next we investigated, under the modified conditions using the most reactive Ac_2O, the effect on the aziridine synthesis of various substituents in *para* position of the benzaldehyde.



8

2.3. Effect of a substituent of aryl aldehydes

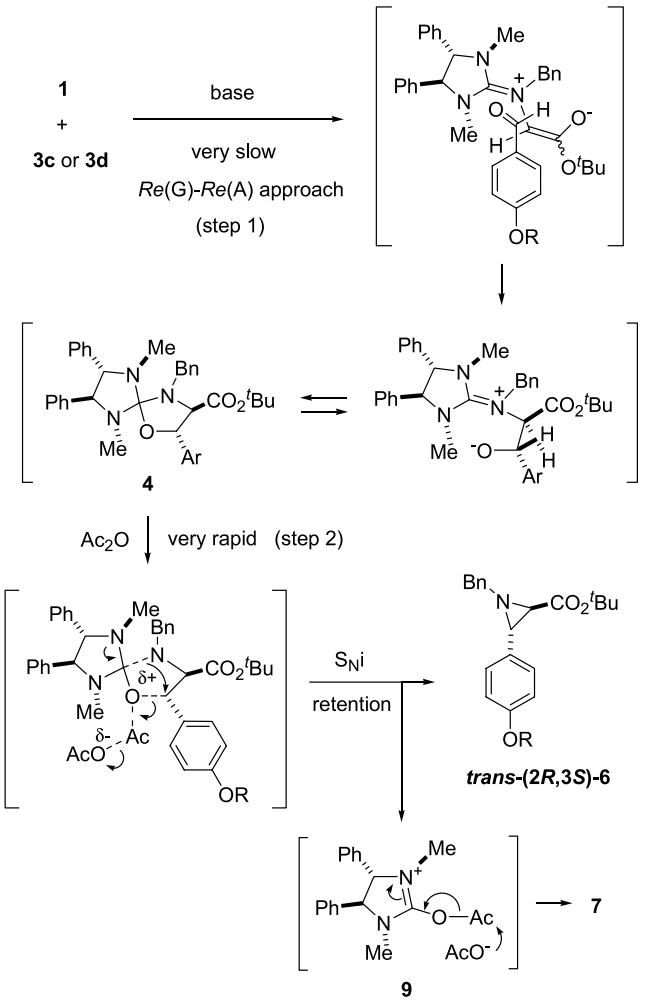
After stirring a mixture of the (*S*,*S*)-guanidinium salt **1** and a benzaldehyde derivative **3** in THF in the presence of TMG at 25 °C for 5 h (step 1), a solution of the intermediate adduct in chloroform is treated with Ac_2O at room temperature until disappearance of the polar intermediate on TLC (step 2). The results are summarized in Table 3.

No reaction occurs when *p*-dimethylaminobenzaldehyde (**3b**) is used, even when an excess (3.5 M equiv) of aldehyde is added (run 1, Table 3). Although in the cases of the alkoxy-substituted benzaldehydes **3c** and **3d** the starting aldehydes remained in step 1, the intermediate adducts were immediately transformed in step 2 (runs 2 and 3, Table 3). Thus, aziridine products **6c** and **6d** were obtained in 67 and 81% yields together with the recovery of the starting benzaldehydes **3c** and **3d** in 29 and 12% yields, respectively, in which the *trans*-derivatives are produced as major aziridine isomers with excellent stereoselectivity in both ee and de. *p*-Tolualdehyde (**3e**) and benzaldehyde (**3f**) smoothly afforded aziridine products **6e** and **6f** with high asymmetric inductions (runs 4 and 5, Table 3). Interestingly, in those cases, the major isomers are the *cis*-derivatives, instead of the *trans*. The nearly same results are obtained in runs 6 and 7 when *p*-chloro- (**3g**) and *p*-methoxycarbonylbenzaldehydes (**3h**) are used. The main difference is the reaction rate of step 2; slow reactions in runs 6 and 7 (about 17 h) compared to runs 4 and 5 (<3 h). On the other hand in the cases of *p*-cyano- (**3i**) and *p*-nitrobenzaldehydes (**3j**), the asymmetric inductions of aziridine products are greatly lowered and, in addition, the predominant formation of *trans*-**6** is again observed (runs 8 and 9, Table 3).

Therefore, these aziridine formation reactions could be categorized into four groups based on the nature of the aldehyde substrates: (i) group A, which includes two benzaldehydes **3c** and **3d** with strong EDG, is characterized by a very slow step 1, a very rapid step 2, and excellent diastereoselectivity and enantioselectivity of *trans*-aziridine; (ii) group B is represented by a slow step 1 and a rapid step 2 with moderate *cis*-diastereoselectivity and excellent to good enantioselectivity in both aziridine products, and contains benzaldehydes **3e** and **3f** which possess a weak EDG or no substituents; (iii) group C shows a similar profile as in group B but with a rapid step 1 and a slow step 2, and comprises benzaldehydes **3g** and **3h** with a weak EWG, and (iv) group D, which includes **3i** and **3j** with a strong EWG, is characterized by a moderate *trans*-diastereoselectivity and low enantioselectivity with a very rapid step 1 and a very slow step 2.

2.4. Reaction mechanisms

We have reported in a preliminary communication[3] that (*S*,*S*)-guanidinium salt **1** produced (2*R*,3*R*)- and (2*R*,3*S*)-3-phenylaziridine-2-carboxylates as *cis*- and *trans*-aziridines after SiO_2 treatment of the intermediate adduct derived from benzaldehyde **3f**. This stereochemical result can be applied to the Ac_2O-triggered reaction because of identical asymmetric induction in both reactions.[‡] The nearly same enantioselectivity in both *cis*- and *trans*-aziridines (see Table 3) indicates that the stereochemistry of products should be strictly controlled in the first C–C bond formation stage (step 1) because of the absence of epimerization of the stereogenic centers between the isomeric aziridines.[3] As



Scheme 4. Mechanistic consideration for the aziridine production in group A.

mentioned above, results in Tables 1 and 2 show that the overall reaction, not only step 1 but also step 2, is controlled by kinetic mode. Thus, we based the reaction mechanisms for the aziridine formation on the four groups categorized above.

Nearly exclusive formation of *trans*-(2*R*,3*S*)-**6** with excellent enantioselectivity in group A with alkoxy-substituents could be explained by the mechanism shown in Scheme 4. An aldehyde approaches to the less hindered *Re*-face [*Re*(G)] of the ylide enolate because the *Si*-face is blocked by the *N*-Me group located in the stable *Z*-configuration of the $C{=}N^{+}$ double bond. Thus, *R* stereochemistry of the stereogenic center in the C–C bond formation (the C-2 of aziridines) should be induced. The step 1 is very slow due to the lowered electrophilicity of the carbonyl function of alkoxy *p*-substituted aldehydes, thus, the less hindered *Re*-face [*Re*(A)] of the aldehyde greatly participates to the bond formation. These situations lead to the production of a sterically stable (4*R*,5*S*)-oxazolidine spiro species **4** with a *trans*-configuration between the ester and the aryl groups. Then, very rapid concerted, S_Ni type, fragmentation can occur with retention at the C-5 benzylic carbon to give *trans*-(2*R*,3*S*)-aziridine.

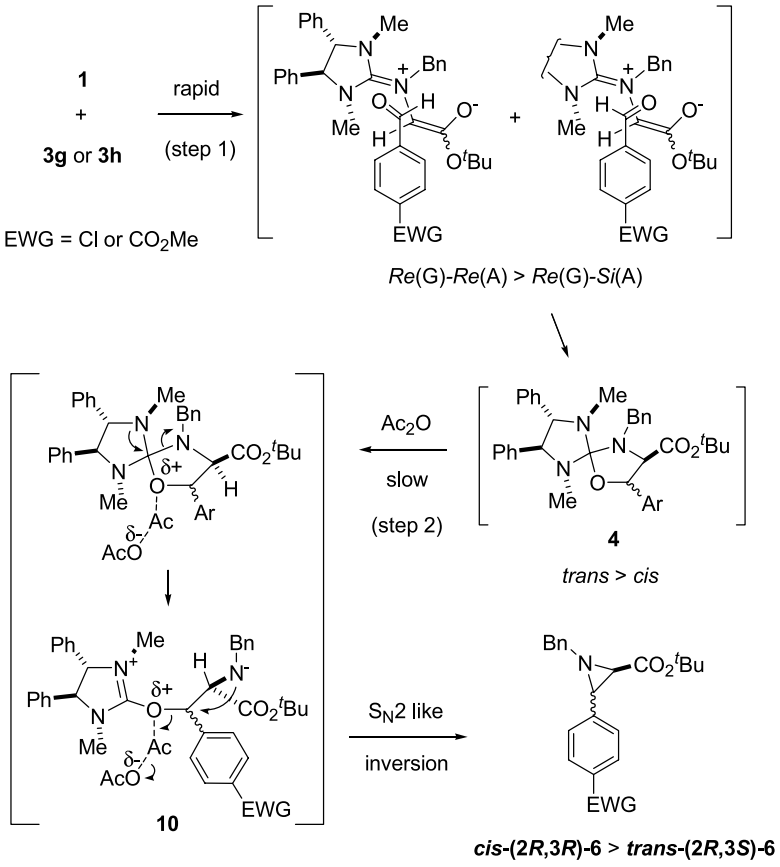
It is reasonable to expect that Ac_2O could coordinate the oxygen atom of the oxazolidine moiety in **4** because of the isolation of *O*-acetyl-β-aminoalcohol **8** as a side product.[¶] The partially cationic character of the oxygen atom in the coordinated species could initiate the fragmentation of the intermediate adduct to *trans*-**6** and a 2-acetoxyamidinium salt **9**,[6] convertible to urea **7**, by simultaneous O–C(Ar) bond cleavage and the N(oxazolidine)–C(Ar) bond formation, in the course of which partially-generated benzyl cationic species could be stabilized by electron-relayed participation of the *p*-substituted alkoxy group. As mentioned in the fragmentation of the piperonal-derived intermediate adduct using various acid anhydrides (see Table 2), the reaction is sensitive to steric demand. Efficient synthesis of *trans*-aziridines with excellent stereoselectivity is due to a judicious combination of activation with a more sterically demanding Ac_2O and the electronic stabilization by the EDG, the former activation acting as a rate-determining step in the fragmentation.[‡‡]

Group C carrying a weak EWG shows moderate diastereoselectivity, but with good enantioselectivity, similar to group B either with a weak EDG or without substituents. For the sake of convenience on discussion, the mechanism of group C (Scheme 5) is considered prior to group B. As mentioned above, the major difference between group B and C is the reaction time needed for the completion of the fragmentation (step 2), a longer time is needed in group C. Since steric environment is all identical around the reaction sites, this fact suggests a different rate-determining step during the fragmentation sequences.

In group C, rapid reaction in step 1 allows an alternative diastereomeric *Re*(G)–*Si*(A) approach in addition to a major *Re*(G)–*Re*(A) approach, resulting in the lowered face selectivity. The oxygen atom of the oxazolidine unit in a diastereomeric mixture of spiro intermediates **4** is similarly activated with Ac_2O; however, the benzyl cationic species supposed in Scheme 4 could not be formed because of the EWG destabilization. Therefore, in this case the cleavage of the C(spiro)–N(oxazolidine) bond involving the nitrogen atom of the imidazolidine unit affords an opened amidinium intermediate **10**, in which an intramolecular S_N2 type reaction occurs, thus, resulting in the predominant production of *cis*-(2*R*,3*R*)-aziridine from a major *trans*-(4*R*,5*S*)-axazolidine spiro species **4** with inversion of the configuration at C-5. This substitution reaction is relatively slow because of a secondary substrate; however, the presence of an EWG accelerates the S_N2 type reaction. The slightly lowered ee of aziridine products could be explained by competitively enantiofacial accesses, such as the *Si*(G)–*Re*(A) approach, in rapid reaction of step 1.

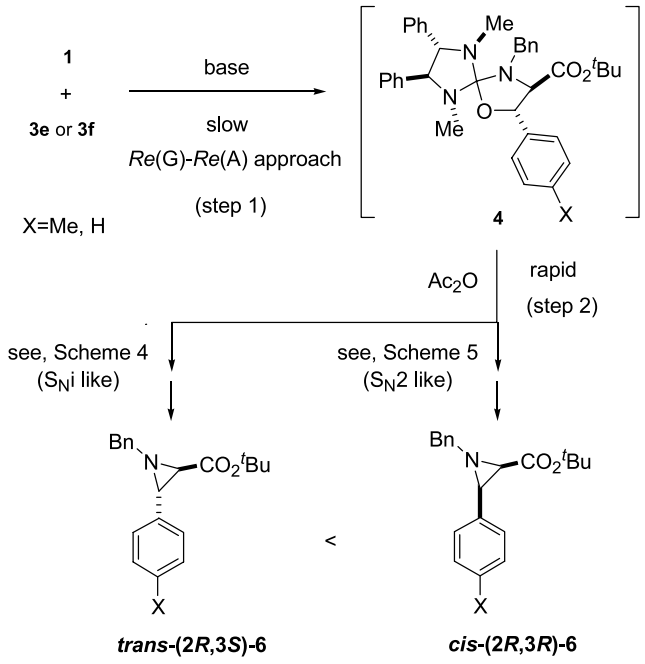
Excellent to good ee observed in group B are explained by the high enantiofacial selectivity of the *Re*(G)–*Re*(A) approach in slow reaction of step 1 as observed in group A (see Scheme 4); however, the lowered diastereoselectivity could not be covered by simple application of the strong

[‡‡] Regeneration of Ac_2O in the final step suggests the possibility of Ac_2O as a catalyst. Actual use of an excess of Ac_2O may be caused by its competitive coordination to other species containing basic nitrogen functions.



Scheme 5. Mechanistic consideration for the aziridine production in group C.

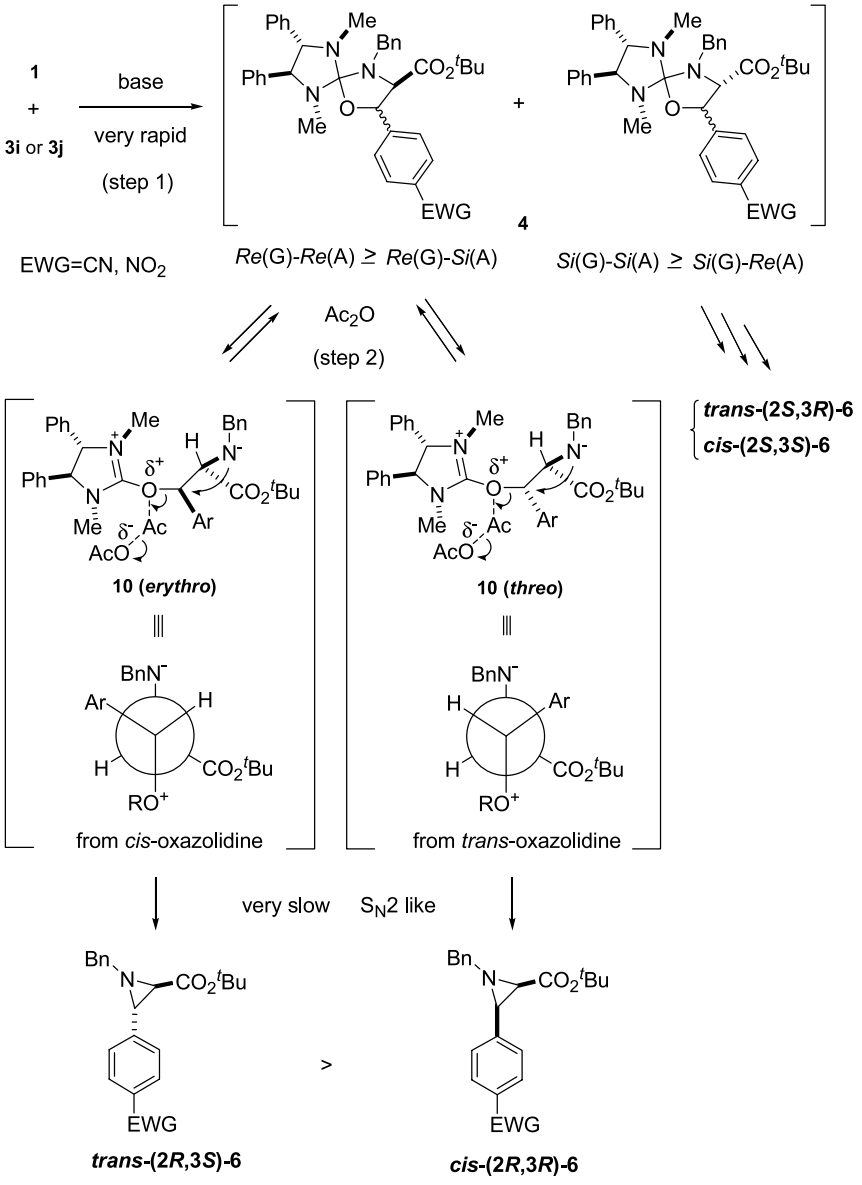
EDG-controlled mechanism proposed for group A. The ratio of *trans*-aziridine diminished in the order of **6d** (95%)≫**6e** (41%)>**6f** (28%) (see runs 3–5, Table 3), which corresponds to a decrease in the electron-donating effect of the *p*-substituents. The reduction of the electron-donating effect and the longer reaction time of step 2 in group B than that in group A allow us to predict the presence of a S_N2 type reaction mechanism. Thus, the contamination of both S_Ni and S_N2 type reactions causes the lower diastereoselectivity in group B (Scheme 6). These speculations can be supported by the fact that a change in mechanism from S_N1 to S_N2 by electronic effect of *p*-substituted benzyl chlorides has been previously reported.[§§] Higher ratio of *trans*-isomer in the case of tolualdehyde (**3e**) compared to benzaldehyde (**3f**) can be rationalized by the ability of the methyl substituent to stabilize a benzyl cationic-like intermediate in the S_Ni type reaction as an EDG.



Scheme 6. Mechanistic consideration for the aziridine production in group B.

In group D with a strong EWG not only the great reduction of stereoselectivity in aziridine production but also the unexpected formation of the *trans*-aziridine can be explained by the mechanism shown in Scheme 7. In a very rapid step 1, four competitive approaches produce all possible isomers with slight differences. On the other hand, in a very slow step 2 amidinium intermediates **10**, substrates of the S_N2 type reaction, are in equilibrium to spiro intermediates **4**, different from the three reactions mentioned above, and a more sterically-favored *anti* conformation **10** (*erythro*) derived from a less stable

[§§] It has been reported that a change in mechanism from S_N1 to S_N2 on a benzylic carbon by electronic substituent effects; see, Swain, C. G.; Langsdorf, W. P., Jr. *J. Am. Chem. Soc.* **1951**, *73*, 2813–2819.



Scheme 7. Mechanistic consideration for the aziridine formation in group D.

cis-oxazolidine preferably act as the substrate for S_N2 type reaction to give *trans*-aziridine.

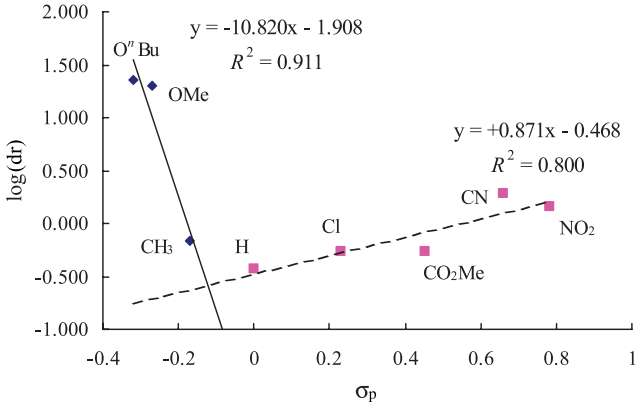


Figure 1. Hammett plot of log(dr) vs σ_p for *p*-substituents of benzaldehydes **3**.

2.5. Hammett studies

As mentioned above, it is clear that substituents on benzaldehydes **3** play a crucial role on the overall reaction of aziridine formation, especially on stereochemical control of the final products. To elucidate the proposed reaction mechanisms, we undertook a Hammett analysis of the diastereoselectivity in aziridine products with substituent constant (σ_p).[7] No equilibrium between *cis*- and *trans*-aziridines allows us to assume that the diastereomeric ratio ($dr = y_{trans}/y_{cis}$) of aziridines reflects the net ratio of their formation rates through the S_Ni and S_N2 type reaction pathways. Since the *trans*-aziridine is mainly afforded as major isomer when conversion rate in step 2 is rapid, the yield of *trans*-aziridine (y_{trans}) is adopted as the numerator of the dr. The logarithm of the dr [log(dr)] was used for these plots because it is directly correlated to the free energy difference between the transition states of the above pathways.[8]

The plot of log(dr) versus the corresponding σ_p reveals two natural groupings depending upon the substituents on the benzaldehyde substrates **3** (Fig. 1). The lines obtained show a good correlation ($R^2=0.911$ for EDGs, $R^2=0.800$ for EWGs) in electronically analogous categories with opposite slopes in sign. Within the EDGs, the log(dr) decreases as the electron-donating effect decreases (the value of σ_p increases), giving a negative slope line with relatively large magnitude (−10.820). The large negative magnitude indicates that the EDG-substituents stabilize a sizable positive charge at the benzylic position[9] developed by the initial coordination of Ac_2O to the oxygen atom of the oxazolidine ring in a spiro intermediate adduct **4** in the transition state of the rate-determining step and leads to high diastereoselectivity in the aziridine products. Thus, the S_Ni type mechanism proposed for the EDG-participated aziridine formation as shown in Scheme 4 can be reasonably supported.

A line with negative slope of relatively small magnitude, leading to a plotting curve with concave upwards,[9] will be expected for the longer reaction time needed for completion in step 2 as the electron-withdrawing ability of the EWGs increases (the value of σ_p also increases). Actually, the log(dr) gradually increases to give a line with positive slope of relatively small magnitude (+0.871). The positive slope can be accounted for the S_N2 type mechanism proposed for the EWG-participated aziridine formation, as shown in Scheme 5, in which the generation of *cis*- and *trans*-aziridines is controlled by diastereofacial selectivity in the formation of spiro intermediate adduct **4** (step 1).

Among the substituents tested, the increase in the electron-withdrawing effect led a gradual diminishment of the ee in the cases of the benzaldehydes **3e–h** with either a weak EDG or a weak EWG and then a dramatic diminishment in the case of benzaldehydes **3i–j** with a strong EWG. This trend allows us to consider another Hammett analysis of the enantioselectivity of the aziridine products to σ_p.[7] The enantiomeric ratio (er)[¶¶] of *trans*-**6** will reflect the net ratio of formation rates between the (2*R*,3*S*)- and (2*S*,3*R*)-aziridines through the possible four approaches in step 1. The logarithm of er [log(er)] is used for these plots because it relates directly to the free energy differences between the diastereomeric transition states.[8] The plot of the log(er) versus the corresponding σ_p leads to two groups of data, a left-hand and a right-hand side (Fig. 2).

The lines obtained show a good-to-excellent correlation ($R^2=0.923$ for the left-hand side, $R^2=0.991$ for the right-hand side) with slightly different negative slopes. For the left-hand side of the plot, the log(er) gradually decreases as the value of σ_p increases, giving a line with a negative slope of relatively small magnitude (−0.764), whereas for the right-hand side of the plot, the log(er) decreases dramatically as the electron-withdrawing ability of the substituents became stronger (the value of σ_p increases), giving a line with negative slope of relatively large magnitude (−2.098). These facts indicate that the C–C bond formation step in the latter case is more electronically controlled, as expected from the low face-selectivity at the rapid addition reaction with reactive aldehydes.

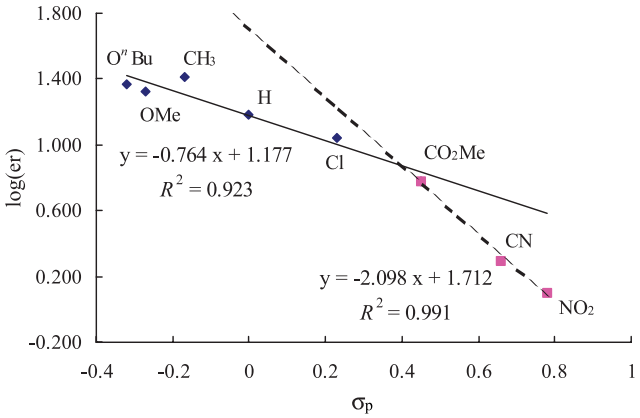


Figure 2. Hammett plot of log(er) in *trans*-**6** versus σ_p for *p*-substituted benzaldehydes **3**.

It should be noted here that the shift of the inflection point, (+0.401 on σ_p) in Figure 2 compared to (−0.123 on σ_p) in Figure 1, clearly reveals that the C–C bond formation in step 1 and the intramolecular nucleophilic substitution in step 2 are independently influenced by electronic effect of the *p*-substitutents of the benzaldehyde substrates.

3. Conclusions

Based on the Ac_2O treatment of the intermediate adduct, two kinds of reaction mechanisms controlled by the nature of the *p*-substituents of aryl aldehydes are proposed for asymmetric aziridine synthesis from guanidinium ylide and aryl aldehydes. A S_Ni-like mechanism via cationic-like transition state is proposed for the asymmetric synthesis using EDG-substituted benzaldehydes, whereas with EWG-substituted benzaldehydes, a S_N2-like mechanism is proposed. Since the SiO_2 treatment of some intermediate adducts show identical diastereo- and enantioselectivity that the Ac_2O treatment, we can assume that the SiO_2 treatment proceeds as well through these two mechanisms by coordination of SiO_2 to the oxazolidine-oxygen atom in the spiro intermediate adduct. Hammett analysis, based on the diastereomeric ratio in the aziridine products, is consistent with the proposed rate-determining steps in these two mechanisms. A second Hammett analysis, based on the enantiomeric ratio of the aziridine products, clearly reveals the difference in the susceptibilities to the electronic substituents effect between the nucleophilic addition (step 1) and the intramolecular nucleophilic substitution (step 2).

We have recognized that vinyl aldehyde derivatives can as well act as electrophiles.[‖‖] Further studies on the scope and limitation of this aziridine synthesis and its application to the synthesis of bioactive nitrogen-containing compounds are at present under way in our laboratory.

[¶¶] The enantiomeric ratio (er) is defined here as the relative amount of the major enantiomer divided by the relative amount of the minor enantiomer (e.g., 80% ee gives an er of 9.0); see Ref. 8.

[‖‖] These works will be published elsewhere in the near future.

4. Experimental

4.1. General

^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in $CDCl_3$ on JEOL JNM-ALPHA 400 or JEOL JNM-AL 400 spectrometers. HPLC was performed on Shimadzu 10A instruments using Daicel Chiralcel OD or OD-H and Chiralpak AD-H columns (4.6 mm×25 cm). For TLC, Merck precoated TLC plates (silica gel 60 F_{254}) were used. The products were purified by column chromatography on silica gel (FL-100D; Fuji Silysia Chemical Ltd.). Low (FABMS) and high-resolution FABMS (HRFABMS) were performed on JEOL JMS-HX 110A and JMS-700-T spectrometers. Dry tetrahydrofuran (THF) was purchased from Kanto Chemical Co. Inc. as 'Dehydrated'. 1,1,3,3-Tetramethylguanidine (TMG) was distilled under reduced pressure prior to use.

4.2. General procedure for the formation of chiral aziridines

Treatment with SiO_2. A solution of TMG (25 μL, 0.202 mM) in THF (35 μL) was dropwise added to a solution of the (*S*,*S*)-guanidinium salt **1** (101 mg, 0.184 mM) and a benzaldehyde **3** (0.180 mM) in THF (365 μL) at 25 °C. The mixture was stirred at 25 °C for 4–5 h under nitrogen atmosphere and then SiO_2 (3.0 g) was added after dilution with $CHCl_3$ (10 mL). The resulting slurry was stirred at room temperature until disappearance of a highly polar intermediate on TLC (ethyl acetate/hexane). After removal of the SiO_2 by filtration, filter cakes were successively washed with $CHCl_3$ (36 mL) and CH_3CN (10 mL). The filtrate and the washings were combined. After evaporation, the residue was purified by column chromatography on SiO_2 (ethyl acetate/hexane as eluent) to give *trans*- *trans*-**6** and *cis*-aziridines *cis*-**6**.

Treatment with carboxylic anhydrides. A solution of TMG (25 μL, 0.202 mM) in THF (35 μL) was dropwise added to a solution of **1** (101 mg, 0.184 mM) and a benzaldehyde **3** (0.180 mM) in THF (365 μL) at 25 °C. The mixture was stirred at 25 °C for 5 h under nitrogen atmosphere. After evaporation of THF with a stream of nitrogen, $CHCl_3$ (10 mL) was added. After addition of carboxylic anhydride (0.405 mM) the mixture was stirred at room temperature until disappearance of a highly polar intermediate on TLC. Evaporation of the solvent followed by purification by column chromatography on SiO_2 (ethyl acetate/hexane as eluent) afforded *trans*-**6** and *cis*-**6**.

Treatment with pivalic acid. A highly polar intermediate was prepared from **1** and piperonal (**3a**) in the same manner described above. A solution of pivalic acid (41.3 mg, 0.405 mM) in $CHCl_3$ (1.0 mL) was added to a solution of the intermediate in $CHCl_3$ (9.0 mL). The mixture was stirred at room temperature for 24 h, washed with saturated $NaHCO_3$ (3.0 mL) and the upper aqueous layer was re-extracted with $CHCl_3$ (2.0 mL×4). The combined organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on SiO_2 using 3–20% ethyl acetate in hexane to give *trans*-**6a** and *cis*-**6a** and urea **7**.

4.2.1. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(3,4-methylenedioxyphenyl)aziridine (6a). *trans*-**6a**. ^{1}H NMR δ 7.36 (2H, d, J=7.1 Hz, Ar-H), 7.30–7.19 (3H, m, Ar-H), 6.79 (1H, dd, J=7.9, 1.5 Hz, Ar-H), 6.75 (1H, s, Ar-H), 6.73 (1H, d, J=7.9 Hz, Ar-H), 5.92 (2H, s, OCH_2O), 4.22 (1H, d, J=13.9 Hz, CH_aH_bPh), 4.06 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.19 (1H, brs, C_3–H), 2.64 (1H, d, J=2.2 Hz, C_2–H), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.5, 147.6, 146.8, 139.2, 132.4, 128.0, 127.9, 126.6, 119.8, 107.9, 106.2, 100.8, 81.6, 54.5, 47.9, 45.5, 28.0. Anal. Calcd for $C_{21}H_{22}NO_4$ C, 71.37; H, 6.56; N, 3.96. Found: C, 71.04; H, 6.55; N, 3.92; HPLC analysis: Daicel Chiralcel OD, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 6.3 min (minor isomer) and 7.1 min (major isomer).

cis-**6a**. ^{1}H NMR δ 7.41 (2H, d, J=7.3 Hz, Ar-H), 7.34–7.30 (2H, m, Ar-H), 7.26–7.23 (1H, m, Ar-H), 6.92 (1H, d, J=1.5 Hz, Ar-H), 6.85 (1H, dd, J=8.1, 1.5 Hz, Ar-H), 6.70 (1H, d, J=8.1 Hz, Ar-H), 5.90 (2H, s, OCH_2O), 3.94 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.54 (1H, d, J=13.9 Hz, CH_aH_bPh), 2.92 (1H, d, J=6.8 Hz, C_3–H), 2.48 (1H, d, J=6.8 Hz, C_2–H), 1.24 (9H, s, $OC(CH_3)_3$); HPLC analysis: Daicel Chiralpak AD-H, mobile phase: hexane/2-propanol=50:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 15.5 min (major isomer) and 17.9 min (minor isomer).

4.2.2. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(4-butoxyphenyl)aziridine (6c) (run 2 in Table 2). *trans*-**6c**. (64% yield, 92% ee); ^{1}H NMR δ 7.37 (2H, d, J=7.3 Hz, Ar-H), 7.30–7.18 (5H, m, Ar-H), 6.82 (2H, d, J=8.5 Hz, Ar-H), 4.24 (1H, d, J=13.9 Hz, CH_aH_bPh), 4.07 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.94 (2H, t, J=6.5 Hz, CH_2), 3.20 (1H, brs, C_3–H), 2.67 (1H, d, J=2.2 Hz, C_2–H), 1.78–1.71 (2H, m, CH_2), 1.52–1.43 (2H, m, CH_2), 1.39 (9H, s, $OC(CH_3)_3$), 0.97 (3H, t, J=7.3 Hz, CH_3); ^{13}C NMR δ 167.8, 158.6, 139.4, 130.3, 128.1, 127.9, 127.3, 126.6, 114.4, 81.6, 67.8, 54.6, 47.8, 45.5, 31.3, 28.0, 19.3, 13.9; HRFABMS m/z 382.2405, calcd for $C_{24}H_{32}NO_3$ 382.2382; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=400:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 18.7 min (minor isomer) and 20.6 min (major isomer).

cis-**6c**. (2.8% yield); ^{1}H NMR δ 7.42 (2H, d, J=7.3 Hz, Ar-H), 7.32–7.22 (5H, m, Ar-H), 6.79 (2H, d, J=8.8 Hz, Ar-H), 3.94 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.92 (2H, t, J=6.6 Hz, CH_2), 3.57 (1H, d, J=13.9 Hz, CH_aH_bPh), 2.94 (1H, d, J=6.8 Hz, C_3–H), 2.48 (1H, d, J=7.1 Hz, C_2–H), 1.77–1.70 (2H, m, CH_2), 1.56–1.42 (2H, m, CH_2), 1.20 (9H, s, $OC(CH_3)_3$), 0.94 (3H, t, J=7.3 Hz, CH_3).

4.2.3. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(4-methoxyphenyl)aziridine (6d) (run 3 in Table 2). *trans*-**6d**. (77% yield, 91% ee); ^{1}H NMR δ 7.37 (2H, d, J=7.3 Hz, Ar-H), 7.30–7.20 (5H, m, Ar-H), 6.84 (2H, d, J=8.3 Hz, Ar-H), 4.24 (1H, d, J=13.9 Hz, CH_aH_bPh), 4.08 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.79 (3H, s, OCH_3), 3.21 (1H, brs, C_3–H), 2.67 (1H, d, J=2.2 Hz, C_2–H), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.7, 159.0, 139.3, 130.5, 128.1, 127.9, 127.3, 126.7, 113.7, 81.6, 55.3, 54.6, 47.8, 45.5, 28.0; HRFABMS m/z 340.1885, calcd for $C_{21}H_{26}NO_3$ 340.1913; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=0.5 mL/min, detection

254 nm, retention time: 14.1 min (minor isomer) and 15.2 min (major isomer).

cis-**6d**. (3.8% yield); ^{1}H NMR δ 7.43 (2H, d, J=7.6 Hz, Ar-H), 7.33–7.21 (5H, m, Ar-H), 6.80 (2H, d, J=8.8 Hz, Ar-H), 3.96 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.77 (3H, s, OCH_3), 3.56 (1H, d, J=13.9 Hz, CH_aH_bPh), 2.95 (1H, d, J=6.8 Hz, C_3–H), 2.49 (1H, d, J=6.8 Hz, C_2–H), 1.20 (9H, s, $OC(CH_3)_3$).

4.2.4. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(*p*-tolyl)aziridine (6e) (run 4 in Table 2). *trans*-**6e**. (31% yield, 93% ee); ^{1}H NMR δ 7.37 (2H, d, J=7.3 Hz, Ar-H), 7.31–7.18 (5H, m, Ar-H), 7.11 (2H, d, J=7.6 Hz, Ar-H), 4.24 (1H, d, J=13.9 Hz, CH_aH_bPh), 4.08 (1H, d, J=13.9 Hz, CH_aH_b-Ph), 3.22 (1H, brs, C_3–H), 2.69 (1H, d, J=2.7 Hz, C_2–H), 2.32 (3H, s, CH_3), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.7, 139.3, 137.0, 135.4, 128.9, 128.1, 127.9, 126.7, 126.1, 81.6, 54.7, 48.0, 45.7, 28.0, 21.2. HRFABMS m/z 324.1945, calcd for $C_{21}H_{26}NO_2$ 324.1964. HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=1000:0.5, flow rate=1.0 mL/min, detection 254 nm, retention time: 22.8 min (minor isomer) and 25.3 min (major isomer).

cis-**6e**. (45% yield, 90% ee); ^{1}H NMR δ 7.43 (2H, d, J=7.6 Hz, Ar-H), 7.43–7.24 (5H, m, Ar-H), 7.06 (2H, d, J=7.8 Hz, Ar-H), 3.96 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.56 (1H, d, J=13.9 Hz, CH_aH_bPh), 2.96 (1H, d, J=7.0 Hz, C_3–H), 2.50 (1H, d, J=7.0 Hz, C_2–H), 2.30 (3H, s, CH_3), 1.19 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.1, 137.9, 136.6, 132.0, 128.2, 128.1, 127.6, 126.9, 81.0, 63.3, 47.1, 46.7, 27.7, 21.1. HRFABMS m/z 324.1970, calcd for $C_{21}H_{26}NO_2$ 324.1964. HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 8.7 min (minor isomer) and 11.0 min (major isomer).

4.2.5. 1-Benzyl-2-*tert*-butoxycarbonyl-3-phenylaziridine (6f) (run 5 in Table 2). *trans*-**6f**. (22% yield, 88% ee); ^{1}H NMR δ 7.38 (2H, d, J=7.3 Hz, Ar-H), 7.31–7.20 (9H, m, Ar-H), 4.26 (1H, d, J=14.2 Hz, CH_aH_bPh), 4.10 (1H, d, J=14.2 Hz, CH_aH_bPh), 3.26 (1H, brs, C_3–H), 2.72 (1H, d, J=2.7 Hz, C_2–H), 1.39 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.6, 139.3, 138.4, 128.3, 128.1, 127.9, 127.4, 126.7, 126.2, 81.7, 54.6, 48.1, 45.8, 28.0. Anal. Calcd for $C_{20}H_{23}NO_2$: C, 77.64; H, 7.49; N, 4.53. Found: C, 77.23; H, 7.51; N, 4.45; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=250:1, flow rate=0.5 mL/min, detection 254 nm, retention time: 13.7 min (minor isomer) and 14.5 min (major isomer).

cis-**6f**. (58% yield, 86% ee); ^{1}H NMR δ 7.46–7.39 (4H, m, Ar-H), 7.34–7.19 (6H, m, Ar-H), 3.97 (1H, d, J=13.8 Hz, CH_aH_bPh), 3.59 (1H, d, J=13.8 Hz, CH_aH_bPh), 3.00 (1H, d, J=7.0 Hz, C_3–H), 2.53 (1H, d, J=7.0 Hz, C_2–H), 1.16 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.0, 137.9, 135.2, 128.3, 127.8, 127.6, 127.1, 127.0, 81.1, 63.3, 47.2, 46.8, 27.8. Anal. Calcd for $C_{20}H_{23}NO_2$: C, 77.64; H, 7.49; N, 4.53. Found: C, 77.69; H, 7.57; N, 4.62; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 10.1 min (minor isomer) and 11.9 min (major isomer).

4.2.6. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(4-chlorophenyl)-aziridine (6g) (run 6 in Table 2). *trans*-**6g**. (33% yield, 84% ee); ^{1}H NMR δ 7.36 (2H, d, J=7.3 Hz, Ar-H), 7.31–7.21 (7H, m, Ar-H), 4.23 (1H, d, J=14.0 Hz, CH_aH_bPh), 4.08 (1H, d, J=14.0 Hz, CH_aH_bPh), 3.22 (1H, brs, C_3–H), 2.67 (1H, d, J=2.7 Hz, C_2–H), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.3, 139.1, 137.0, 133.1, 128.4, 128.2, 127.9, 127.6, 126.8, 81.9, 54.6, 47.2, 45.9, 28.0; HRFABMS m/z 344.1406, calcd for $C_{20}H_{23}ClNO_2$: 344.1417; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=300:1, flow rate=0.3 mL/min, detection 254 nm, retention time: 24.6 min (minor isomer) and 26.4 min (major isomer).

cis-**6g**. (59% yield, 86% ee); ^{1}H NMR δ 7.41 (2H, d, J=7.3 Hz, Ar-H), 7.35–7.22 (7H, m, Ar-H), 3.97 (1H, d, J=13.9 Hz, CH_aH_bPh), 3.56 (1H, d, J=13.9 Hz, CH_aH_bPh), 2.95 (1H, d, J=6.8 Hz, C_3–H), 2.54 (1H, d, J=6.8 Hz, C_2–H), 1.20 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 166.7, 137.7, 133.8, 132.9, 129.2, 128.3, 127.8, 127.7, 127.1, 81.3, 63.2, 46.9, 46.5, 27.8. Anal. Calcd for $C_{20}H_{22}ClNO_2$: C, 69.86; H, 6.45; N, 4.07. Found: C, 69.52; H, 6.44; N, 4.00; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=500:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 27.7 min (major isomer) and 30.4 min (minor isomer).

4.2.7. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(4-methoxycarbonylphenyl)aziridine (6h) (run 7 in Table 2). *trans*-**6h**. (28% yield, 72% ee); ^{1}H NMR δ 7.97 (2H, d, J=8.1 Hz, Ar-H), 7.37 (2H, d, J=8.1 Hz, Ar-H), 7.38–7.21 (5H, m, Ar-H), 4.25 (1H, d, J=13.8 Hz, CH_aH_bPh), 4.10 (1H, d, J=13.8 Hz, CH_aH_bPh), 3.90 (3H, s, $COOCH_3$), 3.29 (1H, brs, C_3–H), 2.73 (1H, d, J=2.2 Hz, C_2–H), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 167.2, 166.8, 143.8, 139.0, 129.6, 129.2, 128.2, 127.9, 126.8, 126.2, 82.0, 54.6, 52.1, 47.5, 46.2, 28.0; HRFABMS m/z 368.1852, calcd for $C_{22}H_{26}NO_4$: 368.1862; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 7.6 min (minor isomer) and 8.9 min (major isomer).

cis-**6h**. (52% yield, 79% ee); ^{1}H NMR δ 7.94 (2H, d, J=8.5 Hz, Ar-H), 7.48 (2H, d, J=8.1 Hz, Ar-H), 7.43 (2H, d, J=7.3 Hz, Ar-H), 7.35–7.24 (3H, m, Ar-H), 3.98 (1H, d, J=13.8 Hz, CH_aH_bPh), 3.89 (3H, s, $COOCH_3$), 3.60 (1H, d, J=13.8 Hz, CH_aH_bPh), 3.02 (1H, d, J=7.1 Hz, C_3–H), 2.59 (1H, d, J=7.1 Hz, C_2–H), 1.16 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 166.9, 166.6, 140.6, 137.7, 129.0, 128.9, 128.3, 127.9, 127.8, 127.2, 81.4, 63.2, 52.0, 47.1, 46.8, 27.8; HRFABMS m/z 368.1870, calcd for $C_{22}H_{26}NO_4$: 368.1862; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 21.4 min (major isomer) and 25.3 min (minor isomer).

4.2.8. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(4-cyanophenyl)-aziridine (6i) (run 8 in Table 2). *trans*-**6i**. (35% yield, 32% ee); ^{1}H NMR δ 7.58 (2H, d, J=8.3 Hz, Ar-H), 7.40 (2H, d, J=8.3 Hz, Ar-H), 7.37–7.21 (5H, m, Ar-H), 4.23 (1H, d, J=13.9 Hz, CH_aH_bPh), 4.09 (1H, d, J=13.9 Hz, CH_aH_b-Ph), 3.29 (1H, brs, C_3–H), 2.70 (1H, d, J=2.4 Hz, C_2–H), 1.40 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 166.8, 144.1, 138.7, 132.1, 128.2, 127.9, 127.0, 118.8, 111.1, 82.3, 54.5, 47.0,

46.5, 28.0; HRFABMS *m/z* 335.1729, calcd for $C_{21}H_{23}N_2O_2$: 335.1760; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=400:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 41.4 min (minor isomer) and 48.6 min (major isomer).

cis-**6i**. (18% yield, 16% ee); ^{1}H NMR δ 7.56 (2H, d, J=8.3 Hz, Ar-H), 7.52 (2H, d, J=8.3 Hz, Ar-H), 7.41 (2H, d, J=7.3 Hz, Ar-H), 7.35–7.25 (3H, m, Ar-H), 4.01 (1H, d, J=13.7 Hz, CH_aH_bPh), 3.56 (1H, d, J=13.7 Hz, CH_aH_b-Ph), 3.01 (1H, d, J=7.1 Hz, C_3–H), 2.63 (1H, d, J=7.1 Hz, C_2–H), 1.18 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 166.3, 140.9, 137.4, 131.5, 128.7, 128.4, 127.8, 127.3, 118.9, 110.9, 81.6, 63.1, 47.2, 46.5, 27.8; HRFABMS *m/z* 335.1758, calcd for $C_{21}H_{23}N_2O_2$: 335.1760; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 26.8 min (major isomer) and 30.8 min (minor isomer).

4.2.9. 1-Benzyl-2-*tert*-butoxycarbonyl-3-(4-nitrophenyl)-aziridine (6j) (run 9 in Table 2).. *trans*-**6j**. (41% yield, 11% ee); ^{1}H NMR δ 8.15 (2H, d, J=8.8 Hz, Ar-H), 7.45 (2H, d, J=8.8 Hz, Ar-H), 7.36 (2H, d, J=7.6 Hz, Ar-H), 7.30 (2H, dd, J=7.6, 7.6 Hz, Ar-H), 7.25–7.22 (1H, m, Ar-H), 4.24 (1H, d, J=13.8 Hz, CH_aH_bPh), 4.10 (1H, d, J=13.8 Hz, CH_aH_bPh), 3.34 (1H, brs, C_3–H), 2.73 (1H, d, J=2.4 Hz, C_2–H), 1.41 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 166.7, 147.2, 146.1, 138.7, 128.3, 127.9, 127.1, 127.0, 123.5, 82.4, 54.5, 46.8, 46.7, 28.0; HRFABMS *m/z* 355.1627, calcd for $C_{20}H_{23}N_2O_4$: 355.1658; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=400:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 37.4 min (minor isomer) and 43.0 min (major isomer).

cis-**6j**. (29% yield, 10% ee); ^{1}H NMR δ 8.13 (2H, d, J=8.8 Hz, Ar-H), 7.57 (2H, d, J=8.8 Hz, Ar-H), 7.41 (2H, d, J=7.3 Hz, Ar-H), 7.35–7.25 (3H, m, Ar-H), 4.03 (1H, d, J=13.7 Hz, CH_aH_bPh), 3.57 (1H, d, J=13.7 Hz, CH_aH_b-Ph), 3.05 (1H, d, J=6.8 Hz, C_3–H), 2.66 (1H, d, J=6.8 Hz, C_2–H), 1.18 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 166.2, 147.1, 142.9, 137.3, 128.8, 128.4, 127.8, 127.4, 122.9, 81.7, 63.1, 47.3, 46.3, 27.9; HRFABMS *m/z* 355.1667, calcd for $C_{20}H_{23}N_2O_4$: 355.1658; HPLC analysis: Daicel Chiralcel OD-H, mobile phase: hexane/2-propanol=100:1, flow rate=1.0 mL/min, detection 254 nm, retention time: 23.2 min (minor isomer) and 25.0 min (major isomer).

4.2.10. *tert*-Butyl 3-acetoxy-3-benzo[1,3]dioxol-5-yl-2-benzylaminopropionate (9): In treatment with diacetyl sulfide (run 11 in Table 1). A highly polar intermediate was prepared from **1** and piperonal (**3a**) in the same manner described above. The solvent was evaporated with a stream of nitrogen and diacetyl sulfide (0.405 mM) was added after dilution with $CHCl_3$ (10 mL). The mixture was stirred at room temperature for 30 min, washed with saturated $NaHCO_3$ (2.5 mL) and brine (2.0 mL), dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography on SiO_2 using 3–20% ethyl acetate in hexane followed by preparative TLC (ethyl acetate/hexane=1:4 as eluent) to give **9** (1.6 mg): ^{1}H NMR δ 7.30–7.19 (5H, m, Ph), 6.83 (1H, d, J=1.5 Hz, Ar-H), 6.80 (1H, dd, J=7.9, 1.5 Hz, Ar-H), 6.74 (1H, d, J=7.9 Hz, Ar-H), 5.96 (2H, s, OCH_2O), 5.78 (1H, d, J=7.2 Hz, CHOCO), 3.82 (1H, d, J=13.4 Hz, CH_aH_bPh), 3.63 (1H, d, J=13.4 Hz, CH_aH_bPh), 3.48 (1H, d, J=7.2 Hz, CHCOO), 2.03 (1H, s, $COCH_3$), 1.45 (9H, s, $OC(CH_3)_3$); ^{13}C NMR δ 171.3, 169.5, 147.5, 139.5, 131.0, 128.3, 128.2, 127.0, 121.4, 107.9, 107.8, 101.1, 81.8, 75.9, 65.0, 51.9, 28.0, 21.0. FABMS *m/z*: 414 (MH^+).

4.2.11. Isolation of a mixture of adduct intermediates in the reaction of 1 and *p*-chlorobenzaldehyde (3g). The highly polar intermediate was prepared from **1** and *p*-chlorobenzaldehyde (**3g**) in the same manner described above. Evaporation of solvent and purification of the residue (214 mg) by short column chromatography on SiO_2 (ethyl acetate/hexane=1:5) gave a ca. 5:1 mixture of adduct intermediates (104 mg) as an oil.

The major isomer. ^{1}H NMR δ 5.18 (1H, d, J=8.5 Hz), 4.52 (1H, d, J=15.0 Hz), 4.06 (1H, d, J=8.1 Hz), 3.90 (1H, d, J=15.0 Hz), 3.83 (1H, d, J=8.1 Hz), 3.75 (1H, d, J=8.5 Hz), 2.52 (3H, s), 2.33 (3H, s), 1.16 (9H, s).

The minor isomer. ^{1}H NMR δ 5.61 (d, J=8.4 Hz), 4.46 (d, J=13.9 Hz), 4.14 (d, J=7.3 Hz), 4.10 (d, J=13.8 Hz), 4.05 (d, J=7.3 Hz), 3.96 (d, J=8.4 Hz), 2.72 (3H, s), 2.49 (3H, s), 0.79 (9H, s). Aromatic protons of both isomers were observed at δ 7.46–7.00 as complex signals.

4.3. Hammett analyses

Diastereomeric (dr) and enantiomeric ratios (er) calculated based on the data in Table 3 and substituent constants (σ_p) are summarized in the following Table 4.

Table 4. The dr, er and σ_p values for Hammett analyses

No.	X in **3**	dr (*trans*/*cis*)[a]	log(dr)	er (*major*/*minor*)[b]	log(er)	σ_p[c]
1	O^{n}Bu	22.857	1.359	23.415	1.369	−0.32
2	OMe	20.184	1.305	21.222	1.327	−0.27
3	Me	0.682	−0.167	26.027	1.415	−0.17
4	H	0.375	−0.425	15.393	1.187	0
5	Cl	0.555	−0.256	11.060	1.044	0.23
6	CO_2Me	0.541	−0.267	6.049	0.782	0.45
7	CN	1.966	0.294	1.950	0.290	0.66
8	NO_2	1.443	0.159	1.257	0.099	0.78

[a] dr=(yield of *trans*-**6**)/(yield of *cis*-**6**).
[b] er=[(the relative amount of the major enantiomer)/(the relative amount of the minor enantiomer)] in *trans*-**6**.[8]
[c] All substituent constants were taken from Ref. 7.

Acknowledgements

This work is supported by a Grant-in-Aid for Scientific Research (14370717) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and notes

1. (a) Aube, J. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 1, p 835. (b) Rosen, T. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 2, p 428. (c) Kemp, J. E. G. In Trost, B. M., Fleming, I., Eds.; Comprehensive Organic Synthesis; Pergamon: Oxford, 1991; Vol. 7, p 469. (d) Tanner, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 599–619. (e) Osborn, H. M. I.; Sweeney, J. *Tetrahedron: Asymmetry* **1997**, *18*, 1693–1715. (f) Atkinson, R. S. *Tetrahedron* **1999**, *55*, 1519–1559. (g) Mitchinson, A.; Nadin, A. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2862–2892.
2. Cardillo, G.; Gentilucci, L.; Tolomelli, A. *Aldrichim. Acta* **2003**, *36*, 39–50.
3. Hada, K.; Watanabe, T.; Isobe, T.; Ishikawa, T. *J. Am. Chem. Soc.* **2001**, *123*, 7705–7706.
4. Isobe, T.; March, J. *March's Advanced Organic Chemistry* 3rd ed.; Wiley: New York, 2001; pp 1231–1237.
5. Smith, M. B.; March, J. *March's Advanced Organic Chemistry* 3rd ed.; Wiley: New York, 2001; pp 1247–1248.
6. Smith, M. B.; Ishikawa, T. *J. Org. Chem.* **1999**, *64*, 5832–5835 see also pp 6984–6988 and 6989–6992.
7. Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165–195.
8. Constantine, R. N.; Kim, N.; Bunt, R. C. *Org. Lett.* **2003**, *5*, 2279–2282.
9. Sykes, P. *A Guidebook to Mechanism in Organic Chemistry*, 5th ed.; Longman: London, 1981; Chapter 13, pp 351–369.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2871–2877

A new route to (±)-*erythro*-roccellic acid and chaetomellic anhydride C through functional rearrangement, promoted by *n*-propylamine or CH_3ONa/CH_3OH, of *N*-propyl-3-chloro-4-dichloromethyl-3-dodecylpyrrolidin-2-one

Laurent De Buyck,[a] Chiara Danieli,[b] Franco Ghelfi,[b,*] Ugo M. Pagnoni,[b] Andrew F. Parsons,[c] Mariella Pattarozzi[b,†] and Fabrizio Roncaglia[b]

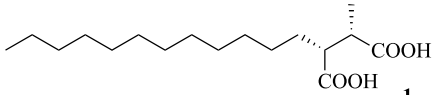
[a]*Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, University of Gent, Coupure Links 653, B-9000 Gent, Belgium*
[b]*Dipartimento di Chimica, Università degli Studi di Modena e Reggio Emilia, via Campi 183, 41100 Modena, Italy*
[c]*Department of Chemistry, University of York, Heslington, York YO10 5DD, UK*

Received 21 October 2004; revised 18 January 2005; accepted 19 January 2005

Abstract—The rearrangement of a trichloro-pyrrolidin-2-one, prepared by the CuCl–TMEDA catalyzed atom transfer radical cyclization of *N*-alkyl-*N*-(3-chloro-2-propenyl)-2,2-dichloromyristamide, with *n*-propylamine or CH_3ONa/CH_3OH, is the key step of a new, short and inexpensive route to chaetomellic anhydride C and (±)-*erythro*-roccellic acid.

1. Introduction

Lichens, the most advanced form of symbiotic life, are a rich source of low-molecular secondary metabolites.[1,2] One of the metabolites is (2*R*,3*S*)-2-dodecyl-3-methylbutanedioic acid, which is better known as (+)-*erythro*-roccellic acid (**1**) (Fig. 1).[3–5] This compound was isolated by von Heeren from *Roccella fuciformis* and *Roccella tinctoria*[2] and has since been observed in many other lichens.[2,6–9] It is a remarkably interesting natural product owing to its numerous synthetic uses and biological properties. This dibasic fatty acid has been used in the preparation of actinonin antibiotic analogues,[10] in the precipitation of human serum albumin[11] and in the assembly of metal complexes.[12] In addition, it displays (as the sodium salt of the ethyl half-ester) antituberculosis activity[13,14] and acts as a plant concentration-dependent growth promoter[15] or inhibitor.[16]



(+)-*erythro*-roccellic acid **1**

Figure 1.

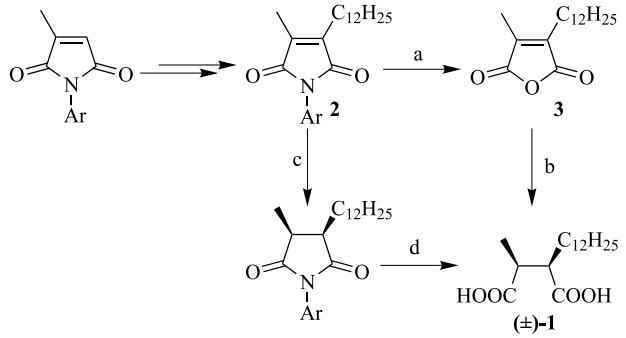
Barry and Twomey were the first to obtain (±)-**1**, albeit as a minor compound of a mixture of *erythro*/*threo* diastereoisomers, by successive alkylations of diethyl malonate.[17] Since then, a related diastereoselective synthesis has been reported by Äkermark and Johansson.[18] This approach, however, involves a large number of steps and the overall yield of (±)-**1** is low. Owing to the remarkable biological activity of **1**, there is a need for the development of more effective methods for preparing **1** and this is an area of current interest.[9] Recently, Argade and Mangaleswaran have developed an efficient route to (±)-*erythro*-roccellic acid **1** starting from *N*-(*p*-tolyl)citraconimide.[9] Also, Fensterbank and Malacria have exploited the conjugate addition of the lithium enolate of 2,6-dimethylphenyl propanoate to a chiral undecylidene bis(sulfoxide), in an enantioselective synthesis of (+)-*erythro*-roccellic acid **1**, although the yield was low.[19]

Recently, we have discovered a novel and efficient route to the pyrrolin-2,5-dione scaffold[20–22] and our attention was drawn to previous work on the synthesis of acid **1**, which

Keywords: Radicals; Cyclizations; Pyrrolidinones; Rearrangements.
* Corresponding author. Fax: +39 059 373543;
e-mail: ghelfi.franco@unimore.it
† Present address: Dipartimento di Scienze Chimiche, Università degli Studi di Padova, via Marzolo 1, 35131 Padova, Italy.

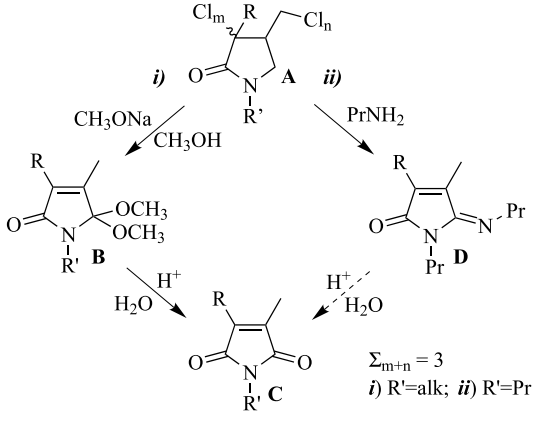
0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.086

involved the elaboration of related heterocycles (Scheme 1).[9] This work, using precursors such as the maleimide **2** and/or the maleic anhydride **3**, showed that catalytic hydrogenation could be used to produce the observed relative configuration of the C(2) and C(3) carbons in acid **1** (Scheme 1).[9,18]



Scheme 1. (a) (1) KOH, THF/MeOH/H_2O, reflux, 2 h, (2) HCl (98%); (b) Adam's catalyst, petroleum ether, H_2, rt, 12 h (60%); (c) Adam's catalyst, petroleum ether, H_2, rt, 10 h (95%); (d) CF_3COOH, HCl 37%, reflux, 48 h (80%).

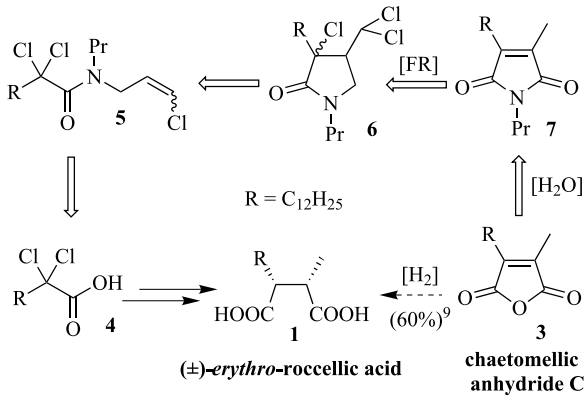
Our approach to the pyrrolin-2,5-dione nucleus starts with the pivotal functional rearrangement (FR) of an appropriate 4-methyl-pyrrolidin-2-one (**A**), which has at least three chlorine atoms at the C(3) and C(6) positions. These compounds, when treated with a solution of alkaline methoxide (in methanol under mild conditions), are converted into the corresponding 5,5-dimethoxy-4-methyl-3-pyrrolin-2-ones (**B**) (Scheme 2).[20,22] The transformation involves a series of eliminations/substitutions in which the oxidation state of the starting molecule is preserved and the functionalities repositioned. In practice, the initial three C–Cl groups (bound at the C(3) and C(6) carbons of **A**) are replaced by a double bond at C(3)–C(4) and by two methoxy groups at C(5). The maleimide nucleus (**C**) can subsequently be formed from **B** using an efficient hydrolysis step (Scheme 2).[20,21]



Scheme 2.

More recently, we have also observed that a similar functional rearrangement can be achieved using *n*-propylamine as the nucleophile/base in place of sodium methoxide in methanol.[22] When using these new conditions, the FR product (**D**) is again a 3-pyrrolin-2-one, but the C(5) carbon bears a propylimino function (Scheme 2). Subsequent acid hydrolysis of **D**, as has been reported previously for related compounds, could afford the 3-pyrrolin-2,5-dione core.[23,24]

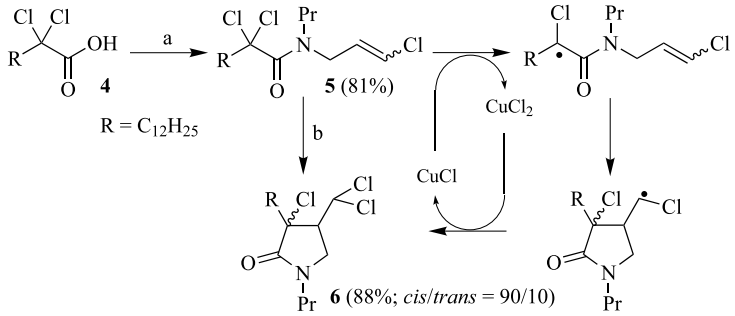
Following our interest in the field, we now report our results on the application of the FR, promoted by *n*-propylamine or CH_3ONa/CH_3OH, to the construction of the disubstituted maleic anhydride **3** (Scheme 3). Both the use of *n*-propylamine and particularly CH_3ONa/CH_3OH has been shown to offer a new and efficient route to (±)-*erythro*-roccellic acid (**1**). Besides, since the maleic anhydride **3** and chaetomellic anhydride C (CAC) have the same structure, this strategy also offers a new approach to the synthesis of this natural inhibitor of the yeast protein farnesyltransferase.[25,26] We have just shown that the FR with CH_3ONa/CH_3OH can be successfully exploited in the synthesis of the naturally-occurring chaetomellic anhydride A (CAA).[21]



Scheme 3.

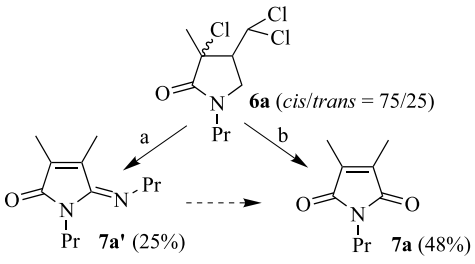
2. Results and discussion

N-Propyl-3-chloro-4-dichloromethyl-3-dodecylpyrrolidin-2-one (**6**) was chosen as the substrate on which to test the two alternative FR promoting systems (*n*-propylamine or CH_3ONa/CH_3OH). This was efficiently prepared by a simple two-step procedure starting from 2,2-dichloromyristic acid (**4**)[21] (Scheme 4). The chlorinated fatty acid **4** was first converted into the corresponding acyl chloride (using oxalyl chloride) and then treated immediately with *N*-propyl-3-chloro-2-propenylamine to furnish, in high yield (81%), the desired amide **5**. Subsequent rearrangement of **5** to the target lactam **6** was accomplished by an atom transfer radical cyclization (ATRC) mediated by a copper(I) complex (Scheme 4).[27] This well-known method of cyclization has some important advantages over other radical methods including: (i) low cost of the catalyst; (ii) ease of work-up; and above all, (iii) preservation of all the carbon-halogen groups in the cyclic product. The reaction is typically mediated by redox catalysts, such as the complex between CuCl and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA).[27–29] The ring closure of **5** was smoothly completed using 10% mol of the CuCl–TMEDA (1:2) complex formed in acetonitrile at 40–50 °C. This gave the expected γ-lactam **6** in 88% yield as an inseparable pair of *cis*-/*trans*-isomers in a ratio of 90:10.

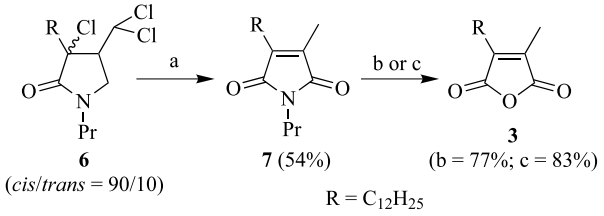


Scheme 4. (a) (1) $(COCl)_2$, DMF, CH_2Cl_2, rt, 3 h, (2) *N*-propyl-3-chloro-2-propenylamine, Py, CH_2Cl_2, rt, 1 h; (b) CuCl/TMEDA, CH_3CN, 40–50 °C, 20 h.

In our previous article[22] we reported that the functional rearrangement of *N*-propyl-3-chloro-4-dichloromethyl-3-methyl-pyrrolidin-2-one (**6a**) using *n*-propylamine at 100 °C gave the 5-imino-3-pyrrolin-2-one **7a′**, which is a precusor to a more simple analogue of **3**, in a moderate 25% yield (Scheme 5). Although it was impossible to separate and characterize the accompanying side-products of the FR reaction of **6a**, analysis of the MS-spectra of the crude suggested that, on acid hydrolysis some of the products, together with **7a′**, could be converted into maleimide **7a**. If this is the case, then including an acid hydrolysis step after the FR of **6a** could produce a higher yield of imide **7a** than the 25% yield expected from the sole **7a′**, which was isolated in the absence of acid hydrolysis. Besides, it was envisaged that with an acidic work-up the rearrangement of **6a** to **7a** could be fruitfully performed even under milder conditions, notwithstanding under these circumstances the yield of **7a′** fell down.[22] Indeed, carrying out the FR of **6a** at 40 °C, followed by heating in acetic acid at 75 °C, resulted in the formation of **7a** in a respectable 48% yield (Scheme 5). Encouraged by this success, the improved procedure was then applied to the rearrangement of *N*-propyl-3-chloro-4-dichloromethyl-3-dodecylpyrrolidin-2-one (**6**) (Scheme 6). Accordingly, pyrrolidinone **6** was



Scheme 5. (a) $CH_3CH_2CH_2NH_2$, 100 °C, 24 h; (b) (1) $CH_3CH_2CH_2NH_2$, 40 °C, 24 h, (2) CH_3COOH, 75 °C, 24 h.



Scheme 6. Functional rearrangement of **6** with *n*-propylamine: (a) (1) $CH_3CH_2CH_2NH_2$, 60 °C, 24 h, (2) CH_3COOH, 75 °C, 24 h; (b) CH_3COOH/MSA (1/1), 140 °C, 20 h; (c) (1) KOH, THF/MeOH/H_2O, reflux, 2 h, (2) HCl.

treated with *n*-propylamine at 60 °C for 24 h, after which time the remaining propylamine was removed (by evaporation) and replaced by glacial acetic acid, and the ensuing reaction mixture heated at 75 °C for an additional 24 h. Analysis of the reaction mixture showed the desired maleimide **7** was formed in a satisfactory 54% yield together with a small amount of the regioisomer **8** (10%), which has the C=C bond outside of the ring (Fig. 2). Other hydrolysis procedures (including HCl_{aq} 18%/CH_3OH, 1/1, 95 °C, 2 h; HCl_{aq} 18%/CF_3COOH, 1/1, reflux, 48 h; and H_2SO_{4aq} 50%/CH_3COOH, 1/1, 100 °C, 20 h) were tested but with no success.

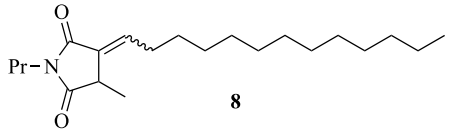
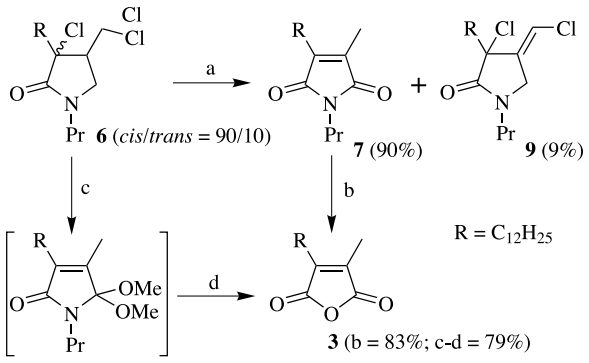


Figure 2.

Finally, on hydrolysis of maleimide **7**, using the KOH method adopted by Argade to convert maleimides into the corresponding maleic anhydrides,[9,30] the desired chaetomellic anhydride C (**3**) was isolated in high yield (83%) (Scheme 6). The same transformation was also achieved under acid conditions (by heating maleimide **7** in acetic acid/methanesulfonic acid (MSA), 1/1) and with similar efficiency (77%). Overall, over the 4 steps from 2,2-dichloromyristic acid (**4**) to anhydride **3**, a moderate yield of 32% was obtained. This represents a new formal synthesis of (±)-**1**, as reduction of anhydride **3** to give (±)-**1** (using hydrogen at 50 psi pressure in the presence of Adam's platinum dioxide catalyst in petroleum ether) has recently been reported by Argade and Mangaleswaran[9] (Schemes 1 and 3).

Our efforts then concentrated on preparing 3-dodecyl-4-methylmaleic anhydride (**3**) by an alternative FR, which is promoted by CH_3ONa/CH_3OH (Scheme 7). We have recently demonstrated the use of this approach in the preparation of CAA,[21] which is a higher homologue of anhydride **3**. Hence, the chlorinated pyrrolidin-2-one **6** was subjected to the FR using sodium in methanol/diethyl ether (at 25 °C) following the literature procedure.[20,21] After acidic work-up of the reaction mixture, the desired intermediate **7** was recovered in excellent yield (90%). As observed in the related CAA synthesis,[21] in addition to the maleimide (**7**), a small amount (9%) of a compound derived from *exo*-dehydrohalogenation of the starting



Scheme 7. Functional rearrangement of **6** with CH_3ONa/CH_3OH: (a) (1) CH_3ONa/CH_3OH, Et_2O, rt, 20 h, (2) HCl, H_2O, rt, 20 h; (b) (1) KOH, THF/MeOH/H_2O, reflux, 2 h, (2) HCl; (c) CH_3ONa/CH_3OH, Et_2O, rt, 20 h, (d) CH_3COOH/MSA (1/1), 140 °C, 20 h.

polychlorinated pyrrolidin-2-one, in this case **9**, was isolated as a side-product (Scheme 7). Since hydrolysis of maleimide **7** can afford anhydride **3** in a respectable 83% yield, this represents a particularly efficient route to CAC. Indeed, the overall yield of anhydride **3** from 2,2-dichloromyristic acid (**4**) is now 53%, which compares to 32% when using *n*-propylamine in the FR.

Since both hydrolysis of the intermediate acetal **10** and maleimide **7** can be performed under acid conditions (Schemes 2 and 6), it seemed possible to shorten the reaction pathway by carrying out the FR and the maleimide hydrolysis in a one-pot process. However, in spite of investigating several different reaction conditions, it was not possible to efficiently convert the crude acetal **10**, obtained after evaporation of the solvent from the reaction mixture, into anhydride **3**. Nevertheless, when we isolated the acetal **10** after a basic work-up of the FR reaction of **6**, the ensuing solvolysis in acetic acid/MSA at 140 °C gave the desired 3-dodecyl-4-methylmaleic anhydride (**3**) in an excellent 79% yield over the two steps (Scheme 7; c and d). In comparison, when **6** was converted into maleimide **7** (yield 90%) and this was hydrolysed to give **3** (yield 83%), the overall efficiency of this approach was slightly lower (75%) (Scheme 7; a and b). This improvement translates into a further rise in the overall yield, from 53% to 56%, over the 4 steps from acid **4** to anhydride **3** (CAC).

3. Conclusion

Two 5-step, simple and convergent formal syntheses of (±)-*erythro*-roccellic acid (**1**) have been developed from inexpensive 2,2-dichloromyristic acid. Both approaches involve the formation of chaetomellic anhydride C (**3**), which has previously been converted into acid **1**. This work represents a further and interesting application of the FR reaction, promoted by nucleophilic/basic reagents, of polychlorinated γ-lactams [generated by the ATRC of *N*-(3-chloro-2-propenyl)-α-perchloroamides] to form furan-2,5-dione target molecules. When the FR reaction of amide **5** was promoted by *n*-propylamine, anhydride **3** was secured in moderate (32%) overall yield (Scheme 6) but this could be improved to 56% by using CH_3ONa/CH_3OH in the FR (Scheme 7). This new approach to acid **1** is of similar efficiency to that reported by Argade and Mangaleswaran (e.g. the carbon efficiency[31] of the two approaches are 51% and 45%, respectively) but it has advantages in that readily available (even in bulk quantities) and much less expensive reagents are used.

4. Experimental

4.1. General

^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions with a Jeol EX 270 and Jeol ECX 400 spectrometers. IR spectra were obtained with an ATI Matteson Genesis FT-IR, and mass spectra were acquired with a Fisons Analytical VG Autospec. Reagents and solvents were standard grade commercial products, purchased from Acros, Aldrich or Fluka, and used without further purification. Acetonitrile (for the radical cyclizations) was dried over three batches of 3 Å sieves (5% w/v, 12 h). The silica gel used for flash chromatography was 60 Merck (0.040–0.063 mm). *N*-Propyl-3-chloro-2-propenylamine was prepared by *N*-alkylation of *n*-propylamine with 1,3-dichloropropene (purchased from Aldrich as a mixture of *E*/*Z* isomers), following the procedure of Shipman.[32] 2,2-Dichlorotetradecanoic acid (2,2-dichloromyristic acid) was obtained by chlorination (Cl_2) of the parent alcohol and oxidation of the intermediate 2,2-dichlorotetradecanal with Br_2/NaOH as described in literature.[21]

4.2. Preparation of *N*-propyl-*N*-(3-chloro-2-propenyl)-2,2-dichlorotetradecanamide (5)

2,2-Dichlorotetradecanoic acid (2.97 g, 10 mmol) was weighed in a Schlenk tube fitted with a perforable septum (blocked by a screw cap), a magnetic stirrer bar and a $CaCl_2$ tube on the side arm. Next, dry CH_2Cl_2 (10 mL) was added under nitrogen. The solution was thermostated at 23 °C, and under stirring, DMF (16 μL) and $(COCl)_2$ (1.69 mL, 20 mmol) were injected using a syringe. The side arm stopcock was opened to vent out any gas (CO, CO_2 and HCl) produced during the reaction. After 3 h, solvent and excess oxalyl chloride were removed under reduced pressure. The crude acyl chloride was diluted with dry CH_2Cl_2 (8 mL), then the solution, thermostated at 23 °C, was treated in sequence with pyridine (1.26 mL, 16 mmol) and *N*-propyl-3-chloro-2-propenylamine (2.14 g, 16 mmol). The reaction mixture was stirred for 1 h and then washed with HCl_{aq} 1 M (2×20 mL) and $NaOH_{aq}$ (5% w/v, 2×20 mL). The acqueous phases were in their turn extracted with CH_2Cl_2 (2×40 mL). The organic extracts were collected and dried over $MgSO_4$. Flash chromatography of the crude product on silica gel, using petroleum ether (bp 40–60 °C)/diethyl ether (9/1) as eluant, gave amide **5** (3.34 g, 81%) as a mixture of *E*-/*Z*- stereoisomers; yellowish oil; [HRMS, calcd for $C_{20}H_{36}Cl_3NO$ ($^{35}M+H^+$): 412.194. Found: 412.193]; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.80–1.00 (3H, m, $NCH_2CH_2CH_3$), 0.88 (3H, t, $J=6.9$ Hz, $CH_2CH_2CH_2CH_3$), 1.10–1.50 (20H, m, $CH_2(CH_2)_{10}CH_3$), 1.52–1.80 (2H, m, NCH_2CH_2), 2.43 (2H, br t, $J=7.8$ Hz, CCl_2CH_2), 3.26+3.70 (2H, 2×br t, $J=8.4$ Hz, NCH_2CH_2), 3.95+4.16 (1H, 2×br d, $J=6.1$ Hz, NCHCH), 4.43+4.65 (1H,

2×br d, J=6.1 Hz, NC*H*CH), 5.80–6.10 (1H, m, C*H*=CHCl), 6.15–6.40 (1H, m, CH=C*H*Cl); IR (film) 1653 (C=O) cm^{-1}; LRMS (CI) *m/z*: 412 (100%, $M+H^+$), 376 (25), 342 (5), 160 (14), 132 (7), 92 (8), 75 (14).

4.3. Cyclization of *N*-propyl-*N*-(3-chloro-2-propenyl)-2,2-dichlorotetradecanamide (5)

CuCl (0.10 g, 1.0 mmol) and the 2,2-dichloro-amide **5** (4.13 g, 10 mmol) were weighted in a Schlenk tube fitted with a perforable septum (blocked by a screw cap) and a magnetic stirrer bar. Dry acetonitrile (15 mL) and TMEDA (302 μL, 2 mmol) were then added under nitrogen. The mixture was stirred at 40–50 °C and after 20 h diluted with HCl_{aq} 1 M (20 mL) and extracted with CH_2Cl_2 (2×30 mL). The combined organic layers were dried over $MgSO_4$. The crude product was purified using flash chromatography on silica gel, eluting with petroleum ether (bp 40–60 °C)/diethyl ether in gradient (from 100:0 to 50:50). This gave the pyrrolidin-2-one **6** (3.63 g, 88%) as a 90/10 mixture of inseparable *cis*-/*trans*-diastereoisomers as indicated by the ^1H NMR spectrum; pale yellow oil; [HRMS, calcd for $C_{20}H_{36}Cl_3NO$ ($^{35}M+H^+$): 412.194. Found: 412.194]; ^1H NMR ($CDCl_3$, 400 MHz): *cis* diastereoisomer (90%) δ 0.88 (3H, t, J=7.2 Hz, $CH_2CH_2CH_2CH_3$), 0.92 (3H, t, J=7.5 Hz, $NCH_2CH_2CH_3$), 1.10–1.45 (20H, m, $CH_2(CH_2)_8CH_2$), 1.46–1.65 (2H, m, NCH_2CH_2), 2.20–2.41 (2H, m, C(3)CH_2), 3.15 (1H, td, J=7.1, 9.2 Hz, C(4)*H*), 3.19–3.52 (4H, m, C(5)H_2NCH_2), 6.06 (1H, d, J=9.2 Hz, C(4)C*H*); *trans* diastereoisomer (10%) δ 0.88 (3H, t, J=7.2 Hz, $CH_2CH_2CH_2CH_3$), 0.95 (3H, t, J=7.5 Hz, $NCH_2CH_2CH_3$), 1.10–1.45 (20H, m, $CH_2(CH_2)_8CH_2$), 1.46–1.65 (2H, m, NCH_2CH_2), 2.20–2.41 (2H, m, C(3)CH_2), 3.19–3.52 (4H, m, C(4)*H*C(5)*H*NCH_2), 3.77 (1H, dd, J=7.0, 11.0 Hz, C(5)*H*), 5.93 (1H, d, J=4.0 Hz, C(4)C*H*); ^{13}C NMR ($CDCl_3$, 100 MHz): *cis* diastereoisomer (90%) δ 11.24 ($CH_2CH_2CH_2CH_3$), 14.29 ($NCH_2CH_2CH_3$), 20.47 ($NCH_2CH_2CH_3$), 22.86, 25.73, 29.42, 29.50, 29.63, 29.75, 29.80, 32.07 (10×CH_2), 37.26 (C(3)CH_2), 44.80, 47.75 (CH_2NCH_2), 49.60 (C(4)H$CHCl_2$), 71.96 (*C*(4)H), 72.29 (*C*(3)), 170.03 (*C*=O); *trans* diastereoisomer (10%) δ 11.44 ($CH_2CH_2CH_2CH_3$), 14.29 ($NCH_2CH_2CH_3$), 24.84 ($NCH_2CH_2CH_3$), 22.86, 25.73, 29.42, 29.50, 29.63, 29.75, 29.80, 32.07 (10CH_2), 33.21 (C(3)CH_2), 44.93, 45.87 (CH_2NCH_2), 54.68 (C(4)H$CHCl_2$), 70.80 (*C*(4)H), 71.83 (*C*(3)), 170.12 (*C*=O); IR (film) 1711 (C=O) cm^{-1}; LRMS (CI) *m/z*: 412 (100%, $M+H^+$), 378 (27), 376 (10), 342 (5), 308 (18), 294 (25), 243 (11), 160 (36), 126 (11).

4.4. Rearrangement of *N*-propyl-3-chloro-4-dichloromethyl-3-dodecylpyrrolidin-2-one (6) with *n*-propylamine

The polychlorinated pyrrolidin-2-one **6** (0.41 g, 1 mmol) was weighted in a Schlenk tube, then *n*-propylamine (2 mL) was added under argon. The mixture was stirred at 60 °C for 24 h. Any remaining *n*-propylamine was evaporated under vacuum and then, glacial HOAc (3 mL) was slowly added while stirring. The solution was then heated at 75 °C for a further 24 h. Finally the reaction mixture was diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (5×5 mL). The organic layers were collected and dried over $MgSO_4$. Flash-chromatography of the crude product on silica gel, using petroleum ether (bp 40–60 °C)/diethyl ether (9/1) as eluant, afforded **7** (0.17 g, 54%) as an orange yellow oil and *N*-propyl-3-dodecilen-4-methyl-5-oxo-pyrrolidin-2-one (**8**) (32 mg, 10%) as a yellowish oil.

4.4.1. *N*-Propyl-4-methyl-3-tetradecylmaleimide (7). [HRMS, calcd for $C_{20}H_{35}NO_2$ ($M+H^+$): 322.275. Found: 322.275]; ^1H NMR ($CDCl_3$, 270 MHz): δ 0.87 (3H, t, J=6.7 Hz, $CH_2CH_2CH_2CH_3$), 0.88 (3H, t, J=7.4 Hz, $NCH_2CH_2CH_3$), 1.20–1.40 (20H, m, $CH_2(CH_2)_8CH_2$), 1.59 (2H, m, NCH_2CH_2), 1.95 (3H, s, C(4)CH_3), 2.36 (2H, t, J=7.6 Hz, C(3)CH_2), 3.43 (2H, t, J=7.2 Hz, NCH_2); ^{13}C NMR (67.9 MHz, $CDCl_3$): δ δ 8.62 ($CH_2CH_2CH_2CH_3$), 11.22 ($NCH_2CH_2CH_3$), 14.09 (C(4)CH_3), 21.95, 22.66, 23.63, 28.20, 29.28, 29.31, 29.48, 29.57, 29.62, 29.68 (11×CH_2), 31.89 (C(3)CH_2), 39.44 (NCH_2), 136.67 (*C*(4)), 140.96 (*C*(3)), 172.15 (*C*(5)=O), 172.45 (*C*(2)=O); IR (film) 1701 (C=O) cm^{-1}; LRMS (CI) *m/z*: 339 (62%, $M+NH_4^+$), 322 (100, $M+H^+$), 167 (12).

4.4.2. *N*-Propyl-3-dodecylen-4-methyl-5-oxo-pyrrolidin-2-one (8). [HRMS, calcd for $C_{20}H_{35}NO_2$ ($M+H^+$): 322.275. Found: 322.275]; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, t, J=6.7 Hz, $CH_2CH_2CH_2CH_3$), 0.91 (3H, t, J=7.5 Hz, $NCH_2CH_2CH_3$), 1.10–1.55 (18H, m, $CH_2(CH_2)_9CH_3$), 1.41 (3H, d, J=7.3 Hz, C(4)CH_3), 1.55–1.80 (2H, m, NCH_2CH_2), 2.10–2.40 (2H, m, C(3)CHCH_2), 3.22–3.32 (1H, m, C(4)*H*), 3.44–3.58 (2H, m, NCH_2), 6.79 (1H, m, C(3)C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.44 ($CH_2CH_2CH_2CH_3$), 14.30 ($NCH_2CH_2CH_3$), 16.47 (C(4)CH_3), 21.32, 22.86, 28.78, 29.37, 29.50, 29.58, 29.59, 29.69, 29.78, 29.79 (10×CH_2), 32.08 (C(3)=CHCH_2), 37.55 (C(4)CH), 40.32 (NCH_2), 131.00 (*C*(3)), 139.44 (C(3)=*C*H), 158.59 (*C*(5)), 178.57 (*C*(2)=O); IR (film) 1703 (C=O) cm^{-1}; LRMS (CI) *m/z*: 322 (100%, $M+H^+$).

4.5. Hydrolysis of *N*-propyl-4-methyl-3-tetradecylmaleimide (7) with KOH.[7]

To a solution of maleimide **7** (0.16, 0.5 mmol) in THF/CH_3OH (1/2, 3 mL), in a 25 mL round bottom flask fitted with a condenser and a magnetic stirrer bar, was added KOH_{aq} 30% (1.5 mL). The resulting two liquid phases were heated to reflux (oil bath temperature 95 °C) under vigorous stirring for 2 h. The reaction mixture was then cooled to room temperature, diluted with HCl_{aq} (1 M, 5 mL) and extracted with ethyl ether (3×5 mL). The organic extracts were then collected, washed with water (5 mL), brine (5 mL) and finally dried over $MgSO_4$. Flash-chromatography of the crude product on silica gel, using diethyl ether as eluant, afforded **3** (0.12 g, 83%) as a light yellow oil; [HRMS, calcd for $C_{17}H_{28}O_3$ ($M+NH_4^+$): 298.238. Found: 298.238]; ^1H NMR ($CDCl_3$, 270 MHz): δ 0.89 (3H, t, J=6.9 Hz, CH_2CH_3), 1.15–1.40 (18H, m, $CH_2(CH_2)_8CH_3$), 1.57 (2H, m, C(3)CH_2CH_2), 2.08 (3H, s, C(4)CH_3), 2.46 (2H, t, J=7.8 Hz, C(3)CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.50 (CH_2CH_3), 14.10 (C(4)CH_3), 22.67, 24.43, 27.59, 29.17, 29.32, 29.41, 29.55, 29.59 (10×CH_2), 31.89 (C(3)CH_2), 140.42 (*C*(4)), 144.77 (*C*(3)), 165.87 (*C*(5)=O), 166.27 (*C*(2)=O); IR (film) 1766 (C=O) cm^{-1}; LRMS (CI) *m/z*: 298 (100%, $M+NH_4^+$), 280 (27, M^+), 252 (8), 150 (10), 95 (8).

4.6. Solvolysis of *N*-propyl-4-methyl-3-tetradecyl-maleimide (7) using CH_3COOH/MSA

The maleimide **7** (0.16, 0.5 mmol) was weighed in a Schlenk tube fitted with a perforable septum (blocked by a screw cap) and a magnetic stirrer bar. Next glacial HOAc (0.5 mL) and MSA (0.5 mL) were added while stirring. The solution was then heated at 140 °C for 20 h. Finally the reaction mixture was diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (5×5 mL). The combined organic layers were dried over $MgSO_4$. Flash chromatography of the crude product on silica gel, using ethyl ether as eluant, gave anhydride **3** as a light yellow oil (0.11 g, 77%).

4.7. Rearrangement of *N*-propyl-3-chloro-4-dichloro-methyl-3-dodecylpyrrolidin-2-one (6) using CH_3ONa/CH_3OH

In a Schlenk tube fitted with a perforable septum (blocked by a screw cap) and a magnetic stirrer bar was added CH_3OH (3 mL) and Na^0 (0.18 g, 8 mmol). When the effervescence ceased, the solution was thermostated at 25 °C and a solution of pyrrolidinone **6** (0.83 g, 2 mmol) in diethyl ether (3 mL) was added by syringe. The reaction mixture was then stirred for 20 h, after which time it was acidified with HCl (5% w/v) and left to stir at 25 °C for a further 20 h. Subsequently, the mixture was extracted with CH_2Cl_2 (3×5 mL) and the combined organic layers dried over $MgSO_4$ and concentrated under reduced pressure. Flash chromatography of the crude product on silica gel, using a petroleum ether (bp 40–60 °C)/diethyl ether gradient, gave maleimide **7** as a yellowish oil (0.58 g, 90%) and the dehydrohalogenated γ-lactam **9** (68 mg, 9%) as a light yellow oil.

4.7.1. (*E*)-*N*-Propyl-3-chloro-4-chloromethylen-3-dodecylpyrrolidin-2-one (9). [HRMS, calcd for $C_{20}H_{35}Cl_2NO$ ($M+H^+$): 376.217. Found: 376.217]; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (3H, t, $J=6.9$ Hz, $CH_2CH_2CH_2CH_3$), 0.93 (3H, t, $J=7.5$ Hz, $NCH_2CH_2CH_3$), 1.03–1.40 (20H, m, $CH_2(CH_2)_{10}CH_3$), 1.54–1.70 (2H, m, NCH_2CH_2), 2.05 (1H, dq, $J=13.3$, 12.0, 4.4 Hz, C(3)CH), 2.29 (1H, dq, $J=13.3$, 11.5, 4.6 Hz, C(3)CH), 3.25–3.50 (2H, m, NCH_2CH_2), 3.98 (1H, dd, $J=15.2$, 2.5 Hz, NCHC(4)), 4.11 (1H, dd, $J=15.2$, 2.5 Hz, NCHC(4)), 6.48 (1H, t, $J=2.5$ Hz, C(4)CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.27 ($CH_2CH_2CH_2CH_3$), 14.28 ($NCH_2CH_2CH_3$), 20.34, 22.84, 25.19, 29.43, 29.49, 29.51, 29.62, 29.71, 29.76, 32.06 (11×CH_2), 39.93 (C(3)CH_2), 44.67, 48.22 (CH_2NCH_2), 67.67 (C(3)), 119.26 (C(4)=CH), 137.19 (C(4)), 170.08 (C(2)); IR (film) 1711 (C=O) cm^{-1}; LRMS (CI) *m/z*: 393 (19%, $M+NH_4^+$), 376 (100, $M+H^+$), 340 (14), 207 (19).

4.8. Direct preparation of 3-dodecyl-4-methylmaleic anhydride (3) by rearrangement of *N*-propyl-3-chloro-4-dichloromethyl-3-dodecylpyrrolidin-2-one (6) using CH_3ONa/CH_3OH

In a Schlenk tube fitted with a perforable septum (blocked by a screw cap) and a magnetic stirrer bar was added CH_3OH (3 mL) and Na^0 (0.18 g, 8 mmol). When the effervescence ceased, the solution was thermostated at 25 °C and a solution of pyrrolidinone **6** (0.83 g, 2 mmol) in diethyl ether (3 mL) was added by syringe. The reaction mixture was then stirred for 20 h, after which time it was diluted with H_2O (20 mL) and extracted with CH_2Cl_2 (4× 10 mL). The combined organic layers, dried over $MgSO_4$, were concentrated in a second Schlenk tube under reduced pressure and the crude, thus obtained, treated with glacial HOAc (3 mL) and MSA (3 mL). The solution was then heated at 140 °C for 20 h. Finally, the reaction mixture was diluted with H_2O (30 mL) and extracted with CH_2Cl_2 (5× 10 mL). The combined organic layers were dried over $MgSO_4$. Flash chromatography of the crude product on silica gel, using a petroleum ether (bp 40–60 °C)/diethyl ether gradient as eluant, gave 0.44 g of anhydride **3** (79%).

Acknowledgements

We thank the Ministero della Università e della Ricerca Scientifica e Tecnologica (MURST) and the EU (under the ERASMUS scheme) for financial assistance.

References and notes

1. Mosbach, K. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 240–250.
2. Honda, N. K.; Vilegas, W. *Quim. Nova* **1999**, *22*, 110–125. (*Chem. Abstr.* **1999**, *130*, 207048).
3. *Beilstein* 2, 734.
4. Huneck, S.; Schmidt, J.; Porzel, A. *Z. Naturforsch. B: Chem. Sci.* **1994**, *49*, 561–568.
5. Rangaswami, S.; Subramanian, S. S. *Indian J. Pharm.* **1952**, *14*, 214–215.
6. Hesse, O. *Ann. Chem.* **1861**, *117*, 297–351.
7. Zopf, W. *Ann. Chem.* **1897**, *295*, 222–300.
8. Hesse, O. *J. Prakt. Chem.* **1898**, *58*, 465–561.
9. Mangaleswaran, S.; Argade, N. P. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1764–1766.
10. Devlin, J. P.; Ollis, W. D.; Thorpe, J. E. *J. Chem. Soc., Perkin Trans. 1* **1975**, 846–848.
11. Armstrong, W. McD. *Proc. R. Irish Acad., Sect. B* **1956**, *58B*, 71–87. (*Chem. Abstr.* **1957**, *51*, 30368).
12. Iskandar, I. K.; Syers, J. K. *J. Soil Sci.* **1972**, *23*, 255–265.
13. Barry, V. C.; Belton, J. G.; Kelly, R. M.; Twomey, D. *Nature* **1950**, *166*, 303–305.
14. Pereira, F. M.; de Sa, J.; Bhatnagar, S. S. *Indian J. Pharm.* **1953**, *15*, 287–289.
15. Garcia, C. F.; Espinoza, G. A.; Coltantes, S. G.; Rios, V. V.; Quilhot, P. W. *J. Hattori Bot. Lab.* **1982**, *53*, 443–447. (*Chem. Abstr.* **1982**, *97*, 212854).
16. Huneck, S.; Schreiber, K. *Phytochemistry* **1972**, *11*, 2429–2434.
17. Barry, V. C.; Twomey, D. *Proc. R. Irish Acad., Sect. B* **1947**, *51*, 145–151. (*Chem. Abstr.* **1947**, *41*, 22293).
18. Akermark, B.; Johansson, N. G. *Ark. Kemi* **1967**, *27*, 1–10. (*Chem. Abstr.* **1967**, *67*, 63671).
19. Brebion, F.; Delouvrié, B.; Nájera, F.; Fensterbank, L.; Malacria, M.; Vaissermann, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 5342–5345.
20. Ghelfi, F.; Stevens, C. V.; Laureyn, I.; Van Meenem, E.; Rogge, T. M.; De Buyck, L.; Nikitin, K. V.; Grandi, R.;

Libertini, E.; Pagnoni, U. M.; Schenetti, L. *Tetrahedron* **2003**, *59*, 1147–1157.
21. De Buyck, L.; Cagnoli, R.; Ghelfi, F.; Merighi, G.; Mucci, A.; Pagnoni, U. M.; Parsons, A. F. *Synthesis* **2004**, 1680–1686.
22. Danieli, C.; Ghelfi, F.; Mucci, A.; Parsons, A. F.; Pagnoni, U. M.; Pattarozzi, M.; Schenetti, L. *Tetrahedron*. **2004**, *60*, 11493–11501.
23. Chasle, M.-F.; Foucaud, A. *Bull. Soc. Chim.* **1971**, 195–205.
24. Fanta, P. E.; Smith, S. *J. Org. Chem.* **1954**, *76*, 2915–2917.
25. Singh, S. B.; Jayasuriya, H.; Silverman, K. C.; Bonfiglio, C. A.; Williamson, J. M.; Lingham, R. B. *Bioorg. Med. Chem.* **2000**, *8*, 571–580.
26. Ratemi, E. S.; Dolence, J. M.; Poulter, C. D.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 6296–6301.
27. Clark, A. J. *Chem. Soc. Rev*. **2002**, *31*, 1–11.
28. Ghelfi, F.; Parsons, A. F. *J. Org. Chem.* **2000**, *65*, 6249–6253.
29. Ghelfi, F.; Bellesia, F.; Forti, L.; Ghirardini, G.; Grandi, R.; Libertini, E.; Montemaggi, M. C.; Pagnoni, U. M.; Pinetti, A.; De Buyck, L.; Parsons, A. F. *Tetrahedron* **1999**, *55*, 5839–5852.
30. Desai, S. B.; Argade, N. P. *J. Org. Chem.* **1997**, *62*, 4862–4863.
31. Constable, D. J. C.; Curzons, A. D.; Cunningham, V. L. *Green Chem.* **2002**, *4*, 521–527.
32. Ince, J.; Ross, T. M.; Shipman, M.; Slawin, A. M. Z. *Tetrahedron* **1996**, *52*, 7037–7044.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2879–2887

Synthesis of 2-aryl-4-chloropyrroles via ring expansion of 2-aryl-1-chlorocyclopropanecarbaldehydes

Guido Verniest, Sven Claessens, Filip Bombeke, Tinneke Van Thienen and Norbert De Kimpe*

Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

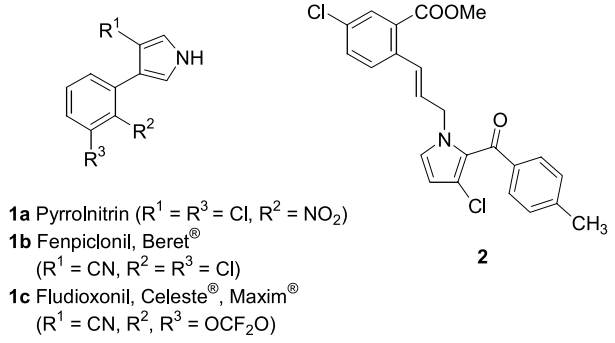
Received 23 September 2004; revised 14 January 2005; accepted 19 January 2005

Abstract—An efficient electrophile-induced ring opening of 2-aryl-1-chlorocyclopropanecarbaldehydes is described towards halogenated butanals, which were converted to the corresponding imines. These α,α,γ-trichloroimines proved to be good substrates for a nucleophile-induced ring closure towards 2-pyrrolines as versatile synthons for the synthesis of pyrroles bearing physiologically interesting substitution patterns.

1. Introduction

In the unabated search for new physiologically active compounds, the study of substituted pyrroles still remains a subject of considerable importance. Halogenated pyrroles isolated from Nature, associated with diverse physiological activities, have served as lead structures to synthesize pyrroles with current use in agrochemistry (e.g., the antifungal pyrrolnitrin (**1a**) derivatives **1b**,**c**)[1] and medicine (e.g., 3-chloropyrrole **2**, a fibrosis inhibitor) (Fig. 1).[2]

Pentabromopseudilin **3** was first isolated from the marine bacterium *Alteromonas luteoviolaceus* and shows antitumor, antibacterial and antifungal activities. This polybrominated pyrrole (**3**) also inhibits various enzyme systems and the cholesterol biosynthesis.[3] Manzacidins A (**4a**) and B (**4b**) are alkaloids isolated from the Okinawan sponge *Hymeniacidon species*.[4] Roseophilin **5** is a 3-chloropyrrole found in *Streptomyces griseoviridis* and displays antibiotic and antileukemic properties.[5] More than 20 compounds of the 'oroidin' (**6**) family of β-brominated pyrroles (i.e., 4-bromo- and 4,5-dibromopyrrole-2-carboxamides) have been isolated from Nature and tested for physiological activities.[6] For instance, clathramides, isolated from *Agelas clathrodes*, possess antifungal properties,[7] while other oroidins show antiserotoninergic (keramadine), cytotoxic (agelastatin), antiviral (sceptrin), antihistaminergic (dispacamide) or antifouling (mauritiamine) activities.[6,8,9] With respect to this diversity of activities related to halogenated pyrroles, various synthetic methods to access these compounds have been developed, where each method displays its own advantages to access pyrroles with specific substitution patterns.[10] Of current interest for agrochemistry is the synthesis of 3-halogenated pyrroles bearing electron withdrawing groups (e.g., COOR, CN or CF_3) (Fig. 2).[11]



1a Pyrrolnitrin ($R^1 = R^3 = Cl$, $R^2 = NO_2$)
1b Fenpiclonil, Beret®
($R^1 = CN$, $R^2 = R^3 = Cl$)
1c Fludioxonil, Celeste®, Maxim®
($R^1 = CN$, R^2, $R^3 = OCF_2O$)

Figure 1.

Structure–activity relationship studies revealed that the presence of an aryl moiety at one pyrrole α-carbon is often responsible for specific biological activities, for example, 2-arylpyrrole **7** and derivatives are insecticidal compounds (100% mortality for *Spodoptera eridania* treated with **7** at 10 mg/L).[12] Related pyrroles **8** were recently patented for the protection of wood from termites.[13] In addition, substituted pyrroles with cyano- or carboxylic acid moieties at the α-carbon are important intermediates in the synthesis of porphyrins and other 'pigments of life'.[14]

Keywords: Ring expansion; Pyrroles; Cyclopropanes.
* Corresponding author. Tel.: +32 9 264 59 51; fax: +32 9 264 62 43; e-mail: norbert.dekimpe@UGent.be

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.087

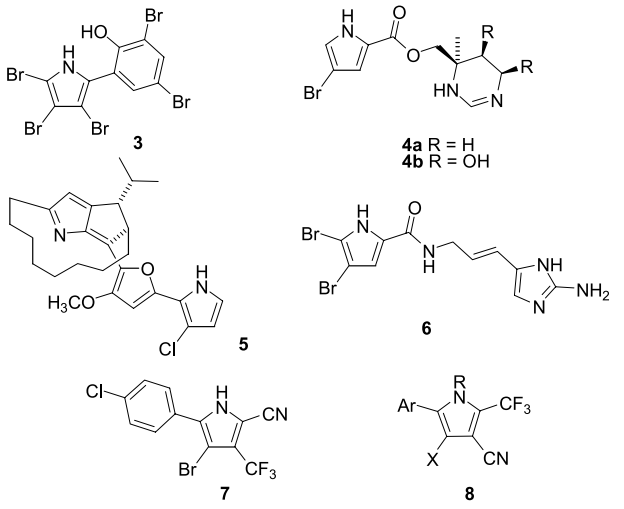


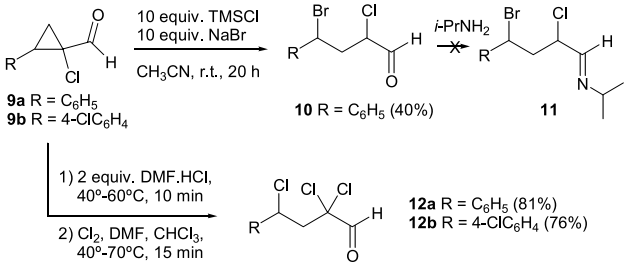
Figure 2.

In this article, an efficient synthesis of halogenated 2-arylpyrroles from 3-aryl-2,2-dichlorocyclobutanones via the intermediacy of 1-chlorocyclopropanecarbaldehydes is disclosed. Only a few publications report the use of cyclopropanecarbaldehydes as building blocks for pyrrole syntheses by thermal rearrangement of the corresponding imines.[15] In contrast, our approach deals with an initial ring opening of appropriate 1-chlorocyclopropane-1-carbaldehydes. Subsequent imination of the resulting γ-haloaldehydes followed by treatment with cyanide to induce a ring closure provides a new entry towards synthetically useful azaheterocyclic compounds.

2. Results and discussion

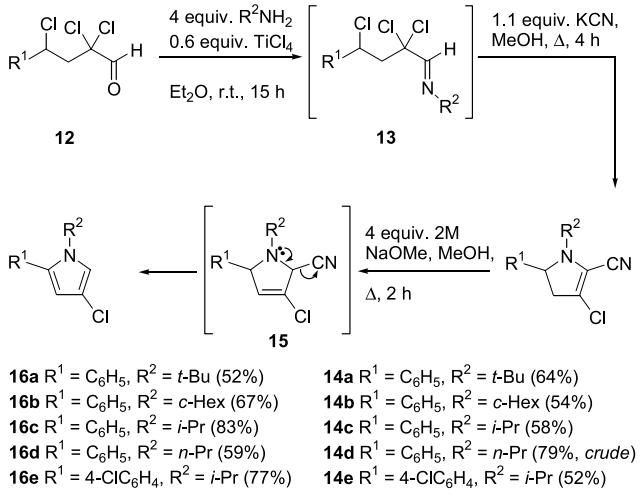
In continuation of the previously reported ring contraction of 2,2-dihalocyclobutanones towards 1-chlorocyclopropanecarbaldehydes **9**, [16] attempts were made to validate the latter compounds as useful synthons for the application in azaheterocyclic synthesis.

Analogous to earlier reported electrophile-induced ring opening reactions of cyclopropylketones under mild conditions,[17] α-chlorocyclopropanecarbaldehyde **9a** was treated with trimethylsilylchloride and sodium bromide to yield a diastereomeric mixture of γ-bromobutanals **10** (ratio 1:1) (Scheme 1). Compounds of this kind could be used to construct five-membered azaheterocycles after imination. Unfortunately, treatment of the aldehyde **10** with isopropylamine in the presence of $MgSO_4$ or $TiCl_4$ under various



Scheme 1.

reaction conditions did not result in the corresponding imines **11**. When an inverse imination procedure was applied, a mixture of cyclopropanecarbaldehyde **9a** and the corresponding imine was obtained due to α-deprotonation of **10** by isopropylamine. In order to eliminate the latter reaction, γ-bromo-α-chlorobutanal **10** was treated with chlorine gas to synthesize the α,α-dichlorinated analogue, which could be used as imine precursor. After chlorination, an inseparable mixture of reaction products was obtained. To overcome this problem an efficient synthesis of 4-aryl-2,2,4-trichlorobutanals **12** was developed by a HCl-induced ring opening and in situ chlorination of the obtained intermediate α-chloroaldehydes.[18] This procedure yielded compounds **12** in almost quantitative yield, which could be purified by distillation. Imination of compound **12** by the use of various amines in the presence of titanium(IV) chloride and subsequent treatment with potassium cyanide in methanol yielded 5-aryl-3-chloro-2-cyano-2-pyrrolines **14** in good yield (Scheme 2). The obtained intermediate imines **13** were not stable enough to purify by distillation or chromatography and were used directly after isolation from the reaction mixture. 2-Pyrrolines **14** proved to be stable at low temperatures (−20 °C) for several days.

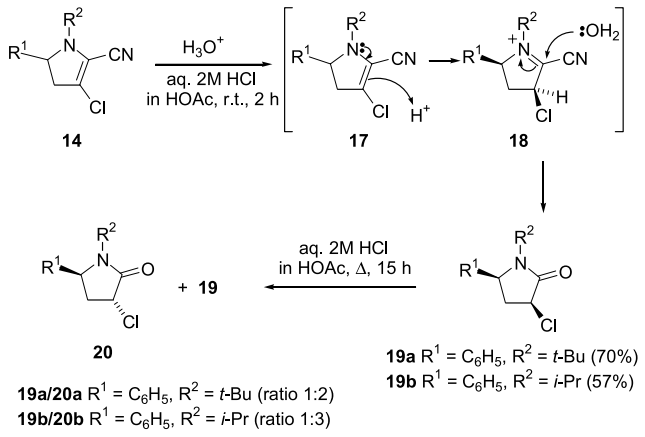


Scheme 2.

The treatment of 2-pyrrolines **14** with 4 equiv of 2 M sodium methoxide in methanol at reflux temperature for 2 h resulted in the formation of 3-chloropyrroles **16**.

The mechanism can be rationalized by an initial base induced isomerization towards 3-pyrrolines **15** and subsequent expulsion of cyanide. Further isomerization results in 2-aryl-4-chloropyrroles **16** in good yield (Scheme 2). With this procedure β-chloropyrroles can be synthesized on a multi-gram scale using cheap reagents and facile chemistry.

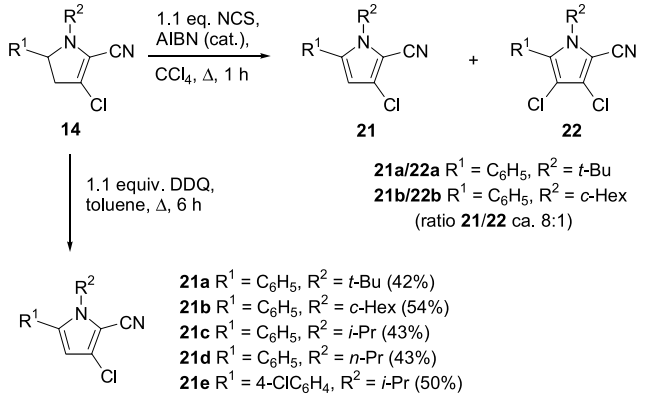
When handling pyrrolines **14** in wet solvents, often some hydrolysis product was formed. These products (**19**) were formed by electrophilic addition of a proton and subsequent attack of water, as shown in Scheme 3. In a more controlled manner, *cis*-substituted pyrrolidinones **19** could be obtained quantitatively by treatment of pyrrolines **14** with aqueous 2 M HCl in acetic acid at room temperature. When higher



Scheme 3.

temperatures were applied (Δ, 15 h), a partial isomerization of the *cis*-substituted pyrrolidinones **19** occurred towards the more stable *trans*-3-chloro-5-phenyl-pyrrolidinones **20** resulting in a mixture of isomers (ratios, see Scheme 3), which were separated by chromatography. The stereochemical assignments of **19** and **20** were performed by analysis of the coupling constants in ^{1}H NMR.

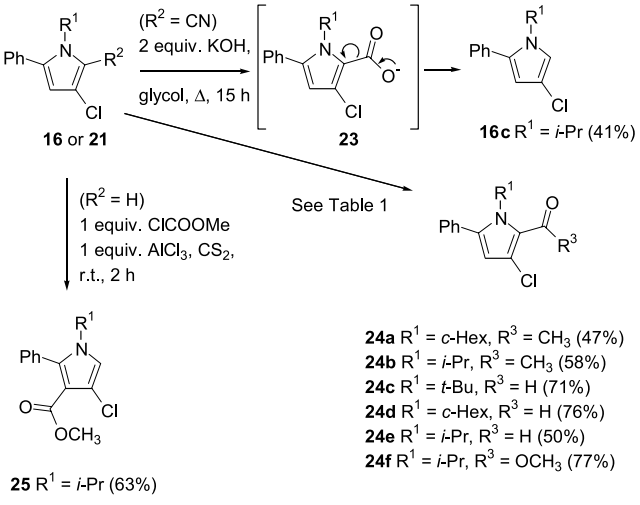
In order to synthesize 2-cyanopyrroles which form a class of compounds with specific physiological activities (e.g., insecticide **7**[12]), the pyrrolines **14** were reacted with NBS in tetrachloromethane to induce a radical bromination yielding halogenated pyrrolines as good precursors for pyrroles. Whereas the use of NBS only resulted in tarry reaction mixtures, the reaction with NCS at reflux temperatures in tetrachloromethane in the presence of a catalytic amount of AIBN yielded 2-cyanopyrroles **21a**,**b** in good yield. In the reaction mixture, also minor amounts of dichlorinated pyrroles **22** were detected (ratio **21**/**22** 8:1), which unfortunately could not be separated from the major pyrroles **21** by flash chromatography. During optimalization of the reaction by evaluation of various reaction conditions and follow up of the formed reaction products by GC-MS, it became clear that the formation of dichloropyrroles **22** started already at the early stage of the reaction, when still starting material is present. No improvement of the ratio **21**/**22** in favor of the monochloropyrrole **21** could be obtained (Scheme 4).



Scheme 4.

In a second approach, 2-pyrrolines **14** were oxidized towards the corresponding pyrroles using DDQ in toluene. This procedure yielded pyrroles in moderate yields. The presence of the cyano functionality at the 2-position leaves opportunities for functional group transformation to various other physiologically interesting pyrroles, for example, 2-acylpyrroles and derivatives. Attempts to hydrolyse the cyano function with aqueous base or acid did not result in pyrrole-2-carboxylate **23**. Starting material accompanied with decomposition products were recovered after treatment of **21c** with aq. 6 M HCl or 48% aq. HBr at reflux overnight. Alkaline treatment with 50% aq. KOH at various temperatures did not result in hydrolysis. Performing the reaction of **21c** with KOH in boiling glycol, hydrolysis of the cyano moiety and immediate decarboxylation occurred towards pyrrole **16c**.

To access 2-acylpyrroles **24a**,**b**, pyrroles **21b**,**c** were treated with methyllithium resulting in the corresponding methylimine, which was hydrolyzed by reaction with aq. 6 M HCl at room temperature (Scheme 5, Table 1). Pyrrole-2-carbaldehydes **24c–e** could be synthesized by electrophilic substitution of 2-unsubsituted pyrroles **16a–c**. Vilsmeier formylation of these pyrroles with DMF-$POCl_3$ yielded pyrrole-2-carbaldehydes **24c–e** together with a minor amount of the isomeric β-formylated pyrroles (10%–35%), which could be easily separated by flash chromatography. Due to the fact that the electrophilic substitution of pyrroles is kinetically driven to take place at the α-position, the major compounds were the 2-formylated pyrroles **24c–e**, as expected. Analogous reactions were evaluated to



Scheme 5.

Table 1. Synthesis of substituted 3-chloropyrroles **24** (Scheme 5)

Entry	Reaction conditions	Product
21b,**c** (R^2=CN)	(1) 1 equiv MeLi, THF, 0 °C, 30 min (2) excess aq. 6 M HCl, rt, 1 h	**24a**, **24b**
16a–c (R^2=H)	(1) 1.2 equiv $POCl_3$, DMF/CH_2Cl_2 (1:1), 0 °C, 5 h (2) excess aq. 1 M NaOH, rt, 15 min	**24c–e**[a]
16a (R^2=H)	1 equiv BuLi, 1 equiv ClCOOMe, THF, 0 °C, 2 h	**24f**

[a] Also the isomeric β-formylated pyrroles were formed (10–35%), which could easily be separated by flash chromatography.

synthesize pyrrole-2-carboxylates. Friedel-Crafts acylation using methyl chloroformate and aluminum(III) chloride in carbon disulfide yielded a mixture of carboxylated pyrroles **25** and **24f** with predominantly pyrrole **25** (ratio 1:4, resp.) Substitution at β-position of pyrroles has previously been observed when the nitrogen atom bears bulky groups.[19] Indeed, the carboxylation of pyrrole **16c** could be seen to be a little more sterically demanding as compared with the formylation, which shifts the ratio towards the 3-substituted pyrrole **25**. Also, under the used reaction conditions, rearrangements of pyrrole substituents are known to result in the thermodynamically most stable compounds (in casu pyrrole **25**).[19] The HSAB-theory as a rationale for selective acylations has only been demonstrated for pyrroles bearing electron withdrawing groups at nitrogen, where the use of a hard Lewis acid such as $AlCl_3$ promotes C-3 acylation.[20] However, *N*-alkyl- or *N*-unsubstituted pyrroles are generally acylated at C-2. To accomplish a carboxylation at C-2, deprotonation of the most acidic hydrogen of pyrrole **16c**, that is, the hydrogen at α-position, with BuLi and subsequent reaction with methyl chloroformate yielded exclusively methyl pyrrole-2-carboxylate **24f** in good yield.

In conclusion it can be stated that various interesting halogenated pyrroles can be synthesized in a straightforward manner from readily available 1-chlorocyclopropanecarbaldehydes.[16] In addition to the presence of a halogen at β-position of the synthesized pyrroles, other interesting substituents in relation to physiological activities, such as an aryl, cyano or carbonyl moiety at α-position were introduced to end up with highly substituted pyrroles with specific substitution patterns.

3. Experimental

^{1}H NMR spectra (270 MHz or 300 MHz) and ^{13}C NMR spectra (68 MHz or 75 MHz) were recorded with a Jeol JNM-EX 270 NMR spectrometer or a Jeol Eclipse FT 300 NMR spectrometer, respectively. Peak assignments were performed with the aid of the DEPT-technique, 2D-COSY and HETCOR spectra. IR assignments were obtained from a Perkin Elmer Spectrum One spectrophotometer. Mass spectra were recorded on an Agilent 1100 Series VL mass spectrometer (ES) or a HP 5973 MSD spectrometer (70 eV). Elemental analysis was performed on a Perkin Elmer 2400 Elemental Analyser and via a Callisto CF-Isotope Ratio Mass Spectrometer. Melting points were measured with a Büchi B-450 apparatus. Flash chromatography was carried out on a glass column with ACROS silica gel (particle size 0.035–0.07 mm, pore diameter ca. 6 nm). HRMS data were obtained with a VG Quattro II, ESI ionization (cone voltage 40 V).

3.1. Synthesis of 4-aryl-2,2,4-trichlorobutanals 12

As a representative example, the synthesis of 2,2,4-trichloro-4-phenylbutanal **12a** is described. A solution of 4.56 g (25.26 mmol) of 1-chloro-2-phenylcyclopropane-1-carbaldehyde **9a**[16] and 5.53 g (50.52 mmol, 2 equiv) of DMF-HCl in 5.00 g of DMF was heated to 40–60 °C. After reaction for 20 min, 10 mL of dry chloroform was added and chlorine gas was bubbled through the solution. During chlorination, the temperature was allowed to reach 65–70 °C. After 15–20 min, when no temperature increase was observed by addition of chlorine, the reaction was stopped and cooled down to room temperature. The reaction mixture was poured in 25 mL of concentrated HCl and extracted with chloroform (3×20 mL). The combined organic extracts were washed with an aqueous solution of sodium bisulfite and subsequently with concentrated HCl (20 mL). After drying ($MgSO_4$), filtration and evaporation of the solvent in vacuo, 2,2,4-trichloro-4-phenylbutanal **12a** was obtained which was purified by distillation (5.45 g, 81%).

3.1.1. 2,2,4-Trichloro-4-phenylbutanal 12a. Bp 80–85 °C, 0.05 mm Hg; yield 81%. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.11 (1H, d×d, J=15.5, 5.6 Hz, CH_aH$_b$), 3.32 (1H, d×d, J=15.5, 7.9 Hz, CH$_a$$H_b$), 5.22 (1H, d×d, J=7.9, 5.6 Hz, CH), 7.29–7.45 (5H, m, C_6H_5), 9.14 (1H, s, CHO). ^{13}C NMR (68 MHz, $CDCl_3$): δ 51.3 (CH_2), 58.0 (CH), 85.9 (CCl_2), 2×127.0 (2×CH_{ar}), 2×128.8 (2×CH_{ar}), 129.0 (CH_{ar}), 139.3 (C_{quat}), 183.5 (C=O). IR (NaCl) ν_{max} 1738 cm^{-1}. MS (70 eV) *m/z* (%) 250/52/54/56 (M^+, 10); 151/53 (31); 138/40 (97); 125/27 (100); 115 (70); 91 (46); 77 (10).

3.1.2. 2,2,4-Trichloro-4-(4-chlorophenyl)butanal 12b. Bp 105–109 °C, 0.05 mm Hg; yield 76%. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.07 (1H, d×d, J=15.2, 5.7 Hz, CH_aH$_b$), 3.28 (1H, d×d, J=15.2, 7.8 Hz, CH$_a$$H_b$), 5.20 (1H, d×d, J=7.8, 5.7 Hz, CH), 7.26–7.36 (4H, m, C_6H_4), 9.17 (1H, s, CHO). ^{13}C NMR (68 MHz, $CDCl_3$): δ 50.9 (CH_2), 57.3 (CH), 85.6 (CCl_2), 2×128.6 (2×CH_{ar}), 2×129.2 (2×CH_{ar}), 135.0 (C_{quat}), 138.1 (C_{quat}), 183.6 (C=O). IR (NaCl) ν_{max} 1742 cm^{-1}. MS (70 eV) *m/z* (%): no M+, 214/16/18 (M^+ −2Cl, 44); 173 (100); 150 (95); 126 (36).

3.2. Synthesis of 1-alkyl-5-aryl-3-chloro-2-cyano-2-pyrrolines 14

As a representative example, the synthesis of 3-chloro-2-cyano-1-isopropyl-5-phenyl-2-pyrroline **14c** is given. To a solution of 1.00 g (3.98 mmol) of 2,2,4-trichloro-4-phenylbutanal **12a** in 10 mL of dry diethyl ether was added 0.45 g (2.38 mmol, 0.6 equiv) of titanium(IV) chloride in 5 mL of dry pentane at 0 °C. After addition, the mixture was stirred for 15 min at 0 °C, followed by addition of 0.94 g (15.92 mmol, 4 equiv) of isopropylamine in 10 mL of dry diethyl ether. Cooling was stopped and the mixture was stirred for 15 h. Subsequently, the mixture was poured in 25 mL of aq. 1 M NaOH and rapidly extracted with diethyl ether (3×25 mL). The extract was dried (K_2CO_3/$MgSO_4$) and the solvent was removed in vacuo at 0–10 °C. The resulting aldimine **13** (R^1= C_6H_5, R^2= *i*-Pr) was used as such (without purification; purity >90%) to minimize decomposition. To 1.16 g (3.98 mmol) of *N*-(2,2,4-trichloro-4-phenyl-1-butylidene)isopropylamine in 20 mL of methanol was added 0.28 g (4.37 mmol, 1.1 equiv) of potassium cyanide and the mixture was heated under reflux for 4 h. After reaction, the mixture was poured in 20 mL of aq. 0.5 M NaOH and extracted with dichloromethane (3×20 mL). After drying ($MgSO_4$) and evaporation of the solvents, pyrroline **14c** was obtained and was purified by flash chromatography (0.57 g, 58%).

3.2.1. 1-*tert*-Butyl-3-chloro-2-cyano-5-phenyl-2-pyrroline 14a. Recrystallization (Et_2O/hexane, 1:3), yield 64%, mp 84–85 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.25 (9H, s, $C(CH_3)_3$), 2.49 (1H, d×d, J=17.8, 6.6 Hz, CH_aH_b), 3.43 (1H, d×d, J=17.8, 11.5 Hz, CH_aH_b), 4.67 (1H, d×d, J=11.5, 6.6 Hz, CH), 7.25–7.39 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 3×28.3 ($C(CH_3)_3$), 45.2 (CH_2), 57.7 ($C(CH_3)_3$), 61.5 (NCH), 113.9 (CN), 119.8 (C_{quat}), 2×125.7 (2×CH_{ar}), 126.1 (C_{quat}), 127.2 (CH_{ar}), 2×128.8 (2×CH_{ar}), 145.7 (C_{quat}). IR (KBr) ν_{max} 2226 cm^{-1}. MS (70 eV) *m/z* (%): 260/62 (M^+, 12); 204/206 (100); 169 (41); 168 (12); 115 (11); 57 (85).

3.2.2. 3-Chloro-1-cyclohexyl-2-cyano-5-phenyl-2-pyrroline 14b. Flash chromatography (hexane/EtOAc 9:1, R_f=0.54), yield 54%. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.01–1.83 (10H, m, 5×CH_2), 2.61 (1H, d×d, J=17.3, 10.9 Hz, CH_aH_b), 2.85–3.07 (1H, m, NCH), 3.23 (1H, d×d, J=17.3, 11.5 Hz, CH_aH_b), 4.57 (1H, d×d, J=11.5, 10.9 Hz, NCH), 7.28–7.36 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 25.4 (CH_2), 25.6 (CH_2), 25.9 (CH_2), 29.6 (CH_2), 30.4 (CH_2), 45.1 (CH_2CCl), 59.4 (NCH), 63.0 (NCH), 112.0 (CN), 120.7 (C_{quat}), 120.9 (C_{quat}), 2×126.5 (2×CH_{ar}), 127.6 (CH_{ar}), 2×128.6 (2×CH_{ar}), 143.1 (C_{quat}). IR (NaCl) ν_{max} 2228 cm^{-1}. MS (70 eV) *m/z* (%): 286/288 (M^+, 35); 243/245 (23); 204/206 (100); 169 (38); 83 (24); 55 (62).

3.2.3. 3-Chloro-2-cyano-1-isopropyl-5-phenyl-2-pyrroline 14c. Flash chromatography (hexane/EtOAc 4:1, R_f=0.60), yield 58%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (3H, d, J=6.9 Hz, CH_3), 1.23 (3H, d, J=6.9 Hz, CH_3), 2.63 (1H, dd, J=17.3, 11.5 Hz, CH_aH_b), 3.21 (1H, d×d, J=17.3, 11.2 Hz, CH_aH_b), 3.33 (1H, sept, J=6.9 Hz, $CH(CH_3)_2$), 4.48 (1H, d×d, J=11.2, 11.5 Hz, CH), 7.25–7.50 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 2×19.3 (2×CH_3), 45.1 (CH_2), 50.8 ($CH(CH_3)_2$), 63.0 (CH), 112.1 (CN), 120.8 (C_{quat}), 121.4 (C_{quat}), 2×126.7 (2×CH_{ar}), 127.7 (CH_{ar}), 2×128.7 (2×CH_{ar}), 142.9 (C_{quat}). IR (NaCl) ν_{max} 2227 cm^{-1}. MS (70 eV) *m/z* (%): 246/48 (M^+, 55); 231/33 (98); 169 (46); 142 (25); 91 (100); 77 (17).

3.2.4. 3-Chloro-2-cyano-5-phenyl-1-propyl-2-pyrroline 14d. This compound was unstable and could not be completely purified by flash chromatography. The spectra still contained impurities (ca. 10%); crude yield 79%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (3H, t, J=7.3 Hz, CH_3), 1.30–7.54 (2H, m, CH_2CH_3), 2.72 (1H, d×d, J=12.7, 17.1 Hz, $NCHCH_aH_b$), 2.83 (1H, t, J=6.6 Hz, NCH_aH_b), 2.85 (1H, t, J=6.6 Hz, NCH_aH_b), 3.15 (1H, d×d, J=11.0, 17.1 Hz, $NCHCH_aH_b$), 4.39 (1H, d×d, J=12.7, 11.0 Hz, $NCHCH_2$), 7.31–7.65 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.5 (CH_3), 20.3 (CH_2), 45.2 (CH_2), 52.7 (NCH_2), 67.8 (NCH), 111.8 (CN), 120.0 (C_{quat}), 2×127.3 (2×CH_{ar}), 127.8 (C_{quat}), 128.3 (CH_{ar}), 2×128.9 (2×CH_{ar}), 141.1 (C_{quat}). IR (NaCl) ν_{max} 2229 cm^{-1}. MS (ES+) *m/z* (%): 238/40 ($M^+ - CN + H_2O$, 100).

3.2.5. 3-Chloro-5-(4-chlorophenyl)-2-cyano-1-isopropyl-2-pyrroline 14e. Flash chromatography (hexane/EtOAc 4:1, R_f=0.60), yield 52%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.98 (3H, d, J=6.9 Hz, CH_3), 1.22 (3H, d, J=6.9 Hz, CH_3), 2.59 (1H, d×d, J=17.5, 11.1 Hz, CH_aH_b), 3.23 (1H, d×d, J=17.5, 11.4 Hz, CH_aH_b), 3.35 (1H, sept, J=6.9 Hz, $CH(CH_3)_2$), 4.49 (1H, d×d, J=11.1, 11.4 Hz, CH), 7.26–7.38 (4H, m, C_6H_4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.3 (CH_3), 19.6 (CH_3), 45.2 (CH_2), 51.1 ($CH(CH_3)_2$), 62.2 (NCH), 112.1 (CN), 121.6 (C_{quat}), 2×128.3 (2×CH_{ar}), 128.5 (C_{quat}), 2×129.0 (2×CH_{ar}), 133.5 (C_{quat}), 141.8 (C_{quat}). IR (NaCl) ν_{max} 2213, 1453 cm^{-1}. MS (70 eV) *m/z* (%): 280/82/84 (M^+, 68); 265/67/69 (100); 238 (34); 203 (34); 125 (57).

3.3. Synthesis of 1-alkyl-2-aryl-4-chloropyrroles 16

As a representative example, the synthesis of 4-chloro-1-isopropyl-2-phenylpyrrole **16c** is described. To 5.00 g (20.28 mmol) of 3-chloro-2-cyano-1-isopropyl-5-phenyl-2-pyrroline **14c** was added 41 mL (81.13 mmol, 4 equiv) of 2 M NaOMe in MeOH at room temperature. The mixture was refluxed for 2 h, cooled and poured in 100 mL of water. After extraction with dichloromethane (3×50 mL), the extract was dried ($MgSO_4$) and the solvents evaporated in vacuo. The resulting pyrrole was purified by flash chromatography (3.96 g, 83%).

3.3.1. 1-*tert*-Butyl-4-chloro-2-phenylpyrrole 16a. Flash chromatography (hexane/EtOAc 95:5, R_f=0.58), yield 52%, mp 77 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.39 (9H, s, $C(CH_3)_3$), 5.95 (1H, d, J=2.3 Hz, NC=CH), 6.82 (1H, d, J=2.3 Hz, NCH=C), 7.32–7.36 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 3×31.8 ($C(CH_3)_3$), 58.0 ($C(CH_3)_3$), 109.5 (C_{quat}), 111.5 (NC=CH), 116.0 (NCH=C), 2×127.5 (2×CH_{ar}), 127.9 (CH_{ar}), 2×131.7 (2×CH_{ar}), 133.8 (C_{quat}), 136.1 (C_{quat}). IR (KBr): ν_{max} 1368 cm^{-1}. MS (70 eV) *m/z* (%): 233/235 (M^+, 16); 178/180 (12); 177/179 (100); 57 (27). Anal. Calcd for $C_{14}H_{16}NCl$: C, 71.94; H, 6.90; N, 5.99. Found: C, 72.12; H, 7.09; N, 5.86.

3.3.2. 4-Chloro-1-cyclohexyl-2-phenylpyrrole 16b. Flash chromatography (hexane/EtOAc 95:5, R_f=0.56), yield 67%. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.14–1.98 (10H, m, 5×CH_2), 3.88–3.40 (1H, m, NCH), 6.05 (1H, d, J=1.9 Hz, NC=CH), 6.77 (1H, d, J=1.9 Hz, NCH=C), 7.29–7.44 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 25.3 (CH_2), 2×25.8 (2×CH_2), 2×34.8 (2×CH_2), 55.6 (NCH), 108.1 (NC=CH), 111.6 (C_{quat}), 115.2 (NCH=C), 127.4 (CH_{ar}), 2×128.5 (2×CH_{ar}), 2×129.2 (2×CH_{ar}), 132.7 (C_{quat}), 133.7 (C_{quat}). IR (NaCl): ν_{max} 1495, 1448, 1395 cm^{-1}. MS (70 eV) *m/z* (%): 259/61 (M^+, 38); 178/180 (22); 177/179 (100); 115 (10); 55 (29); 41 (22). Anal. Calcd for $C_{16}H_{18}NCl$: C, 73.98; H, 6.98; N, 5.39. Found: C, 73.75; H, 7.11; N, 5.28.

3.3.3. 4-Chloro-1-isopropyl-2-phenylpyrrole 16c. Flash chromatography (hexane/EtOAc 95:5, R_f=0.50), yield 83%, mp 42–43 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.35 (6H, d, J=6.6 Hz, $CH(CH_3)_2$), 4.40 (1H, sept, J=6.6 Hz, $CH(CH_3)_2$), 6.05 (1H, d, J=1.9 Hz, NC=CH), 6.78 (1H, d, J=1.9 Hz, NCH=C), 7.30–7.43 (5H, m, C_6H_5). ^{13}C NMR (68 MHz, $CDCl_3$): δ 2×23.9 (2×CH_3), 47.7 (CH), 108.2 (NC=CH), 111.8 (C_{quat}), 114.3 (NCH=C), 127.6 (CH_{ar}), 2×128.5 (2×CH_{ar}), 2×129.3 (2×CH_{ar}), 132.7 (C_{quat}), 133.8 (C_{quat}). IR (KBr) ν_{max} 1497, 1463, 1394 cm^{-1}. MS (70 eV) *m/z* (%): 219/21 (M^+, 53); 178/80 (12); 177/79 (100); 115 (21). Anal. Calcd for $C_{13}H_{14}NCl$: C, 71.07; H,

6.42; N, 6.38. Found: C, 71.06; H, 6.48; N, 6.34. HRMS: Calcd for $C_{13}H_{14}NCl$, 220.0888; Found 220.0901.

3.3.4. 4-Chloro-2-phenyl-1-propylpyrrole 16d. Flash chromatography (hexane/EtOAc 98:2, R_f=0.35), yield 59%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (3H, t, J=7.4 Hz, CH_3), 1.64 (2H, sext, J=7.4 Hz, CH_2CH_3), 3.80 (3H, t, J=7.4 Hz, NCH_2), 6.10 (1H, d, J=1.9 Hz, NC=CH), 6.69 (1H, d, J=1.9 Hz, NCH=C), 7.30–7.42 (5H, m, C_6H_5). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.3 (CH_3), 24.9 (CH_2), 49.2 (NCH_2), 108.8 (NC=*C*H), 111.7 (C_{quat}), 118.9 (NCH), 127.7 (CH_{ar}), 2×128.8 (2×CH_{ar}), 2×129.3 (2×CH_{ar}), 132.8 (C_{quat}), 134.5 (C_{quat}). IR (NaCl) ν_{max} 1603, 1500, 1475, 1324 cm^{-1}. MS (70 eV) *m/z* (%): 219/21 (M^+, 100); 190/92 (84); 177/79 (39); 155 (37); 142 (15); 115 (20). Anal. Calcd for $C_{13}H_{14}NCl$: C, 71.07; H, 6.42; N, 6.38. Found: C, 71.18; H, 6.60; N, 6.21. HRMS: Calcd for $C_{13}H_{14}NCl$, 220.0888; Found 220.0887.

3.3.5. 4-Chloro-2-(4-chlorophenyl)-1-isopropylpyrrole 16e. Flash chromatography (hexane/EtOAc 97:3, R_f=0.42), yield 77%, mp 61 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (6H, d, J=6.7 Hz, $CH(CH_3)_2$), 4.34 (1H, sept, J=6.7 Hz, $CH(CH_3)_2$), 6.04 (1H, d, J=1.9 Hz, NC=CH), 6.78 (1H, d, J=1.9 Hz, NCH=C), 7.21–7.26 (2H, m, 2×CH_{ar}), 7.34–7.39 (2H, m, 2×CH_{ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ 2×24.0 (2×CH_3), 47.9 (CH), 108.6 (NC=*C*H), 112.1 (C_{quat}), 114.8 (N*C*H=C), 2×128.9 (2×CH_{ar}), 2×130.6 (2×CH_{ar}), 131.1 (C_{quat}), 132.6 (C_{quat}), 133.7 (C_{quat}). IR (KBr) ν_{max} 1495, 1415, 1288 cm^{-1}. MS (70 eV) *m/z* (%) 253/55/57 (M^+, 76), 211/13/15 (100), 176 (27), 149 (19). Anal. Calcd for $C_{13}H_{13}NCl_2$: C, 61.43; H, 5.16; N, 5.51. Found: C, 61.59; H, 5.30; N, 5.37.

3.4. Hydrolysis of 1-alkyl-5-aryl-3-chloro-2-cyano-2-pyrrolines 14

As a representative example, the hydrolysis of 3-chloro-2-cyano-1-isopropyl-5-phenyl-2-pyrroline **14c** is described. 3-Chloro-2-cyano-1-isopropyl-5-phenyl-2-pyrroline **14c** (0.50 g, 2.03 mmol) was dissolved in 10 mL of a 1:1 mixture of acetic acid and aq. 2 M HCl. The solution was stirred at room temperature in a well vented fume hood (CAUTION: formation of HCN!) for 2 h and subsequently poured in 25 mL of water and extracted with dichloromethane (3×25 mL). After drying ($MgSO_4$) and evaporation of the solvent in vacuo, *cis*-substituted pyrrolidinone **19b** was obtained after flash chromatography (0.27 g, 57%). When the reaction temperature was raised to reflux temperatures (15 h) a mixture of *cis*- (**19b**) and *trans*-substituted pyrrolidinones (**20b**) was obtained in a ratio 1:3, respectively. The two isomers were easily separated by flash chromatography.

3.4.1. *cis*-1-*tert*-Butyl-3-chloro-5-phenyl-2-pyrrolidinone 19a. Recrystallization (Et_2O/hexane 1:1), yield 75%, mp 130 °C. ^{1}H NMR ($CDCl_3$): δ 1.35 (9H, s, $C(CH_3)_3$), 2.09 (1H, d×t, J=14.8, 2.5 Hz, CH_aH_b), 2.99 (1H, d×t, J=14.8, 9.0 Hz, CH_aH_b), 4.37 (1H, d×d, J=9.0, 2.5 Hz, CH), 4.90 (d×d, J=9.0, 2.5 Hz, CHCl), 7.27–7.36 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 3×27.9 (3×CH_3), 38.4 (CH_2), 54.6 (CH), 56.3 ($C(CH_3)_3$), 61.2 (CHCl), 2×126.2 (2×CH_{ar}), 127.8 (CH_{ar}), 2×128.8 (2×CH_{ar}), 143.9 (C_{quat}), 171.1 (C=O). IR (KBr) ν_{max} 1677, 1495, 1457 cm^{-1}. MS (ES+) *m/z* (%) 252/54 ($M+H^+$, 60), 196/98 (100). Anal. Calcd for $C_{14}H_{18}NOCl$: C, 66.79; H, 7.21; N, 5.56. Found: C, 66.62; H, 7.38; N, 5.42.

3.4.2. *cis*-3-Chloro-1-isopropyl-5-phenyl-2-pyrrolidinone 19b. Flash chromatography (hexane/EtOAc 4:1, R_f=0.31), yield 57%, mp 110–112 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (3H, d, J=6.9 Hz, CH_3), 1.25 (3H, d, J=6.9 Hz, CH_3), 2.20 (1H, d×t, J=14.3, 6.3 Hz, CH_aH_b), 3.06 (1H, d×d×d, J=14.3, 9.1, 7.8 Hz, CH_aH_b), 3.85 (1H, sept, J=6.9 Hz, $CH(CH_3)_2$), 4.45 (1H, d×d, J=9.1, 6.3 Hz, CH), 4.62 (1H, d×d, J=7.8, 6.3 Hz, CH), 7.32–7.42 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 19.6 (CH_3), 20.3 (CH_3), 39.5 (CH_2), 46.8 ($CH(CH_3)_2$), 54.3 (NCH), 60.3 (CHCl), 2×127.5 (2×CH_{ar}), 128.6 (CH_{ar}), 2×129.0 (2×CH_{ar}), 141.2 (C_{quat}), 170.6 (C=O). IR (KBr) ν_{max} 1677, 1419 cm^{-1}. MS (70 eV) *m/z* (%) 237/39 (M^+, 9), 222/24 (30), 202 (100), 117(84). Calcd for $C_{13}H_{16}NOCl$: C, 65.68; H, 6.78; N, 5.89. Found: C, 65.84; H, 6.95; N, 5.70.

3.4.3. *trans*-1-*tert*-Butyl-3-chloro-5-phenyl-2-pyrrolidinone 20a. Flash chromatography (hexane/EtOAc 9:1, R_f=0.31), yield 70%, mp 81–82 °C. ^{1}H NMR ($CDCl_3$): δ 1.37 (9H, s, $C(CH_3)_3$), 2.41 (1H, d×d×d, J=12.7, 7.9, 1.4 Hz, CH_aH_b), 2.58 (1H, d×d×d, J=12.7, 10.2, 8.5 Hz, CH_aH_b), 4.67 (1H, d×d, J=10.2, 7.9 Hz, CH), 4.92 (1H, d×d, J=8.5, 1.4 Hz, CHCl), 7.17–7.40 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 3×27.8 (3×CH_3), 41.0 (CH_2), 55.0 (CH), 56.0 ($C(CH_3)_3$), 59.8 (CHCl), 2×125.26 (2×CH_{ar}), 127.9 (CH_{ar}), 2×129.2 (2×CH_{ar}), 142.7 (C_{quat}), 170.6 (C=O). IR (KBr) ν_{max} 1690, 1455, 1398 cm^{-1}. MS (ES+) *m/z* (%) 252/54 ($M+H^+$, 75), 196/98 (100). Calcd for $C_{14}H_{18}NOCl$: C, 66.79; H, 7.21; N, 5.56. Found: C, 66.67; H, 7.43; N, 5.39.

3.4.4. *trans*-3-Chloro-1-isopropyl-5-phenyl-2-pyrrolidinone 20b. Flash chromatography (hexane/EtOAc 4:1, R_f=0.06), yield 32%. ^{1}H NMR ($CDCl_3$): δ 0.99 (3H, d, J=6.8 Hz, CH_3), 1.27 (3H, d, J=6.8 Hz, CH_3), 2.44 (1H, d×d×d, J=14.0, 7.4, 5.3 Hz, CH_aH_b), 2.60 (1H, d×d×d, J=14.0, 7.4, 5.3 Hz, CH_aH_b), 3.89 (1H, sept, J=6.8 Hz, $CH(CH_3)_2$), 4.60 (1H, d×d, J=7.4, 5.3 Hz, CH), 4.79 (1H, d×d, J=7.4, 5.3 Hz, CH), 7.33–7.42 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 19.7 (CH_3), 20.0 (CH_3), 40.7 (CH_2), 46.4 ($CH(CH_3)_2$), 55.1 (NCH), 59.7 (CHCl), 2×126.8 (2×CH_{ar}), 128.5 (CH_{ar}), 2×129.1 (2×CH_{ar}), 140.8 (C_{quat}), 170.6 (C=O). IR (NaCl) ν_{max} 1699, 1495, 1456, 1222 cm^{-1}. MS (70 eV) *m/z* (%) 237/39 (M^+, 13), 222/24 (30), 202 (100), 117(88). Anal. Calcd for $C_{13}H_{16}NOCl$: C, 65.68; H, 6.78; N, 5.89. Found: C, 65.90; H, 6.95; N, 6.03.

3.5. Synthesis of 1-alkyl-5-aryl-3-chloro-2-cyanopyrroles 21

To a solution of 0.54 g (2.19 mmol) of 3-chloro-2-cyano-1-isopropyl-5-phenyl-2-pyrroline **14c** in 20 mL of dry toluene was added 0.55 g (2.41 mmol, 1.1 equiv) of DDQ. The resulting mixture was refluxed for 6 h. After reaction, the mixture was diluted with 20 mL of pentane and the formed heterogeneous mixture was filtered over Celite®. The filtrate was diluted with 50 mL of water and extracted with pentane (3×50 mL). The combined extracts were

dried ($MgSO_4$), filtered and the solvent was removed in vacuo. Purification of the synthesized pyrrole was performed by a fast flash chromatography over a short (5 cm) column of silica gel (0.23 g, 43%).

3.5.1. 1-*tert*-Butyl-3-chloro-2-cyano-5-phenylpyrrole 21a. Flash chromatography (hexane/EtOAc 9:1, R_f= 0.43), yield 42%, mp 119–120 °C. ^{1}H NMR ($CDCl_3$): δ 1.56 (9H, s, $C(CH_3)_3$), 5.96 (1H, s, CH), 7.26–7.46 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 3×32.3 (3×CH_3), 62.2 ($C(CH_3)_3$), 102.1 (CN), 112.4 (CH), 114.4 (C_{quat}), 125.4 (C_{quat}), 2×127.9 (2×CH_{ar}), 128.8 (CH_{ar}), 2×130.3 (2×CH_{ar}), 134.7 (C_{quat}), 139.8 (C_{quat}). IR (KBr) ν_{max} 2211, 1325 cm^{-1}. MS (70 eV) *m/z* (%) 258/60 (M^+, 5), 202/04 (100), 57 (20). Calcd for $C_{15}H_{15}N_2Cl$: C, 69.63; H, 5.84; N, 10.83. Found: C, 69.75; H, 5.96; N, 10.68.

3.5.2. 3-Chloro-1-cyclohexyl-2-cyano-5-phenylpyrrole 21b. Flash chromatography (hexane/EtOAc 4:1, R_f= 0.26), yield 54%, mp 125 °C. ^{1}H NMR ($CDCl_3$): δ 1.12–2.32 (10H, m, 5×CH_2), 4.05 (1H, t×t, J=12.4, 3.7 Hz, CHN), 6.12 (1H, s, CH), 7.26–7.32 and 7.41–7.51 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 24.7 (CH_2), 2×25.9 (2×CH_2), 2×32.6 (2×CH_2), 58.2 (CHN), 100.6 (CN), 109.5 (CH), 113.3 (C_{quat}), 124.7 (C_{quat}), 2×128.9 (2×CH_{ar}), 129.0 (CH_{ar}), 2×129.3 (2×CH_{ar}), 130.7 (C_{quat}), 139.4 (C_{quat}). IR (NaCl) ν_{max} 2212, 1333 cm^{-1}. MS (ES+) *m/z* (%) 285/87 ($M+H^+$, 100), 203/04 (15). Anal. Calcd for $C_{17}H_{17}N_2Cl$: C, 71.70; H, 6.02; N, 9.84. Found: C, 71.51; H, 6.14; N, 9.88.

3.5.3. 3-Chloro-2-cyano-1-isopropyl-5-phenylpyrrole 21c. Flash chromatography (hexane/EtOAc 95:5, R_f= 0.25), yield 43%, mp 68–71 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (6H, d, J=6.9 Hz, $CH(CH_3)_2$), 4.52 (1H, sept, J=6.9 Hz, $CH(CH_3)_2$), 6.11 (1H, s, NC=CH), 7.28–7.34 (2H, m, 2×CH_{ar}), 7.43–7.49 (3H, m, 3×CH_{ar}). ^{13}C NMR ($CDCl_3$): δ 2×22.5 (2×CH_3), 50.3 (NCH), 100.0 (CN), 109.6 (NC=*C*H), 113.2 (C_{quat}), 124.9 (C_{quat}), 2×128.8 (2×CH_{ar}), 129.2 (CH_{ar}), 2×129.6 (2×CH_{ar}), 130.7 (C_{quat}), 139.4 (C_{quat}). IR (KBr) ν_{max} 2211, 1454 cm^{-1}. MS (70 eV) *m/z* (%) 244/46 (M^+, 38), 202/4 (100), 166 (9), 140 (16). Calcd for $C_{14}H_{13}N_2Cl$: C, 68.71; H, 5.35; N, 11.45. Found: C, 68.88; H, 5.49; N, 11.30.

3.5.4. 3-Chloro-2-cyano-5-phenyl-1-propylpyrrole 21d. Flash chromatography (hexane/EtOAc 9:1, R_f=0.35), yield 43%. ^{1}H NMR ($CDCl_3$): δ 0.79 (3H, t, J=7.5 Hz, CH_3), 1.70 (2H, sext, J=7.5 Hz, CH_3CH_2), 3.98 (2H, t, J=7.5 Hz, NCH_2), 6.18 (1H, s, NC=*C*H), 7.32–7.49 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 10.9 (CH_3), 24.5 (CH_2), 48.7 (NCH_2), 103.5 (CN), 110.1 (NC=*C*H), 112.4 (C_{quat}), 123.1 (C_{quat}), 2×129.0 (2×CH_{ar}), 3×129.2 (2×CH_{ar} and C_{quat}), 130.6 (CH_{ar}), 139.7 (C_{quat}). IR (NaCl) ν_{max} 2217, 1460, 1337 cm^{-1}. MS (70 eV) *m/z* (%) 244/46 (M^+, 83), 202/4 (100), 190 (23), 180 (24), 166 (13), 140 (20). Anal. Calcd for $C_{14}H_{13}N_2Cl$: C, 68.71; H, 5.35; N, 11.45. Found: C, 68.59; H, 5.47; N, 11.37.

3.5.5. 3-Chloro-5-(4-chlorophenyl)-2-cyano-1-isopropylpyrrole 21e. Flash chromatography (hexane/EtOAc 97:3, R_f=0.19), yield 50%, mp 102 °C. ^{1}H NMR ($CDCl_3$): δ 1.59 (6H, d, J=6.9 Hz, $CH(CH_3)_2$), 4.46 (1H, sept, J=6.9 Hz, $CH(CH_3)_2$), 6.11 (1H, s, NC=CH), 7.22–7.28 (2H, m, 2×CH_{ar}), 7.40–7.48 (2H, m, 2×CH_{ar}). ^{13}C NMR ($CDCl_3$): δ 2×22.5 (2×CH_3), 50.5 (CH), 100.4 (CN), 109.8 (NC=*C*H), 113.0 (C_{quat}), 124.9 (C_{quat}), 129.0 (C_{quat}), 2×129.3 (2×CH_{ar}), 2×130.8 (2×CH_{ar}), 135.5 (C_{quat}), 138.1 (C_{quat}). IR (KBr) ν_{max} 2215, 1449, 1337 cm^{-1}. MS (70 eV) *m/z* (%) 278/80/82 (M^+, 54), 236/38/40 (100), 201/3 (16), 174 (21). Calcd for $C_{14}H_{12}N_2Cl_2$: C, 60.23; H, 4.33; N, 10.03. Found: C, 60.12; H, 4.50; N, 9.89.

3.6. Synthesis of 2-acetylpyrroles 24a,b

A solution of 1.6 M MeLi in diethyl ether (2.60 mL, 4.09 mmol, 1 equiv) was added to a solution of 1.00 g (4.09 mmol) of 3-chloro-2-cyano-1-isopropyl-5-phenylpyrrole **21c** in 50 mL of dry THF under N_2-atmosphere. After stirring for 2 h at room temperature, the reaction mixture was diluted with 50 mL of water and subsequently extracted with diethyl ether (3×25 mL). Drying of the solvents ($MgSO_4$/K_2CO_3), filtration and evaporation of the solvents in vacuo yielded a mixture of 2-acetylpyrrole **21c** and the corresponding imine. Separation of these two products was not possible, because the imine hydrolyzed towards **21c** during flash chromatography. The mixture was dissolved in 25 mL of dichloromethane and 10 mL of aq. 6 M HCl was added. The resulting biphasic solution was stirred at room temperature for 2 h and subsequently extracted with dichloromethane (3× 25 mL). Standard work up yielded 2-acetylpyrrole **24b**, which was purified by flash chromatography (0.62 g, 58%).

3.6.1. 2-Acetyl-3-chloro-1-cyclohexyl-5-phenylpyrrole 24a. Flash chromatography (hexane/EtOAc 4:1, R_f= 0.70), yield 47%. ^{1}H NMR ($CDCl_3$): δ 1.07–2.06 (10H, m, 5×CH_2), 2.66 (3H, s, CH_3), 4.32–4.34 (1H, m, NCH), 6.11 (1H, s, CH), 7.32–7.45 (5H, m, C_6H_5). ^{13}C NMR ($CDCl_3$): δ 25.0 (CH_2), 2×26.3 (2×CH_2), 31.7 (CH_3), 2×32.4 (2×CH_2), 60.5 (CHN), 112.0 (CH), 121.2 (C_{quat}), 2×128.3 (2×CH_{ar}), 128.7 (CH_{ar}), 129.0 (C_{quat}), 2×129.8 (2×CH_{ar}), 132.9 (C_{quat}), 141.5 (C_{quat}), 189.2 (C=O). IR (NaCl) ν_{max} 1654 cm^{-1}. MS (ES+) *m/z* (%) 302/04 ($M+H^+$, 65), 220/22 (100). Anal. Calcd for $C_{18}H_{20}NOCl$: C, 71.63; H, 6.68; N, 4.64. Found: C, 71.86; H, 6.52; N, 4.60.

3.6.2. 2-Acetyl-3-chloro-1-isopropyl-5-phenylpyrrole 24b. Flash chromatography (hexane/EtOAc 97:3, R_f= 0.20), yield 58%, mp 42 °C. ^{1}H NMR ($CDCl_3$): δ 1.42 (6H, d, J=7.0 Hz, $CH(CH_3)_2$), 2.67 (3H, s, CH_3CO), 4.73 (1H, sept, J=7.0 Hz, $CH(CH_3)_2$), 6.12 (1H, s, NC=*C*H), 7.34–7.38 (2H, m, 2×CH_{ar}), 7.41–7.47 (3H, m, 3×CH_{ar}). ^{13}C NMR ($CDCl_3$): δ 2×22.4 (2×CH_3), 31.7 (CH_3), 51.8 (CH), 111.9 (NC=*C*H), 121.5 (C_{quat}), 2×128.5 (2×CH_{ar}), 128.9 (CH_{ar}), 2×129.9 (2×CH_{ar}), 132.7 (C_{quat}), 141.4 (C_{quat}), 189.0 (C=O). IR (KBr) ν_{max} 1651, 1445 cm^{-1}. MS (70 eV) *m/z* (%) 261/63 (M^+, 58), 219/21 (50), 204/6 (100), 149 (25). Anal. Calcd for $C_{15}H_{16}NOCl$: C, 68.83; H, 6.16; N, 5.35. Found: C, 69.01; H, 6.31; N, 5.54.

3.7. Synthesis of 2-formylpyrroles 24c,d,e

To a solution of 1.00 g (4.56 mmol) of 3-chloro-1-isopropyl-5-phenylpyrrole **16c** in 20 mL of DMF and 20 mL of dry dichloromethane was added a solution of 0.84 g (5.47 mmol, 1.2 equiv) of $POCl_3$ in 5 mL of dry

dichloromethane at 0 °C under N_2-atmosphere. After stirring for 5 h, the reaction mixture was diluted with 25 mL of aq. 1 M NaOH at 0 °C and stirred for 15 min at the same temperature. The mixture was poured in 25 mL of water and extracted with dichloromethane (3×30 mL). The extracts were dried over $MgSO_4$ and after filtration, the solvent was removed in vacuo. To remove residual DMF, additional evaporation at high vacuum (0.01 mm Hg) was applied. This procedure yielded a mixture of 2- and 4-formylated pyrroles (ratio 65:35, calculated from ^{1}H NMR spectra, which were easily separated by flash chromatography.

3.7.1. 1-*tert*-Butyl-3-chloro-2-formyl-5-phenylpyrrole 24c. Flash chromatography (hexane/EtOAc 9:1, R_f=0.36), yield 71%, mp 103 °C. ^{1}H NMR ($CDCl_3$): δ 1.52 (9H, s, $C(CH_3)_3$), 6.04 (1H, s, CH), 7.25–7.42 (5H, m, C_6H_5), 9.80 (1H, s, CHO). ^{13}C NMR ($CDCl_3$): δ 32.2 ($C(CH_3)_3$), 62.5 ($C(CH_3)_3$), 113.9 (CH), 2×128.1 (2×CH_{ar}), 128.3 (C_{quat}), 128.7 (CH_{ar}), 2×129.5 (2×CH_{ar}), 131.0 (C_{quat}), 135.6 (C_{quat}), 144.7 (C_{quat}), 177.3 (C=O). IR (KBr) ν_{max} 1655 cm^{-1}. MS (ES+) *m/z* (%) 262/64 ($M+H^+$, 5), 206/08 (100). Calcd for $C_{15}H_{16}NOCl$: C, 68.83; H, 6.16; N, 5.35. Found: C, 68.71; H, 6.32; N, 5.24.

3.7.2. 3-Chloro-1-cyclohexyl-2-formyl-5-phenylpyrrole 24d. Flash chromatography (hexane/EtOAc 9:1, R_f=0.35), yield 76%, mp 142-143 °C. ^{1}H NMR ($CDCl_3$): δ 0.81–2.42 (10H, m, 5×CH_2), 4.16–4.28 (1H, m, CHN), 6.19 (1H, s, CH), 7.29–7.36 (2H, m, 2×CH_{ar}), 7.44–7.48 (3H, m, 3×CH_{ar}), 9.79 (1H, s, CHO). ^{13}C NMR ($CDCl_3$): δ 24.5 (CH_2), 2×26.0 (2×CH_2), 2×31.0 (2×CH_2), 59.1 (CHN), 111.2 (CH), 126.1 (C_{quat}), 2×128.6 (2×CH_{ar}), 129.1 (CH_{ar}), 2×129.4 (2×CH_{ar}), 130.9 (C_{quat}), 131.7 (C_{quat}), 143.0 (C_{quat}), 176.9 (C=O). IR (KBr) ν_{max} 1667 cm^{-1}. MS (ES+) *m/z* (%) 288/90 ($M+H^+$, 100). Calcd for $C_{17}H_{18}NOCl$: C, 70.95; H, 6.30; N, 4.87. Found: C, 71.11; H, 6.48; N, 4.76.

3.7.3. 3-Chloro-2-formyl-1-isopropyl-5-phenylpyrrole 24e. Flash chromatography (hexane/EtOAc 9:1, R_f=0.54), yield 50%, mp 59 °C. ^{1}H NMR ($CDCl_3$): δ 1.49 (6H, d, J=6.9 Hz, $CH(CH_3)_2$), 4.64 (1H, sept, J=6.9 Hz, $CH(CH_3)_2$), 6.18 (1H, s, NC=CH), 7.33–7.37 (2H, m, 2×CH_{ar}), 7.43–7.50 (3H, m, 3×CH_{ar}), 9.80 (1H, s, CHO). ^{13}C NMR ($CDCl_3$): δ 2×21.4 (2×CH_3), 50.9 (CH), 111.1 (NC=*C*H), 126.1 (C_{quat}), 2×128.8 (2×CH_{ar}), 129.2 (C_{quat}), 129.3 (CH_{ar}), 2×129.5 (2×CH_{ar}), 131.6 (C_{quat}), 142.8 (C_{quat}), 176.7 (C=O). IR (KBr) ν_{max} 2845, 2806, 1655, 1450, 1207 cm^{-1}. MS (70 eV) *m/z* (%) 247/49 (M^+, 83), 205 (100), 149 (31), 140 (18), 115 (16). Anal. Calcd for $C_{14}H_{14}NOCl$: C, 67.88; H, 5.70; N, 5.65. Found: C, 68.01; H, 5.82; N, 5.67. HRMS: Calcd for $C_{14}H_{14}NOCl$, 248.0837; Found 248.0831.

3.8. Synthesis of methyl (3-chloro-1-isopropyl-5-phenylpyrrol-2-yl)carboxylate 24f

3-Chloro-1-isopropyl-5-phenylpyrrole **16c** (0.50 g, 2.28 mmol) was dissolved in 25 mL of dry THF and cooled to −78 °C. Under N_2-atmosphere, 0.92 mL (2.28 mmol, 1 equiv) of a 2.5 M BuLi solution in hexane was added and the mixture was stirred at 0 °C. After 30 min, 0.21 g (2.28 mmol, 1 equiv) of methyl chloroformate in 5 mL of dry THF was added via a syringe and the mixture was stirred for 2 h at 0 °C. After reaction, 25 mL of water was added, the organic phase was separated and the aqueous phase was extracted with diethyl ether (3×25 mL). After drying ($MgSO_4$) and evaporation of the solvents, compound **24f** was recrystallized yielding 0.49 g (77%) of pure compound.

3.8.1. Methyl (3-chloro-1-isopropyl-5-phenylpyrrol-2-yl)carboxylate 24f. Recrystallization (pentane), yield 77%, mp 66 °C. ^{1}H NMR ($CDCl_3$): δ 1.47 (6H, d, J=7.0 Hz, $CH(CH_3)_2$), 3.90 (3H, s, OCH_3), 4.71 (1H, sept, J=7.0 Hz, $CH(CH_3)_2$), 6.11 (1H, s, NC=CH), 7.33–7.38 (2H, m, 2×CH_{ar}), 7.40–7.47 (3H, m, 3×CH_{ar}). ^{13}C NMR ($CDCl_3$): δ 2×22.4 (2×CH_3), 51.4 and 51.5 (NCH and OCH_3), 111.5 (NC=*C*H), 119.3 (C_{quat}), 120.9 (C_{quat}), 2×128.5 (2×CH_{ar}), 128.8 (CH_{ar}), 2×129.9 (2×CH_{ar}), 132.7 (C_{quat}), 140.1 (C_{quat}), 161.5 (C=O). IR (KBr) ν_{max} 1697, 1454, 1223 cm^{-1}. MS (70 eV) *m/z* (%) 277/79 (M^+, 100), 246/48 (36), 235/37 (47), 204/6 (96). Calcd for $C_{15}H_{16}NO_2Cl$: C, 64.87; H, 5.81; N, 5.04. Found: C, 64.99; H, 6.03; N, 4.91.

3.9. Synthesis of methyl (4-chloro-2-phenylpyrrol-3-yl)-carboxylate 25

3-Chloro-1-isopropyl-5-phenylpyrrole **16c** (0.10 g, 0.45 mmol) was dissolved in 10 mL of carbon disulfide and cooled to 0 °C. To the cold solution, 45 mg (0.48 mmol, 1.05 equiv) of methyl chloroformate and 64 mg (0.48 mmol, 1.05 equiv) of aluminum(III) chloride were added under N_2-atmosphere and the mixture was allowed to reach room temperature. After stirring for 4 h, the mixture was diluted with 20 mL of water and extracted with dichloromethane (3×25 mL). After drying ($MgSO_4$), the solvents were removed in vacuo, in a fume hood (CS_2!) yielding a mixture of methoxycarbonylated pyrroles **25** and **24f** (ratio 1:4, resp.), which were separated by flash chromatography.

3.9.1. Synthesis of methyl 4-chloro-2-phenylpyrrol-3-yl carboxylate 25. Flash chromatography (hexane/EtOAc 9:1, R_f=0.23), yield 63%, mp 109 °C. ^{1}H NMR ($CDCl_3$): δ 1.30 (6H, d, J=6.7 Hz, $CH(CH_3)_2$), 3.60 (3H, s, OCH_3), 4.10 (1H, sept, J=6.7 Hz, $CH(CH_3)_2$), 6.79 (1H, s, NCH=C), 7.26–7.30 (2H, m, 2×CH_{ar}), 7.42–7.46 (3H, m, 3×CH_{ar}). ^{13}C NMR ($CDCl_3$): δ 2×23.7 (2×CH_3), 48.2 (OCH_3), 50.9 (CH), 110.4 (C_{quat}), 113.2 (C_{quat}), 115.3 (N*C*H=C), 2×128.2 (2×CH_{ar}), 128.7 (CH_{ar}), 2×130.5 (2×CH_{ar}), 131.7 (C_{quat}), 138.1 (C_{quat}), 164.1 (C=O). IR (KBr): ν_{max} 1695, 1543, 1478, 1256 cm^{-1}. MS (70 eV) *m/z* (%) 277/79 (M^+, 68), 246/48 (13), 235/37 (45), 203/5 (100), 140 (64). Calcd for $C_{15}H_{16}NO_2Cl$: C, 64.87; H, 5.81; N, 5.04. Found: C, 64.71; H, 5.95; N, 4.88.

Acknowledgements

The authors are indebted to the IWT (Flemish Institute for the Promotion of Scientific-Technological Research in Industry), the FWO-Flanders and Ghent University (GOA) for financial support.

References and notes

1. (a) Arima, K.; Imanaka, H.; Kousaka, M.; Fukuda, A.; Tamura, G. *J. Antibiot.* **1965**, *18*, 201. (b) Leroux, P. *Pestic. Sci.* **1996**, *47*, 191. (c) Di Santo, R.; Costi, R.; Artico, M.; Massa, S.; Lampis, G.; Deidda, D.; Pompei, R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2931.
2. Tokunaga, T.; Hume, W. E.; Kitoh, M.; Nagata, R.; Kishino, M.; Nakagawa, T.; Nagamine, J., Taiji, M. *PCT Int. Appl.* **2003**, WO 2003063861 A1 20030807; *Chem. Abstr.* **2003**, *139*, 159972.
3. Laatsch, H.; Pudleiner, H. *Liebigs Ann. Chem.* **1989**, 863.
4. (a) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574. (b) Namba, K.; Shinada, T.; Teramoto, T.; Ohfune, Y. *J. Am. Chem. Soc.* **2000**, *122*, 10708.
5. (a) Hayakawa, Y.; Kawakami, K.; Seto, H.; Furihata, K. *Tetrahedron Lett.* **1992**, *33*, 2701. (b) Fürstner, A.; Weintritt, H. *J. Am. Chem. Soc.* **1998**, *120*, 2817.
6. Hoffmann, H.; Lindel, T. *Synthesis* **2003**, *12*, 1753.
7. Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron* **1996**, *52*, 13713.
8. Cafieri, F.; Carnuccio, R.; Fattorusso, E.; Taglialatela-Scafati, O.; Vallefuoco, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2283.
9. Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 501.
10. (a) De Kimpe, N.; Abbaspour Tehrani, K.; Stevens, C; De Cooman, P. *Tetrahedron* **1997**, *53*, 3693 and references cited therein. (b) *Pyrroles, Part II, The Synthesis, Reactivity and Physical Properties of Substituted Pyrroles*; Jones, R. A., Ed.; Wiley: New York, 1992. (c) Ferreira, V. F.; de Souza, M. C. B. V.; Cunha, A. C.; Pereira, L. O. R.; Fereirra, M. L. G. *Org. Prep. Proc. Int.* **2001**, *33*, 411. (d) Abbaspour Tehrani, K.; Borremans, D.; De Kimpe, N. *Tetrahedron* **1999**, 4133 and references cited therein. (e) Knight, D. W.; Redfern, A. L.; Gilmore, J. *J. Chem. Soc., Perkin Trans. 1* **2002**, *5*, 622.
11. *Agrochemicals from Natural Products*; Godfrey, C. R. A., Ed.; Marcel Dekkers: New York, 1995.
12. Laatsch, H.; Renneberg, B.; Hanefeld, U.; Kellner, M.; Pudleiner, H.; Hamprecht, G.; Kraemer, H.-P.; Anke, H. *Chem. Pharm. Bull.* **1995**, *43*, 537.
13. (a) Reid, B. L.; Farlow, R. A. U.S. Patent US 6077863 Appl. PV55054, 2000; *Chem. Abstr.* **2000**, *133*, 27665. (b) Reid, B. L.; Farlow, R. A. *U.S. Patent* US 6071951 Appl. PV55069, 2000; *Chem. Abstr.* **2000**, *133*, 1766.
14. (a) Quiclet-Sire, B.; Wendeborn, F.; Zard, S. Z. *Chem. Commun.* **2002**, 2214. (b) Quiclet-Sire, B.; Thévenot, I.; Zard, S. Z. *Tetrahedron Lett.* **1995**, *36*, 9469. (c) Adamczyk, M.; Reddy, R. E. *Tetrahedron Lett.* **1995**, *36*, 7983.
15. (a) Nadim, A. M.; Romashin, Y. N.; Kulinkovich, O. G. *Zh. Org. Khim.* **1991**, *27*, 1621. *Chem. Abstr.* **1991**, *116*, 128565. (b) Wasserman, H. H.; Dion, R. P. *Tetrahedron Lett.* **1983**, *24*, 3409. (c) Brinker, U. H.; Boxberger, M. *J. Chem. Res., Synopses* **1983**, *99*, 87973. (d) Toshibe, S.; Kawai, O.; Wada, K. Jpn Kokai Tokkyo Koho JP05001027 A2 19930108, 1993; *Chem. Abstr.* **1993**, *119*, 117097. (e) Kagabu, S.; Tsuji, H.; Kawai, I.; Ozeki, H. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 341. (f) Barluenga, J.; Tomas, M.; Lopez-Pelegrin, J. A.; Rubio, E. *J. Chem. Soc., Chem. Commun.* **1995**, 665.
16. Verniest, G.; Bombeke, F.; Kulinkovich, O. G.; De Kimpe, N. *Tetrahedron Lett.* **2002**, *43*, 599.
17. (a) Dieter, R. K.; Pounds, S. *J. Org. Chem.* **1982**, *42*, 3174. (b) Huang, H.; Forsyth, C. J. *Tetrahedron* **1997**, *53*, 16341.
18. De Buyck, L.; Menke, N.; Schamp, N. *Bull. Soc. Chim. Belg.* **1990**, *99*, 121.
19. Kakushima, M.; Frenette, R. *J. Org. Chem.* **1984**, *49*, 2025.
20. Xiao, D.; Schreier, J. A.; Cook, J. H.; Seybold, P. G.; Ketcha, D. M. *Tetrahedron Lett.* **1996**, *37*, 1523.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2889–2896

Synthesis and photoresponsive study of azobenzene centered polyamidoamine dendrimers

Samaresh Ghosh,[a] Ajit K. Banthia[a,*] and Zhu Chen[b]

[a]*Materials Science Centre, Indian Institute of Technology, Kharagpur 721302, India*
[b]*Advanced Materials Laboratory, 1001 University Blvd. SE Suite-100, Albuquerque, NM 87106, USA*

Received 4 October 2004; revised 16 December 2004; accepted 14 January 2005

Abstract—A new series of novel polyamidoamine (PAMAM) dendrimers **4**, **5** and **6** possessing azobenzene units specifically at the core were prepared and their reversible *trans*/*cis* photoisomerization properties were studied. PAMAM dendritic wedges as well as azo-based PAMAM dendrimers were fully characterized by means of FT-IR, NMR (^{1}H and ^{13}C), mass spectrometry (MALDI-MS), thermogravimetric and elemental analysis.

1. Introduction

Dendrimers[1] are a new class of macromolecules characterized by their highly branched, compartmentalized, three dimensional architecture. In particular, there is considerable current interest in the synthesis of photoresponsive dendritic macromolecular systems[2] with precisely placed photostimulable units within their structures. In this context, azobenzene-type compounds undergo efficient and fully reversible photoisomerisation reaction (*trans*/*cis*) and have been the subject of intensive research in particular for the construction of photoswitchable devices.[3,4] Therefore, precise placement of photochromic azobenzene moieties within a dendrimer interior can be considered to represent an intriguing scaffold for photoresponsive materials.[5–7] Furthermore, more predictable control of well-defined photoinduced configurational as well as constitutional changes in the dendritic system should be possible, allowing reversible alteration of function.

The polyamidoamine (PAMAM) family of starburst dendrimers[8,9] are a class of macromolecules that comprise the fastest growing areas of research within the diverse pool of branched polymeric architectures because of their definite molecular composition and constitution. Constructing a PAMAM dendrimer around a azobenzene core could profitably control the photoinduced configurational as well as conformational changes in the macromolecular structure and reversibly alter its function.

Even though McGrath et al. and others have recently reported[5–7] several examples of azobenzene based dendrimers, azobenzene-based photoresponsive polyamidoamine (PAMAM) dendrimers are little explored. Therefore, continuing our investigations of photoresponsive PAMAM dendritic macromolecules,[9a,b,c] we report herein in detail the synthesis and photoresponsive study of our previously reported PAMAM dendrimers possessing an azobenzene core unit.

2. Results and discussion

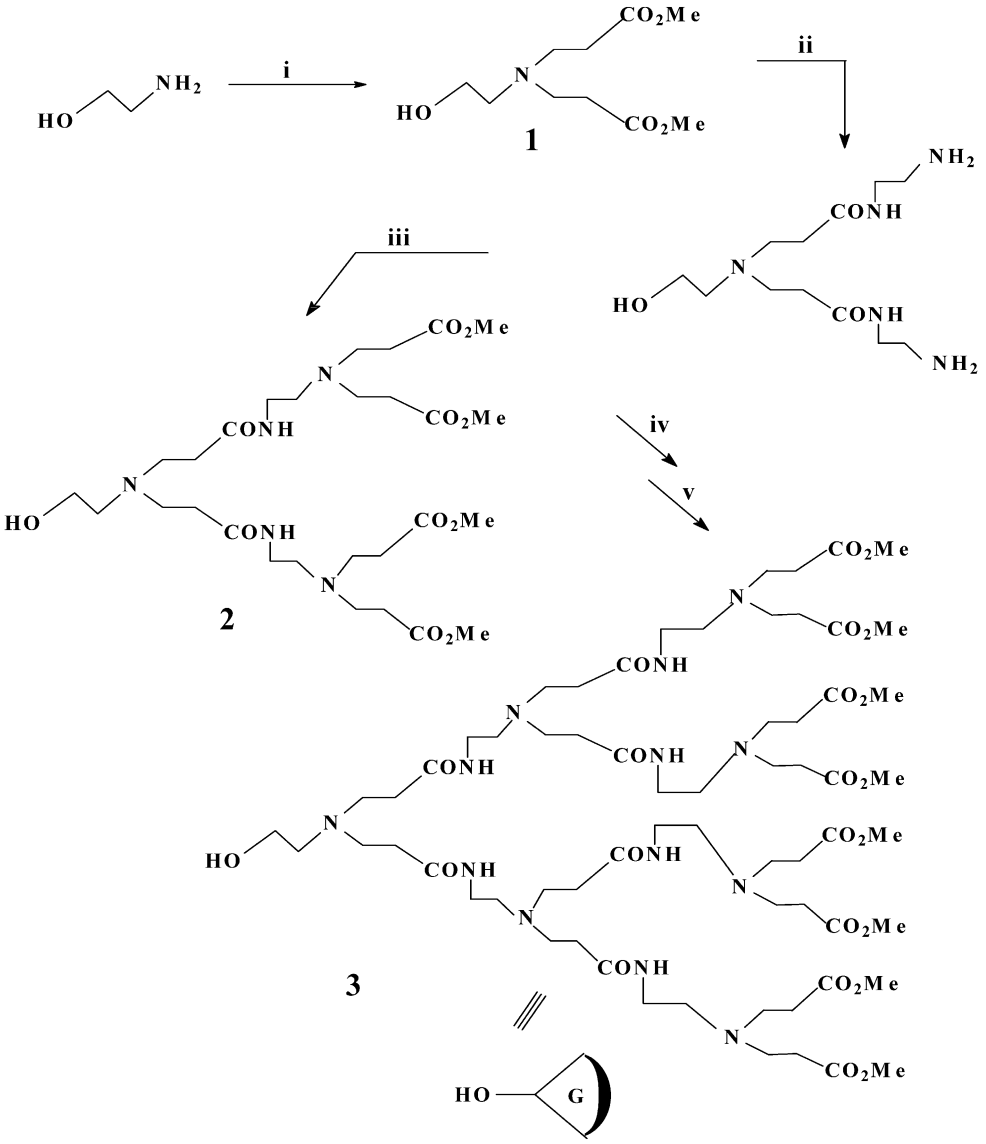
2.1. Synthesis of azobenzene centered PAMAM dendrimers

Our strategy for the divergent synthesis of ester-terminated PAMAM dendritic wedges (**1**, **2**, **3**) involves the initial Michael reaction of ethanolamine with methyl acrylate followed by exhaustive amidation of the resulting esters with a large excess of ethylenediamine to afford the next generation with reactive amine groups. Repetition of this two-step procedure ultimately leads to PAMAM dendritic wedges (Scheme 1) with one hydroxyl group at the focal point.

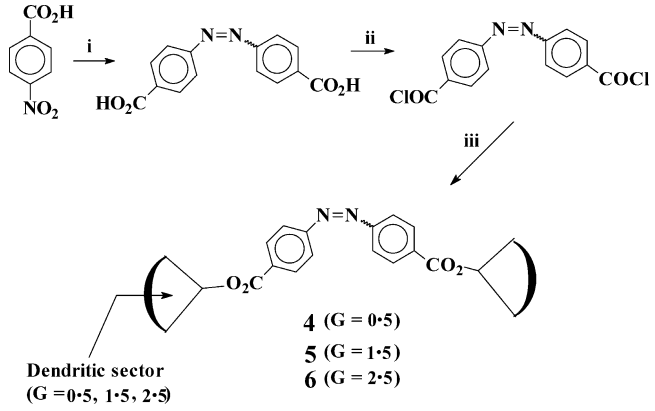
The synthesized dendritic wedges having one hydroxyl group at the focal point thus offer a myriad of possibilities for designing unique structures with specific properties by

Keywords: Polyamidoamine; Dendrimer; Azobenzene; Photoresponsive.
* Corresponding author. Tel.: +91 3222281660; fax: +91 3222 255303; e-mail: ajitbanthia2000@yahoo.co.in

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.052



Scheme 1. (i) Methyl acrylate, MeOH, rt; (ii) ethylenediamine, MeOH, rt; (iii) methyl acrylate, MeOH, rt; (iv) ethylenediamine, MeOH, rt; (v) methyl acrylate, MeOH, rt.

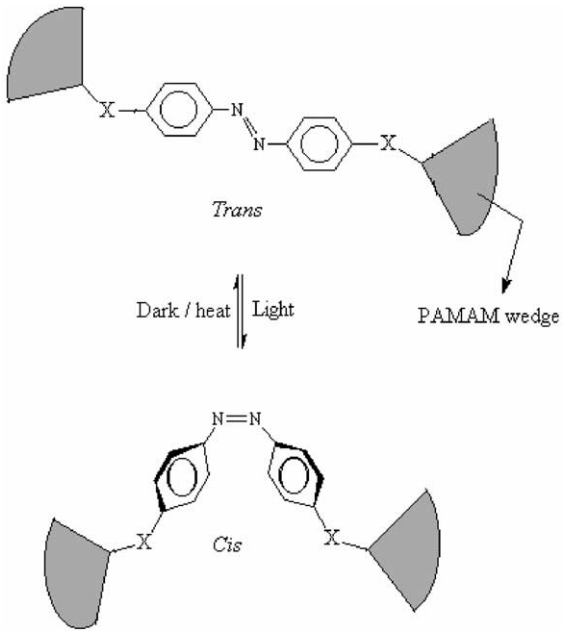


Scheme 2. (i) Glucose, NaOH, 25 °C; (ii) PCl_5 (iii) dendron (**1**, **2** or **3**), CH_2Cl_2, Et_3N, rt, 24 h.

coupling them to various polyfunctional core molecules. Photoresponsive dendrimers **4**, **5**, **6** were synthesized from 4,4′-bis(chlorocarbonyl)azobenzene[10] by coupling with these dendritic wedges (Scheme 2).

Dendrimers **4**, **5**, **6** are honey colored and gummy in nature, and were isolated in 60–70% yield. The dendrimers are soluble in halogenated organic solvents like chloroform, dichloromethane etc. The dendritic wedges as well as the dendrimers have been fully characterized by means of spectroscopic techniques and elemental analysis.

The FT-IR spectra of both the dendritic wedges and dendrimers show broad absorption bands at ~3410–3280 cm^{-1} which are characteristics of the O–H, N–H stretching frequencies. The bands occurring in the range 2800–2950 cm^{-1} are associated with symmetric and asymmetric C–H stretching vibrations of the aliphatic CH_2



Scheme 3.

and CH_3 groups. Other spectral features include the ester C=O stretching at 1740 cm^{-1}, ν (C=O) amide-I at 1655 cm^{-1}, δ (NH) with ν (CO–N) amide-II at 1590 cm^{-1} and the fingerprint region below 1500 cm^{-1}. However, the peak attributed to –N=N– of azo-based dendrimers overlaps with amide-I stretching at ~1655 cm^{-1}. The bands at ~1207 and ~1040 cm^{-1} are assigned to the stretching vibrations of the ester group (–CO–O–C–).

^{1}H NMR spectral data further substantiated the expected molecular structures of both the dendrons and azo-based dendrimers. ^{1}H NMR spectra of azo-based PAMAM dendrimers show two new doublets of aromatic protons at δ 8.21 (J=8 Hz) and δ 8.0 (J=8 Hz) for the azobenzene unit providing strong evidence of its placement into the dendritic center. Meanwhile, both the dendritic wedges (ester terminated) as well as the azo-based PAMAM dendrimers show the singlet peak of methylester protons at δ 3.67, a broad singlet at δ 3.29–3.27 for –CONHCH_2– protons, and complex multiplates in the range δ 2.85–2.33 for the other CH_2 protons of PAMAM units. These conclusions are consistent with the ^{13}C NMR spectroscopic characterization and supported by elemental analysis. The ^{13}C NMR spectra display the expected carbon signals. The peaks in the region δ 174–170 are observed for both of these structures due to the ester as well as amide carbonyl carbons. The resonance peaks associated with aromatic carbons of the azobenzene unit appear at δ 152.4, 129.0, 124.6, 121.5, etc.

The data from elemental analyses of **3** and **6** show the expected C/H/N percentages. The MALDI-TOF mass spectrometry clearly confirms the idealized structures of **3** and **6**. The calculated mass numbers of **3** and **6** are in good agreement with that derived from the MALDI-MS analysis.

The described azo-based PAMAM dendrimers possess moderate thermal stability as observed by a thermogravimetric analysis (TGA) under argon atmosphere at the heating rate 10 °C/min. For example, the thermogram of **6** indicated that the degradation takes place in two distinct stages. At the first stage weight loss (42%) occurs in the

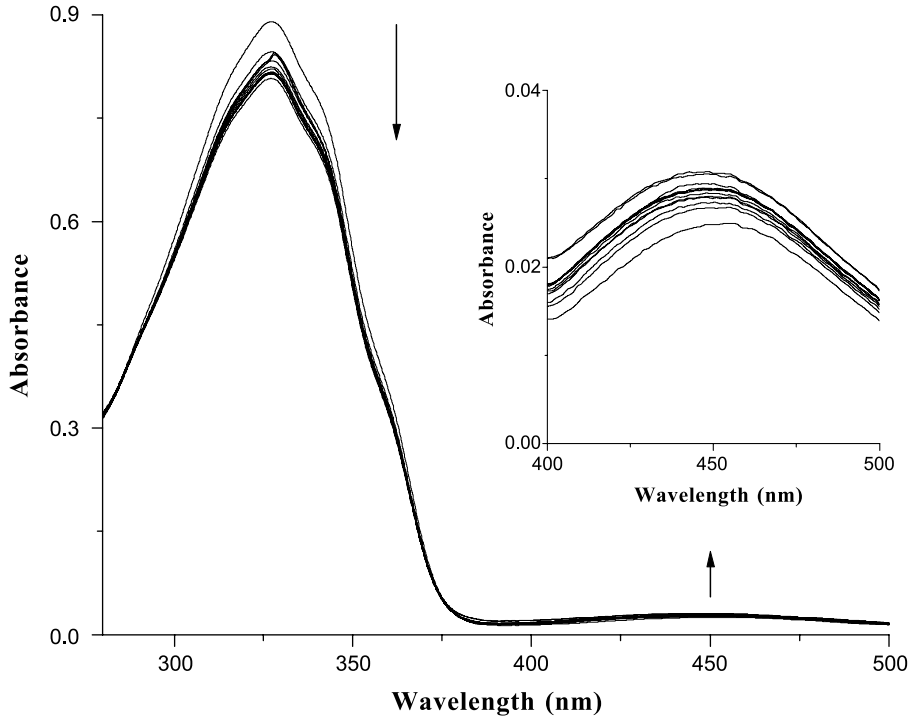


Figure 1. UV–vis absorption spectral change of acetonitrile solution of **4** (5.22×10^{-5} M) under irradiation (λ_{ex}=260–320 nm, 25 °C, *trans*-to-*cis*).

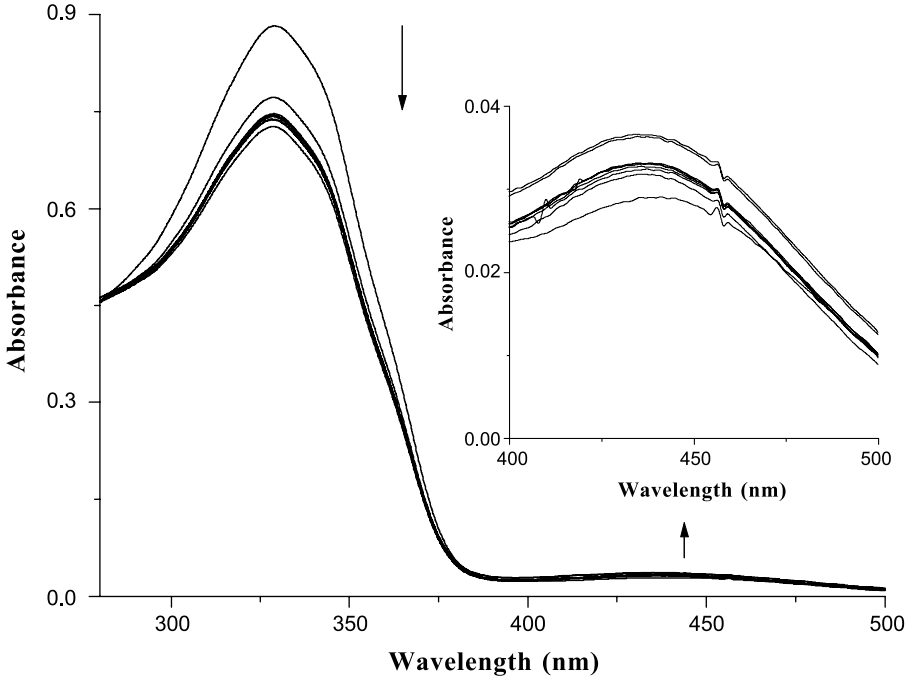


Figure 2. UV–vis absorption spectral change of acetonitrile solution of **5** (6.75×10^{-5} M) under irradiation ($\lambda_{ex}=260$–320 nm, 25 °C, *trans*-to-*cis*).

temperature range 174–239 °C. The weight loss (37%) at the second stage takes place between 240 and 450 °C.

2.2. Photoresponsive study

We have established the photoresponsive behavior of the azobenzene-centered dendrimers **4**, **5**, **6** to be essentially identical to that of small molecule azobenzenes. The dark incubation of acetonitrile solutions of **4**, **5**, **6** served to maximize the absorption at 330 nm corresponding to the π–π* transition of *trans* azobenzene chromophore and a weak band at 450 nm corresponding to the *n*–π* transition of the azo unit. On irradiation of these dark incubated solutions with 260–320 nm UV light, the energetically preferred ground state *trans*-form goes to the *cis*-form via a photochemical isomerization process (Scheme 3), as

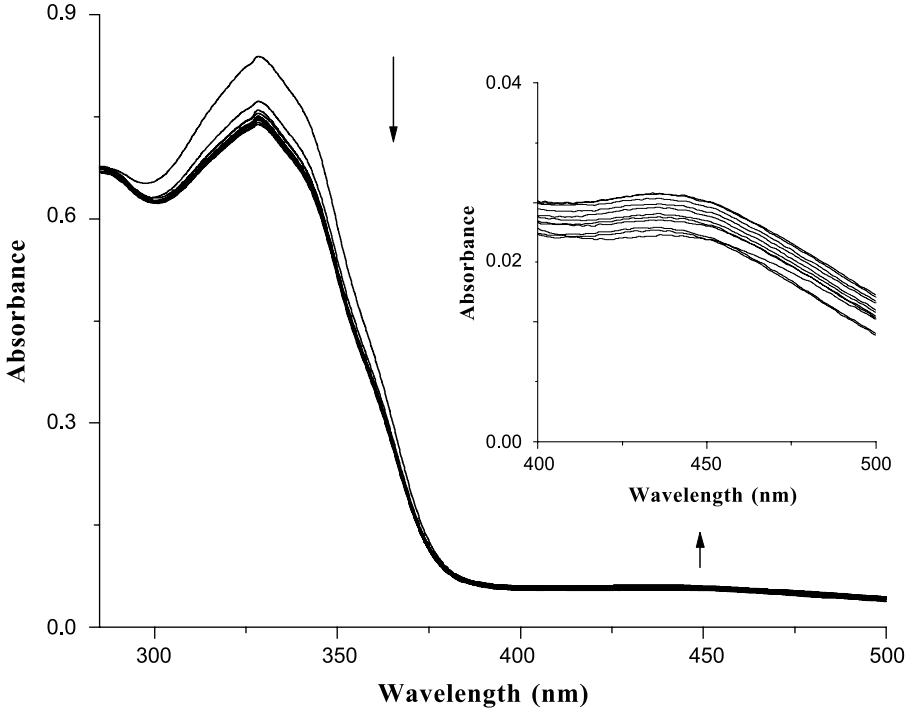
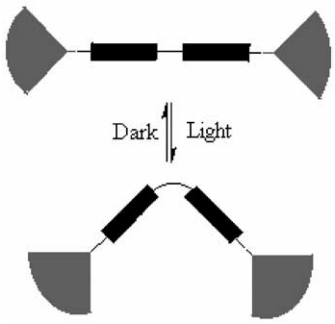


Figure 3. UV–vis absorption spectral change of acetonitrile solution of **6** (6.21×10^{-5} M) under irradiation ($\lambda_{ex}=260$–320 nm, 25 °C, *trans*-to-*cis*).



Scheme 4.

evidenced by a gradual decrease in absorbance at 330 nm and concomitant increase in absorbance at 450 nm with time (Figs. 1–3).

In a previous recent paper,[9a] we also reported the efficient *trans*-to-*cis* photoisomerization of **6**. In this present paper, we present the full account of the photoinduced reversible *cis*/*trans* isomerization behavior of this series of azobenzene centered PAMAM dendrimers of increasing size. We placed the azobenzene unit at the core to allow the control of its photochromic isomerization [*trans* (rod-shaped)/*cis* (V-shaped)] thereby imposing concomitant reorientation of the dendritic sectors as proposed in Scheme 4. These 15 min irradiated solutions of **4**, **5**, and **6** were then subjected to back isomerization (*cis*-to-*trans*) (Figs. 4–6), at 298 K in the dark.

In the dark, the back isomerization to *trans*- was slow and reversion to the original dark incubated spectrum was observed over the course of approximately 6 h. However, this back isomerization was much faster when exposed to visible light.

These results demonstrate that the azobenzene units in the core of the PAMAM dendrimers do isomerize reversibly by photochemical procedures. These isomerizations were reproducible upon further irradiation cycles. These observations indicate that the configurational/conformational changes of azo-based PAMAM dendrimers might be controllable by UV irradiation and dark adaptation.

3. Conclusion

We have thus synthesized azobenzene centered PAMAM dendrimers ($G=0.5$, 1.5, 2.5) and demonstrated the reversible photoswitching property due to the isomerism of azobenzene moiety on irradiation with 260–320 nm UV light. Since these phoresponsive azo-based PAMAM dendrimers possess amide, amine and ester functionalities, they may be utilized in the photomodulated binding of guest molecules bearing specific functional groups. Along this direction we anticipate that the photoswitchable dendrimers of these types will have applications in the field of stimuli-responsive transport process. Further studies in our laboratory are in progress.

4. Experimental

4.1. Materials

Methylacrylate was shaken with a 5% NaOH solution, washed with water, dried over Na_2SO_4 and distilled. Ethylenediamine was used as received without further

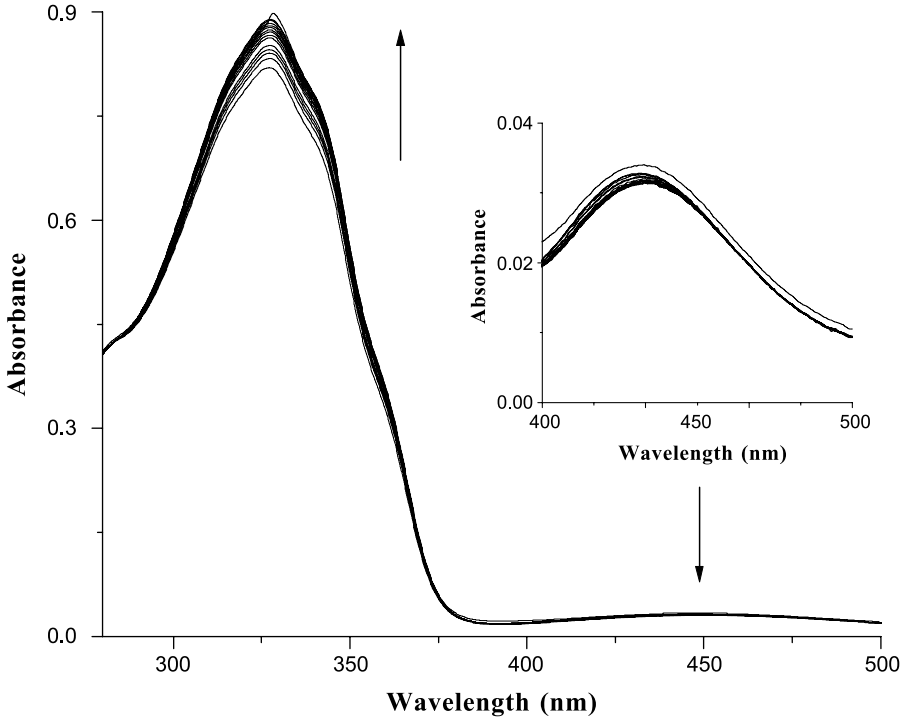


Figure 4. UV–vis absorption spectral change of irradiated acetonitrile solution of **4**, kept in dark at 25 °C.

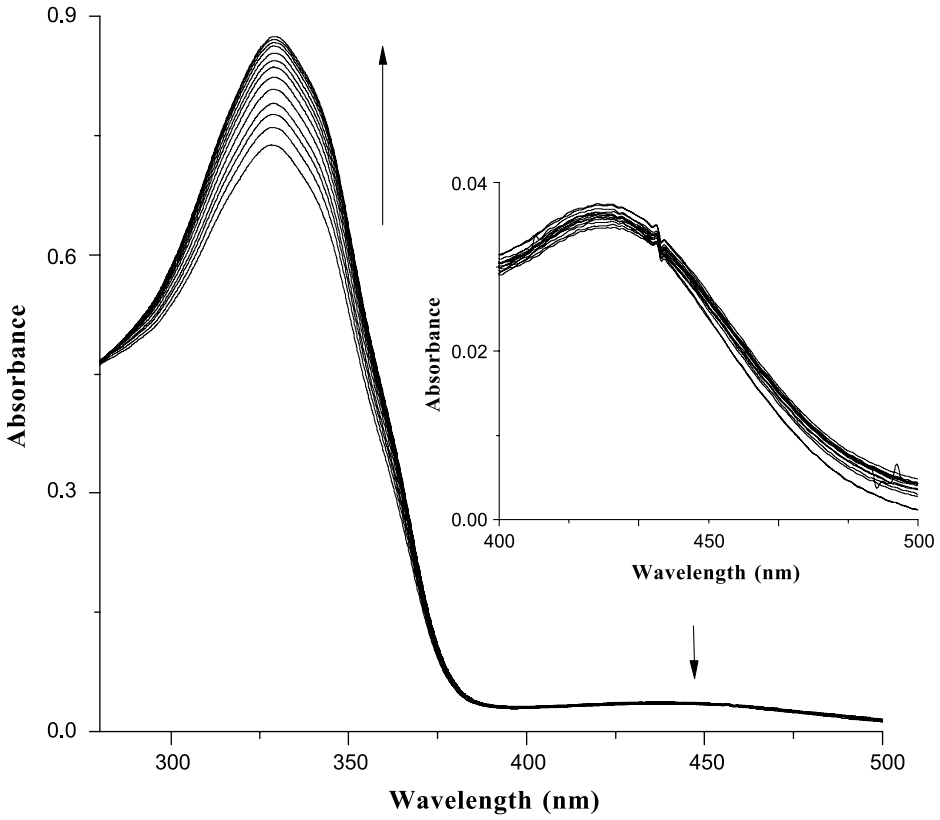


Figure 5. UV–vis absorption spectral change of irradiated acetonitrile solution of **5**, kept in dark at 25 °C.

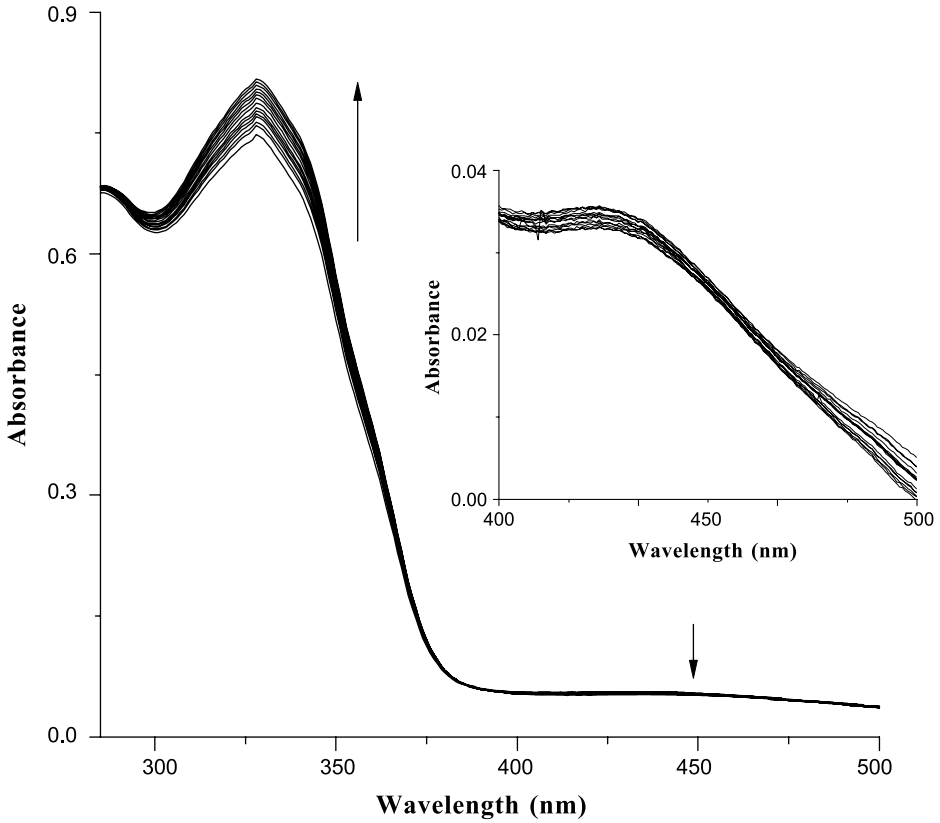


Figure 6. UV–vis absorption spectral change of irradiated acetonitrile solution of **6**, kept in dark at 25 °C.

purification. 4,4′-Bis(chlorocarbonyl)azobenzene synthesis was carried out following the literature procedure.[10]

FT-IR spectroscopic measurements as KBr pellets were carried out using. Thermo-Nicolate Nexus-870 FT-IR spectrometer. NMR spectra were recorded on a Bruker AC200 spectrometer using $CDCl_3$ solvent. CHN-analysis was obtained from a 2400 series II CHN-analyser, Perkin Elmer, USA, using helium as the carrier gas and oxygen as combustion gas. Thermogravimetric analysis (TGA) were conducted under argon atmosphere on a STA 625 STANTON-REDCROFT thermal analyzer at a heating rate of 10 °C/min. MALDI-TOF mass spectra of **3** and **6** were obtained on a Biospectrometry Voyager-DE PROs instrument by using cinnamic acid matrix. UV–vis absorption spectra were recorded with a Perkin–Elmer Lamda 45 spectrophotometer. Photochemical isomerization (*trans*/*cis*) was carried out by exposing 260–320 nm UV light, obtained from a 200 watt HgXe lamp.

4.2. Synthesis of PAMAM dendritic wedges (1, 2, 3)

4.2.1. General. Divergent synthesis (Scheme 1) of the ester-terminated PAMAM dendritic wedges was carried out by initial Michael addition of methanolic solution of ethanolamine (2.0 g, 0.03 mol) with excess methyl acrylate (28.2 g, 0.3 mol) (1:10 molar ratio). The reaction mixture was stirred for three days at room temperature. The excess methylacrylate was removed under vacuum at 60–70 °C temperature to afford the ester-functionalized derivative **1**. The reaction mixture was next submitted to the reaction sequence leading to the next generation PAMAM dendritic wedge **2**, consisting of the exhaustive amidation of the ester functionalized ethanolamine **1** to ethylenediamine (1:30 molar ratio), followed by Michael addition of the resulting amine with methylacrylate (20 equiv of **1**). Excess reagents were removed under vacuum at 60–70 °C temperature. Repetition of this two-step procedure ultimately leads to the next generation PAMAM dendritic wedge **3**. The dendritic wedges **2** and **3**, isolated in 85–90% yield, were gummy in nature.

4.2.2. Selected data for dendritic wedge 2. FT-IR (KBr): cm^{-1}: 3378, 3269 (broad, OH, NH, both free and H-bonded), 2929, 2840 (C–H of CH_3 and CH_2 groups), 1738 (ester C═O), 1649 (amide-I), 1546 (amide-II), 1437, 1207, 1040 (CO–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.14 (bs, 2H, all –CON*H*–), 3.68 (s, 14H, –CO_2CH_3, HO–CH_2–), 3.28–3.25 (bm, 4H, –CONH–CH_2–), 2.83–2.70 (bm, 12H, $CONHCH_2CH_2N$, $NCH_2CH_2CO_2Me$), 2.60–2.35 (m, 19H, all other –CH_2–, *H*O–CH_2–). Elemental analysis for $C_{28}H_{51}N_5O_{11}$: Calcd: C, 53.07; H, 8.11; N, 11.04; Found: C, 52.67, H, 7.88; N, 10.7%.

4.2.3. Selected data for dendritic wedge 3. FT-IR (KBr): cm^{-1}: 3410, 3250 (broad, OH, NH, both free and H-bonded), 2955, 2846 (C–H of CH_3 and CH_2 groups), 1738 (ester C═O), 1655 (amide-I), 1591 (amide-II), 1437, 1373, 1207, 1040 (CO–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.66 (s, 26H, –CO_2CH_3, HO–CH_2–), 3.29–3.27 (m, 12H, –CONH–CH_2), 2.84– 2.70 (bm, 28H, $CONHCH_2CH_2N$, $NCH_2CH_2CO_2Me$), 2.58–2.37 (m, 43H, all other –CH_2–, *H*OCH_2–). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 177.1 (NC═O; out), 174.1 (NC═O, in), 172.3 (C═O), 56.8, 56.1, 52.2, 51.2, 50.2, 49.9, 49.2, 45.1, 42.4, 37.7, 36.9, 32.5, 32. MALDI-TOF MS: *m*/*z* Calcd for $C_{64}H_{115}N_{13}O_{23}$ 1472.6 $[M+K]^+$, found. 1473.0 $[M+K]^+$; Elemental analysis for $C_{64}H_{115}N_{13}O_{23}$: Calcd: C, 53.58; H, 8.08; N, 12.69; Found: C, 53.31, H, 7.83; N, 12.43%.

4.3. Synthesis of azo-based PAMAM dendrimers (4, 5, 6)

4.3.1. General. Photoresponsive azo-based PAMAM dendrimers **4**, **5**, **6** were synthesized as shown in Scheme 2 by condensing a stoichiometric amount of 4,4′-*bis*(chlorocarbonyl) azobenzene with the PAMAM dendritic wedges (1:2 mole ratio) in presence of Et_3N in dichloromethane for 24 h at room temperature and then the mixture was washed with aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 solution. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give a honey coloured gummy liquid. Purification was achieved by column chromatography ($CHCl_3$:MeOH = 10:1) to obtain dendrimers **4**, **5**, **6** as gummy like products in 60–70% yield.

4.3.2. Dendrimer 4. Column chromatograph ($CHCl_3$: MeOH = 10:1; R_f = 0.95); FT-IR (KBr) cm^{-1}: 2952, 2840 (C–H of CH_3 and CH_2 groups), 1732 (ester C═O), 1630 (amide-I; –N═N–; amide-II),1457, 1247, 1000 (CO–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.18 (d, 4H, J = 8 Hz), 7.97(d, 4H, J = 8 Hz), 3.66 (bs, 16H), 2.80–2.30 (m, 20H). Elemental analysis for $C_{34}H_{44}N_4O_{12}$: Calcd C, 58.28; H, 6.33; N, 7.99; Found: C, 57.95; H, 6.15; N, 7.57%.

4.3.3. Dendrimer 5. Column chromatograph ($CHCl_3$: MeOH = 10:1; R_f = 0.90); FT-IR (KBr) cm^{-1}: 3410 (broad, NH, both free and H-bonded), 2942, 2830 (C–H of CH_3 and CH_2 groups), 1732 (ester C═O), 1640 (broad amide-I, –N═N–, amide-II), 1427, 1386, 1203, 1050 (CO–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.95 (d, 4H, J = 8 Hz), 7.52 (d, 4H, J = 8 Hz), 3.68 (bs, 28H), 3.27 (bs, 8H), 2.92–2.38 (bm, 60H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 173.0–171.5 (unresolved, *C*ONH, *C*OOCH_3, –*C*OOCH_2), 130.5, 127.5, 123.1 (unresolved), 76.4, 53.0, 51.5, 49.8, 49.6, 49.1, 36.5, 32.4.

Elemental analysis for $C_{70}H_{108}N_{12}O_{24}$: Calcd C, 55.99; H, 7.25; N, 11.19; Found: C, 55.71; H, 7.16; N, 10.93%.

4.3.4. Dendrimer 6. Column chromatograph ($CHCl_3$: MeOH = 10:1; R_f = 0.83); FT-IR (KBr) cm^{-1}: 3410, 3245 (broad, NH, both free and H-bonded), 2950, 2840 (C–H of CH_3 and CH_2 groups), 1740 (ester C═O), 1654 (broad amide-I, –N═N–), 1590 (amide-II), 1435, 1374, 1206, 1040 (CO–O–C); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.94 (d, 4H, J = 9 Hz), 7.52 (d, 4H, J = 9 Hz), 6.21 (bs, 12H, –CONH–), 3.65 (s, 52H), 3.28 (bm, 24H), 2.73–2.35 (m, 140H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 173.0 (broad and unresolved, –*C*ONH–, –*C*OOCH_3, –*C*OOCH_2–), 152.4 (ArC), 129.0 (ArC), 124.6 (ArCH), 121.5 (ArCH), 79.3, 75.1, 55.9, 53.0, 50.1, 49.0, 47.2, 46.5, 39.8, 36.9, 35.0, 32.5, 29.9. MALDI-TOF MS: *m*/*z* Calcd 3103.55 $[MH^+]$, found. 3101.96 $[MH^+]$; Elemental analysis for $C_{142}H_{236}N_{28}O_{48}$: Calcd C, 54.95; H, 7.66; N, 12.64; Found: C, 54.72; H, 7.41; N, 12.42%.

Acknowledgements

We thank Professor C. Jeff Brinker of Advanced Materials Laboratory, 1001 University Blvd. SE Suite-100, Albuquerque, NM 87106, USA for providing the facilities for UV experiments.

References and notes

1. (a) Vogtle, F.; Gestermann, S.; Hesse, R.; Schwierz, H.; Windisch, B. *Prog. Polym. Sci.* **2000**, *25*, 987–1041. (b) Smith, D. K.; Diederich, F. *Top. Curr. Chem.* **2000**, *210*, 183–227. (c) Zeng, F.; Zimmerman, S. C.; Kolotuchin, S. V.; Reichert, D. E. C.; Ma, Y. *Tetrahedron* **2002**, *58*, 825–843. (d) Gittins, P. J.; Twyman, L. J. *Supramol. Chem.* **2003**, *15*(1), 5–23 and references therein. (e) Ghosh, S.; Banthia, A. K. *Supramol. Chem.* **2004**, *16*(7), 487–494.
2. (a) Jiang, D.-L.; Aida, T. *Nature* **1997**, *388*, 454–456. (b) Adronov, A.; Gilat, S. L.; Frechet, J. M.; Ohta, K.; Neuwahl, F. V. R.; Fleming, G. R. *J. Am. Chem. Soc.* **2000**, *122*, 1175–1185. (c) Balzani, V.; Ceroni, P.; Gestermann, S.; Gorka, M.; Kauffman, C.; Vogtle, F. *Tetrahedron* **2002**, *58*(4), 629–637 and references therein.
3. (a) Archut, A.; Vogtle, F.; De Cola, L.; Azzelini, G. C.; Balzani, V.; Ramanujam, P. S.; Berg, R. H. *Chem. Eur. J.* **1998**, *4*, 699–706. (b) Norikane, Y.; Kitamoto, K.; Tamaoki, N. *J. Org. Chem.* **2003**, *68*, 8291–8304 and references therein.
4. Schenning, A. P. H. J.; Elissen-Roman, C.; Weener, J. W.; Baars, M. W. P. L.; van der Gaast, S. J.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 8199–8208.
5. (a) Junge, D. M.; McGrath, D. V. *J. Am. Chem. Soc.* **1999**, *121*, 4912–4913. (b) McGrath, D. V.; Junge, D. M. *Macromol. Symp.* **1999**, *137*, 57–65. (c) Liao, L.-X.; Junge, D. M.; McGrath, D. V. *Macromolecules* **2002**, *35*, 319–322 and references therein. (d) Villavicencio, O. F.; McGrath, D. V. In Newkome, G. R., Ed.; Advances in Dendritic Macromolecules; JAI: Greenwich, CT, 2002; Vol. 5, p 1.
6. (a) Nagasaki, T.; Tamagaki, S.; Ogino, K. *Chem. Lett.* **1997**, 717–718. (b) Archut, A.; Vogtle, F. *Chem. Soc. Rev.* **1998**, *27*, 233–240.
7. (a) Tanaka, S.; Itoh, S.; Kurita, N. *Chem. Phys. Lett.* **2000**, *323*, 407. (b) Sebastian, R. M.; Blais, J.-C.; Caminade, A.-M.; Majoral, J.-P. *Chem. Eur. J.* **2002**, *8*, 2172–2183 and references therein.
8. (a) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138–175. (b) Pistolis, G.; Malliaris Tsiouravas, D.; Paleos, C. *Chem. Eur. J.* **1999**, *5*, 1440–1444. (c) Grabchev, I.; Chovelon, J.-M.; Bojinov, V.; Ivanova, G. *Tetrahedron* **2003**, *59*, 9551 and references therein.
9. (a) Ghosh, S.; Banthia, A. K. *Tetrahedron Lett.* **2001**, *42*, 501–503. (b) Ghosh, S.; Banthia, A. K. *J. Polym. Sci., Part—A, Polym. Chem.* **2001**, *39*, 4182–4188. (c) Ghosh, S.; Banthia, A. K.; Maiya, B. G. *Org. Lett.* **2002**, *4*, 3603–3606 and references therein. (d) Takaguchi, Y.; Sako, Y.; Yanagimoto, Y.; Tsuboi, S.; Motoyoshiya, J.; Aoyama, H.; Wakahara, T.; Akasaka, T. *Tetrahedron Lett.* **2003**, *44*, 5777–5780.
10. Ameerunisha, S.; Zacharis, P. S. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1679–1682.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 2897–2905
Tetrahedron

Synthesis of new polyaza heterocycles. Part 42: Diazines

Mircea Darabantu,[a] Ludovic Boully,[b] Alain Turck[b] and Nelly Plé[b,*]

[a]*Department of Organic Chemistry, 'Babes-Bolyai' University 11 Aranyi Janos Str., 400028 Cluj-Napoca, Romania*
[b]*Laboratoire de Chimie Organique Fine et Hétérocyclique, UMR 6014, IRCOF-INSA, B.P. 08, 76131 Mont St Aignan Cedex, France*

Received 8 November 2004; revised 6 January 2005; accepted 13 January 2005

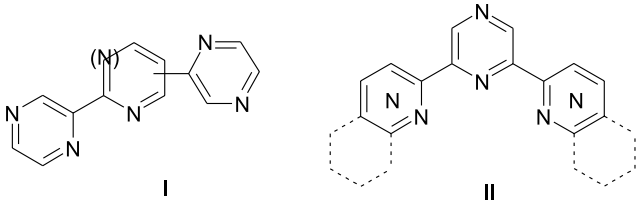
Abstract—Using Pd-catalyzed Stille cross-coupling reactions, we report here the synthesis of various mono- or bis(tri-*n*-butylstannyl)diazines which were reacted with various halogenated diazines to access to various polyaza heterocyclic derivatives.

Polyaromatic compounds containing N-heterocyclic subunits have received considerable attention due to their wide use in various fields such as molecular recognition, metal cryptates, supramolecular devices and self-assembly. Among them, oligopyridines have been extensively studied during the last two decades and many well-defined supramolecular architectures with 2,2′-bipyridines (bpy) and 2,2′6′,2″-terpyridines (tpy) units as building blocks have found applications in catalysis,[1] electrochemistry,[2] photochemistry[3] or new materials.[4]

More recently, synthesis of polydentate nitrogen ligands based on pyridine and 1,3-pyrimidine[5] or 3,6 pyridazine[6] units have been reported. Interest in this class of molecules incorporating several coordination sites is mostly due to their use in supramolecular chemistry, and as building blocks for self-assembled polynuclear coordination arrays.[7]

We report here the synthesis of a new family of polyaromatic compounds containing a pyridine or pyrimidine ring as central unit substituted by two pyrazinyl groups (type I) and symmetrical structures with a pyrazine central unit substituted at the 2 and 6 positions by π-deficient heterocycles such as pyridine, quinoline, diazine or benzodiazine (type II) (Scheme 1).

Synthetic approaches to such polyaza heterocyclic compounds are based either on palladium-catalyzed cross-coupling procedures: Suzuki,[8] Negishi[9] and Stille[10] coupling reactions or by generation of the aza-heterocyclic rings with the help of ring-closure reactions.



Scheme 1.

The Suzuki reaction involves the palladium-catalyzed cross-coupling of heteroarylboronic acids with heteroaryl halides (or triflates). It is remarkable that whereas halopyridines have often been employed in Suzuki reactions there are only few examples in the literature of use of pyridylboronic acids or esters.[11] In diazine series only the 5-pyrimidylboronic acids or esters have been described[12] while to our knowledge, they are unknown with pyrazine or pyridazine rings probably because of their unstability.

Some Negishi reactions have been achieved with organozinc derivatives of diazines to give cross-coupling reactions with iodo or bromo aromatics leading to diazine-aryl or (heteroaryl) bound.[13]

Stille-type coupling provides another efficient way for the formation of aryl–aryl bonds in particular between π-deficient N-heterocycles. This process has been used to prepare a wide variety of functionalized 2,2′-bipyridines and 2,2′6′,2″-terpyridines.[14]

Using a synthetic strategy based on Stille coupling reaction, we report here the synthesis of mono- or bis(tri-*n*-butylstannyl) diazines which were reacted with various halogenated diazines to obtain polyaza heterocyclic derivatives.

Keywords: Stille cross-coupling reaction; Diazines; Polyaza heterocycles.
* Corresponding author. Tel.: +33 2 35 52 29 02; fax: +33 2 35 52 29 62; e-mail: nelly.ple@insa-rouen.fr

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.059

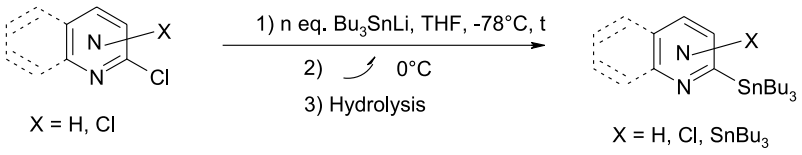
1. Results

The synthesis of diazinylstannanes could be performed either using the lithiation reaction of diazine followed by a transmetalation step with tributyltin chloride[15] or by use of the nucleophilic substitution of a chlorine atom by the stannyl anion.[16]

We have investigated this last procedure to synthesize the tri-(*n*-butyl)organostannanes (**1**–**9**) with a diazine moiety (Scheme 2) the results of which are given in Table 1.

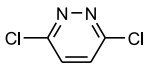
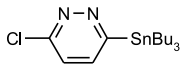
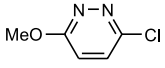
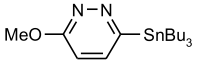
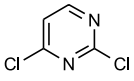
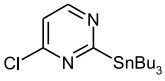
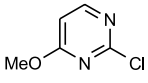
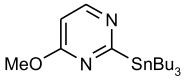
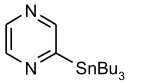
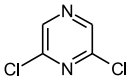
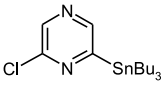
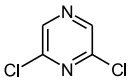
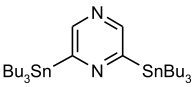
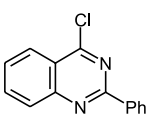
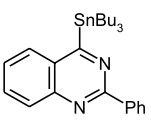
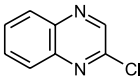
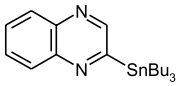
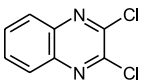
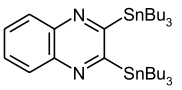
One can observe a monosubstitution with very low yields for dichloro-pyridazine and pyrimidine (entries 1 and 3). These low yields could be explained by competitive radical reactions, since some dimers and bistributyltin were observed besides the expected compounds. Good results were obtained for chloropyrazine (entry 5) and 2,6-dichloropyrazine (entry 6). For this last compound, mono or distannylation has been achieved in good and moderate yield (entries 6 and 7). The nucleophilic substitution was performed successfully with good yield with 2-chloro-4-methoxypyrimidine (entry 4) whereas the yield was low with 2,4-dichloropyrimidine (entry 3). Such a result could suggest that the presence of a methoxy group makes the substitution reaction more easy, however, it can be noticed that stannylation failed with 3-chloro-6-methoxypyridazine (entry 2). In benzodiazine series good yields with mono-chloro-quinazoline (entry 8) or quinoxaline (entry 9) were observed. With 2,3-dichloroquinoxaline, a disubstitution can be obtained with a very good yield (entry 10) despite the steric hindrance of the tri-*n*-butylstannyl group.

Coupling reactions of the stannyl derivatives **3**, **7**–**9** were

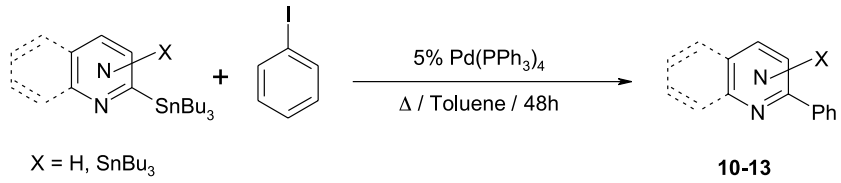


Scheme 2.

Table 1. Synthesis of tri-*n*-butyl-*N*-heteroarylstannanes

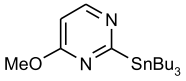
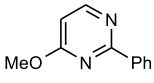
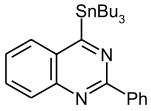
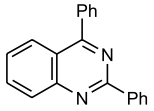
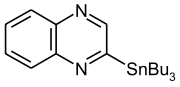
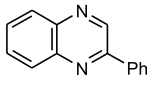
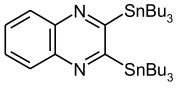
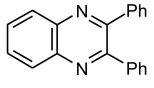
Entry	Het-Cl	Product	*n* (equiv)	*t* (h)	Compound	Yield (%)
1			1.1	5	**1**	9
2			1.1	5	—	—
3			1.1	6	**2**	4
4			1.1	1	**3**	70
5			1.1	8	**4**	85[a]
6			1.1	21	**5**	95
7			2.1	24	**6**	56
8			1.1	1	**7**	97
9			1.1	1	**8**	54
10			2.1	1	**9**	98

[a] Hydrolysis was performed at −40 °C.

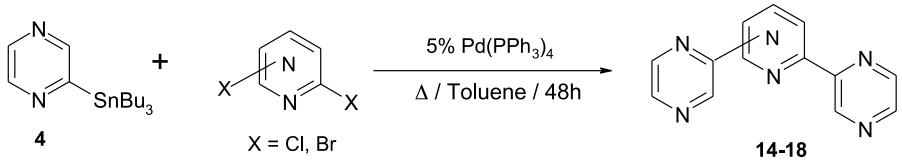


Scheme 3.

Table 2. Cross-coupling reaction of $HetSnBu_3$ with iodobenzene

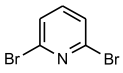
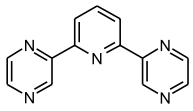
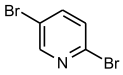
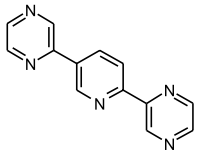
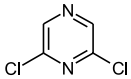
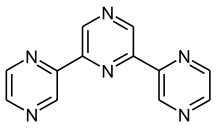
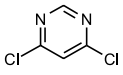
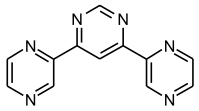
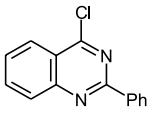
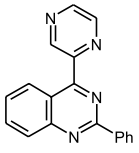
Entry	$HetSnBu_3$	Product	Compound[a]	Yield (%)
1			**10**[17]	78
2			**11**[18]	67
3			**12**[18]	64
4			**13**[19]	39

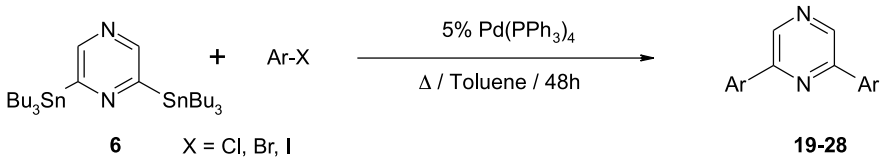
[a] All products **10–13** have been characterized and comparison with already published data are in agreement.



Scheme 4.

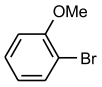
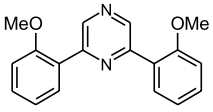
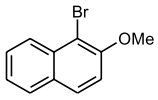
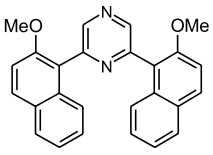
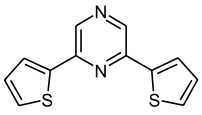
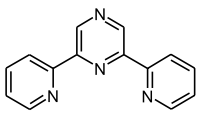
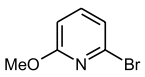
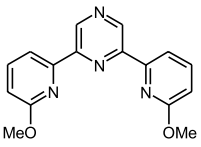
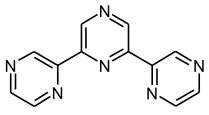
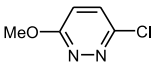
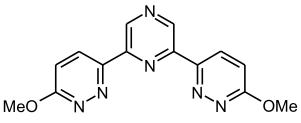
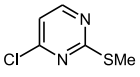
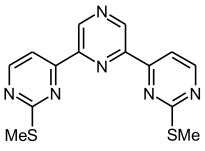
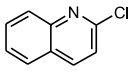
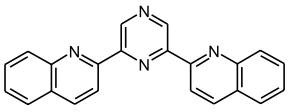
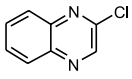
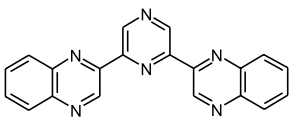
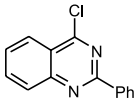
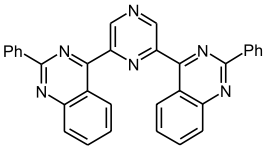
Table 3. Cross-coupling reaction of tri-(*n*-butyl)stannylpyrazine **4** with halogeno-N-heteroaryl compounds

Entry	N-$HetX_2$	Product	*t* (h)	Compound	Yield (%)
1			15	**14**	66
2			27	**15**	64
3			26	**16**	75
4			27	**17**	61
5			48	**18**	70



Scheme 5.

Table 4. Cross-coupling reaction of the 2,6-distannylpyrazine **6** with halogenoaromatics

Entry	ArX	Product	Compound	Yield (%)
1			**19**	82
2			**20**	51
3			**21**	76
4			**22**	30
5			**23**	60
6			**16**	65
7			**24**	21
8			**25**	59
9			**26**	91
10			**27**	65
11			**28**	71

tested with iodobenzene according to the general procedure for Stille reaction in toluene with $Pd(PPh_3)_4$ as catalyst. Under these conditions, the expected phenyl or diphenyl compounds **10–13** were obtained in moderate yields (Scheme 3, Table 2).

We then extended this procedure to the coupling reaction of 2-tri-*n*-butylstannylpyrazine **4** with various mono or dihalogeno N-heteroaryl compounds leading to various π-deficient heterocycles appended to a pyrazinyl group (Scheme 4, Table 3).

Compounds **14–18** were obtained in fairly good yields and present a bi- or tridentate binding system which make them potential metal cryptands. Compounds **15** and **18** may be viewed as aza-analogues of bipyridine (bpy), **17** as aza-analogue of two bpys and **14** and **16** as aza-analogues of terpyridine (tpy). Compounds **16** and **17** belong to a family of symmetrical tridiazines, whereas **14** and **15** are di-pyrazinylpyridines.

In the aim to synthesize symmetrical polydentates with a pyrazine moiety as central unit, the cross-coupling reaction of the 2,6-bis-tri-*n*-butylstannylpyrazine **6** was performed with various monohalogenoaryl compounds. Pyrazine linked to various aromatic rings (benzene, naphthalene, thiophene or π-deficient N-heterocycles) were obtained (Scheme 5, Table 4).

This general methodology allowed us to access to a wide range of compounds which constitute a new family of triaromatic strands with a diazine as central unit, most of them (**16**, **22–28**) are tri-π-deficient N-heterocycles linked by direct aryl–aryl bounds and could be seen as aza-analogues of terpyridines. These structures are potential trinucleating ligands due to the presence of coordinating atoms such as nitrogen, oxygene or sulfur.

Compounds **19** and **20** have two phenyl or naphthyl ring, *ortho* substituted by a methoxy group which could allow complexation, whereas for the compound **21**, the sulfur of the thiophene ring could play this role. All the other compounds **16**, **22–28** are obtained in moderate to good yields. One can notice an exception observed with a pyridazine unit as lateral rings (**24**).

2. Conclusion

In conclusion, a simple general procedure for the synthesis of new polyaza heterocycles has been developed on the basis of palladium-mediated coupling of mono- or bis-tribuylstannanes of diazines. Among the compounds prepared here some are aza-analogues of terpyridines (tpy) and will be new potential ligands for metal chelation and new building blocks for synthesis of supramolecular arrays. Further studies on this new family of polyaza heterocycles are underway and will include a careful assessment of metal coordination, photo- and electrochemical properties. Variation of functionalization of lateral units will be performed to access new ligand-bridged supramolecular structures.

3. Experimental

Melting points were determined on a Kofler hot-stage. The ^1H, and ^{13}C spectra were recorded on a Bruker AC 300 (300 MHz ^1H, 75 MHz ^{13}C) instrument. Microanalyses were performed on a Carlo Erba CHNOS 1160 apparatus. The IR spectra were obtained as potassium bromide pellets with a Perkin–Elmer Paragon 500 spectrophotometer. Mass spectra were recorded on an ATI-Unicam Automass® apparatus.

3.1. General procedure A for the nucleophilic substitution of a chlorine atom by the tributylstannyl anion

A solution of *n*-butyllithium (1.6 or 2.5 M in hexane) was added to cold (0 °C), stirred and anhydrous mixture of THF (50 mL) and diisopropylamine (DIPAH) under an atmosphere of dry nitrogen. After 15 min, tributyltinhydride was introduced, the yellow pale solution was stirred at 0 °C for 15 min and the temperature was decreased to at −78 °C. Then a solution of chloro- or dichlorodiazine in THF at −78 °C was added, after *t* hours of stirring, mixture was warmed to 0 °C. Hydrolysis was then carried out at this temperature, using a saturated aqueous solution of potassium fluoride. The aqueous layer was extracted with dichoromethane or ethylacetate (3×20 mL), the combined organic extracts were then dried over magnesium sulfate, filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel.

3.2. Procedure B for cross-coupling of heteroaryl halides with tributylstannylheteroarene under Stille conditions

A solution of tributylstannylheteroarene, arylhalide and $Pd(PPh_3)_4$ (0.05 equiv) in degassed toluene (15 mL) was heated under reflux under nitrogen atmosphere for a time *t*. After cooling, water (20 mL) was added. The aqueous phase was extracted with dichloromethane (3×20 mL). The combined organic extracts were then dried over magnesium sulfate, filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel.

3.3. Procedure C for cross-coupling of heteroaryl halides with 2,6-bis(tributylstannyl)pyrazine under Stille conditions

A mixture of 2,6-bis(tributylstannyl)pyrazine used as crude product (constituted with 77% of **6** and 21% of hexabutylditin) (1.010 g, 1.17 mmol of **6**), heteroaryl halide (2–3 mmol), and $Pd(PPh_3)_4$ (0.10 equiv) in degassed toluene (25 mL) was heated under reflux under nitrogen atmosphere for a time *t*. After cooling, dichloromethane (150 mL) was added and the mixture was filtered. The organic phase was washed with aqueous ammonia (2×25 mL). The combined organic extracts were then dried over magnesium sulfate, filtered and evaporated in vacuo. The crude product was purified by column chromatography on silica gel.

3.3.1. 3-Chloro-6-tri-*n*-butylstannylpyridazine (1). Substitution of 3,6-dichloropyridazine (2.0 g, 13.02 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 2.5 M (14.7 mmol, 5.88 mL), DIPAH (14.1 mmol, 2.00 mL), tributyltinhydride (14.4 mmol, 4.00 mL), $t=5$ h,

gave after purification by column chromatography (silica, eluent petroleum ether/ethyl acetate (9/1)) 480 mg of **1** (9.2%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.63 (m, 9H), 0.93 (m, 6H), 1.04 (m, 6H), 133 (m, 6H), 7.07 (dd, J= 8.67, 2.64 Hz, 1H), 7.22 (dd, J=8.67, 2.64 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 10.63, 13.70, 27.60, 29.24, 126.0, 136.6, 156.7, 174.4. MS (CI) m/z 403 (M^+, 40), 347 (M−Bu, 10), 269 (M−2Bu, 10), 235 (M−3Bu, 5).

3.3.2. 4-Chloro-2-tri-*n*-butylstannylpyrimidine (2). Substitution of 2,4-dichloropyrimidine (1.0 g, 6.7 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (14.1 mmol, 8.80 mL), DIPAH (14.1 mmol, 1.99 mL), tributyltinhydride (14.1 mmol, 3.91 mL), t=6 h, gave after purification by column chromatography (silica, eluent petroleum ether/ethyl acetate (25/1)) 120 mg of **2** (4%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.80 (t, J= 7.1 Hz, 9H), 1.11 (m, 6H), 1.26 (m, 6H), 1.51 (m, 6H), 7.09 (d, J=5.65 Hz, 1H), 8.46 (d, J=5.65 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 10.8, 14.0, 27.6, 29.2, 120.1, 155.9, 159.9, 191.3.

3.3.3. 4-Methoxy-2-tri-*n*-butylstannylpyrimidine (3). Substitution of 2-chloro,4-methoxypyrimidine (0.650 g, 4.5 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (5 mmol, 3.12 mL), DIPAH (5 mmol, 0.71 mL), tributyltinhydride (5 mmol, 1.35 mL), t=1 h, gave after purification by column chromatography (silica, eluent petroleum ether/ethyl acetate (25/1)) 1.20 g of **3** (70%) as a colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.80 (t, J= 7.1 Hz, 9H), 1.11 (m, 6H), 1.26 (m, 6H), 1.51 (m, 6H), 3.73 (s, 3H), 6.32 (d, J=6.0 Hz, 1H), 8.15 (d, J=6.0 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 10.3, 14.0, 27.8, 31.0, 54.8, 117.1, 153.9, 158.9, 188.6. MS (EI) m/z 343 (M−Bu, 100).

3.3.4. Tri-*n*-butylstannylpyrazine (4). Substitution of chloropyrazine (1.50 g, 12.8 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (13.5 mmol, 8.5 mL), DIPAH (13.47 mmol, 1.90 mL), tributyltinhydride (13.5 mmol, 3.75 mL), t=8 h. Hydrolysis was carried out at −40 °C. Purification by column chromatography (silica, eluent petroleum ether/ethyl acetate (10/1)) gave 4.05 g of **4** (85%) as a yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.80 (t, J= 7.5 Hz, 9H), 1.10 (m, 6H), 1.25 (m, 6H), 1.48 (m, 6H), 8.29 (d, J=2.64 Hz, 1H), 8.48 (d, J=1.86 Hz, 1H), 8.63 (dd, J= 2.64, 1.88 Hz); ^{13}C NMR ($CDCl_3$): δ 10.2, 14.0, 27.6, 29.3, 143.3, 147.1, 151.7, 170.2. MS (CI) m/z 371 (M^+, 100).

3.3.5. 6-Chloro-2-tri-*n*-butylstannylpyrazine (5). Substitution of 2,6-dichloropyrazine (0.75 g, 5.0 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (5.30 mmol, 3.30 mL), DIPAH (5.30 mmol, 0.75 mL), tributyltinhydride (5.30 mmol, 1.47 mL), t= 21 h, gave after purification by column chromatography (silica, eluent petroleum ether/ethyl acetate (30/1)) 456 mg of **5** (56%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.85 (t, J=7.1 Hz, 9H), 1.16 (m, 6H), 1.31 (m, 6H), 1.54 (m, 6H), 8.34 (s, 1H), 8.41 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 10.5, 14.0, 27.6, 29.3, 143.2, 149.2, 151.6, 170.7. HRMS (FAB) calcd for $C_{16}H_{29}ClN_2Sn$ 403.58394; found 403.57764).

3.3.6. 2,6-Bis(tri-*n*-butylstannyl)pyrazine (6). Substitution of 2,6-dichloropyrazine (0.75 g, 5.0 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (10.60 mmol, 6.60 mL), DIPAH (10.60 mmol, 1.50 mL), tributyltinhydride (10.60 mmol, 2.95 mL), t= 4 h. gave 2.7 g as a yellow oil which contains 77% of **6** and 21% of hexabutylditin. The product was not purified by column chromatography because its unstability on silica gel. It will be used as crude product for further reactions; ^{1}H NMR ($CDCl_3$): δ 0.85–1.70 (m 76H, (H_{Bu} of **6** and $(SnBu_3)_2$), 8.07 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 10.3, 14.1, 27.7, 29.4, 149.0, 171.7. MS (CI) m/z 658 (M^+, 100).

3.3.7. 2-Phenyl-4-tri-*n*-butylquinazoline (7). Substitution of 2-phenyl-4-chloroquinazoline (1.08 g, 4.5 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (4.5 mmol, 2.81 mL), DIPAH (4.5 mmol, 0.64 mL), tributyltinhydride (4.5 mmol, 1.25 mL), t=1 h, gave after purification by column chromatography (silica, eluent cyclohexane) 2.15 g of **7** (97%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.91 (m, 9H), 1.16 (m 6H), 1.32 (m, 6H), 1.47 (m, 6H), 7.77 (m, 3H), 7.92 (t, J=8.3 Hz, 1H), 8.19 (t, J=8.3 Hz, 1H), 8.35 (d, J=8.3 Hz, 1H), 8.51 (d, J=8.3 Hz, 1H), 8.85 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 11.7, 14.1, 27.8, 29.6, 127.0, 128.9, 129.1, 129.6, 130.0, 130.6, 131.4, 133.4, 139.1, 148.6, 159.0, 193.0. MS (EI) m/z 455 (90, M−Bu).

3.3.8. 2-Tri-*n*-butylquinoxaline (8). Substitution of 2-chloroquinoxaline (0.740 g, 4.5 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (4.5 mmol, 2.81 mL), DIPAH (4.5 mmol, 0.64 mL), tributyltinhydride (4.5 mmol, 1.25 mL), t=1 h, gave after purification by column chromatography (silica, eluent cyclohexane) 1.01 g of **8** (54%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.82 (m, 9H), 1.17 (m 6H), 1.30 (m, 6H), 1.55 (m, 6H), 7.63 (m, 2H), 7.96 (m, 1H), 8.06 (m, 1H), 8.52 (s, 1H). MS (EI) m/z 363 (10, M−Bu).

3.3.9. 2,6-Bis(tri-*n*-butylstannyl)quinoxaline (9). Substitution of 2,3-dichloroquinoxaline (0.445 g, 4.5 mmol) by Bu_3SnLi according to the general procedure A with *n*-BuLi 1.6 M (4.5 mmol, 2.81 mL), DIPAH (4.5 mmol, 0.64 mL), tributyltinhydride (4.5 mmol, 1.25 mL), t=1 h, gave after purification by column chromatography (silica, eluent cyclohexane) 3.12 g of **9** (98%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 0.86 (m, 18H), 1.10 (m 12H), 1.27 (m, 12H), 1.55 (m, 12H), 7.59 (dd, J=6.4 Hz, 3.3 Hz, 2H), 7.82 (dd, J=6.4 Hz, 3.3 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 11.2, 13.7, 27.4, 29.2, 128.4, 129.7, 142.0, 179.2. MS (EI) m/z 708 (M^+, 1), 651 (M−Bu, 5), 413 (M−$SnBu_3$, 90).

3.3.10. 2-Phenyl-4-methoxypyrimidine (10). Cross-coupling reaction of iodobenzenze (408 mg, 225 μL, 2 mmol) with **3** (400 mg, 1 mmol) according to the general procedure B (t=48 h) gave after purification by column chromatography (silica, eluent: dichloromethane) 145 mg (76%) of **10** as a colorless solid, mp 44–45 °C (lit.[16] 45 °C); ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 3H, H_{OMe}); 6.80 (d, J=6.0 Hz, 1H, H_5') 7.51 (m, 3H, H_{Ph}'); 8.40 (m, 2H, H_{Ph}); 8.60 (d, J= 6.0 Hz, 1H, H_6')). Anal. Calcd for $C_{11}H_{10}N_2O$ (186.21): C, 70.95; N, 15.04; H, 5.41. Found: C, 71.02; N, 15.15; H, 5.35. MS (EI) m/z 186 (M^+, 100).

3.3.11. 2,4-Diphenylquinazoline (11). Cross-coupling reaction of iodobenzenze (408 mg, 225 μL, 2 mmol) with

7 (500 mg, 1 mmol) according to the general procedure B ($t=48$ h) gave after purification by column chromatography (silica, eluent: dichloromethane) 189 mg (67%) of **11** as a colorless solid, mp 115–116 °C (lit.[17] 118 °C); ^{1}H NMR ($CDCl_3$): δ 7.58 (m, 7H), 7.90 (m, 3H), 8.15 (d, $J=8.3$ Hz, 1H), 8.17 (d, $J=8.3$ Hz, 1H), 8.73 (m, 2H). NMR ^{13}C ($CDCl_3$) δ 121.7, 127.0, 128.5, 128.7, 129.2, 129.9, 130.2, 130.5, 133.5, 137.8, 138.2, 152.0, 160.2, 168.3. Anal. Calcd for $C_{20}H_{14}N_2$ (282.12): C, 85.08; N, 9.92; H, 5.00. Found: C, 85.46; N, 9.75; H, 4.78. MS (EI) *m/z* 282 (M^+, 100).

3.3.12. 2-Phenylquinoxaline (12). Cross-coupling reaction of iodobenzenze (408 mg, 225 μL, 2 mmol) with **8** (420 mg, 1 mmol) according to the general procedure B ($t=48$ h) gave after purification by column chromatography (silica, eluent: dichloromethane) 131 mg (64%) of **12** as a yellow solid, mp 74–75 °C (lit.[17] 73–75 °C); ^{1}H NMR ($CDCl_3$): δ 7.54 (m, 3H), 7.75 (m, 2H), 8.15 (m, 4H), 9.32 (s, 1H). NMR ^{13}C ($CDCl_3$) δ 127.5, 129.1, 129.5, 129.6, 130.1, 130.2, 136.8, 141.5, 142.3, 143.3, 151.8. Anal. Calcd for $C_{14}H_{10}N_2$ (206.24): C, 81.53; N, 13.58; H, 4.89. Found: C, 81.46; N, 13.75; H, 4.78. MS (EI) *m/z* 206 (M^+, 100).

3.3.13. 2,3-Diphenylquinoxaline (13). Cross-coupling reaction of iodobenzenze (612 mg, 340 μL, 3 mmol) with **9** (708 mg, 1 mmol) according to the general procedure B ($t=48$ h) gave after purification by column chromatography (silica, eluent: dichloromethane) 110 mg (39%) of **13** as a yellow solid, mp 123–124 °C (lit.[18] 124.5 °C); ^{1}H NMR ($CDCl_3$): δ 7.44 (m, 2H), 7.48 (m, 4H), 7.64 (dd, $J=7.2$, 1.5 Hz, 4H), 7.88 (d, $J=6.4$ Hz, 2H), 8.31 (d, $J=6.4$ Hz, 2H). NMR ^{13}C ($CDCl_3$) δ 128.7, 129.2, 129.6, 130.2, 130.4, 139.4, 141.6. Anal. Calcd for $C_{20}H_{14}N_2$ (282.34): C, 85.08; N, 9.92; H, 5.00. Found: C, 85.52; N, 9.65; H, 5.38. MS (EI) *m/z* 235 (M^+, 100).

3.3.14. 2,6-Bispyrazinylpyridine (14). Cross-coupling reaction of 2,6-dibromopyridine (237 mg, 1 mmol) with **4** (738 mg, 2 mmol) according to the general procedure B ($t=$ 15 h) gave after purification by column chromatography (silica, eluent: ethyl acetate) 155 mg (66%) of **14** as a colorless solid, mp 215–216 °C; ^{1}H NMR ($CDCl_3$): δ 7.90 (t, $J=7.9$ Hz, 1H), 8.35 (d, $J=7.9$ Hz, 2H), 8.55 (m, 4H), 9.72 (d, $J=0.75$ Hz, 2H). NMR ^{13}C ($CDCl_3$) δ 122.4, 138.7, 143.8, 143.9, 145.1, 151.0, 154.1. MS (EI) *m/z* 235 (M^+, 100). Anal. Calcd for $C_{13}H_9N_5$ (235.25): C, 66.37; N, 29.77; H, 3.86. Found: C, 66.36; N, 29.77; H, 3.85.

3.3.15. 2,5-Bispyrazinylpyridine (15). Cross-coupling reaction of 2,5-dibromopyridine (237 mg, 1 mmol) with **4** (1.107 g, 3 mmol) according to the general procedure B ($t=$ 27 h) gave after purification by recrystallization in ethyl acetate 150 mg (64%) of **15** as a colorless solid, mp 265–266 °C; ^{1}H NMR ($CDCl_3$): δ 8.39 (d, $J=8.0$ Hz, 1H), 8.53 (dd, $J=8.4$, 2.0 Hz, 1H), 8.56 (d, $J=2.4$ Hz, 1H), 8.58 (d, $J=2.8$ Hz, 1H), 8.75 (d, $J=2.4$ Hz, 1H), 8.76 (d, $J=$ 2.4 Hz, 1H), 9.03 (d, $J=1.6$ Hz, 1H), 9.27 (d, $J=1.6$ Hz, 1H), 9.37 (d, $J=0.8$ Hz, 1H). NMR ^{13}C ($CDCl_3$) δ 124.2, 135.0, 138.7, 143.6, 144.3, 145.1, 145.8, 146.7, 147.1, 149.9, 152.7, 153.2, 156.3. MS (EI) *m/z* 235 (M^+, 100). Anal. Calcd for $C_{13}H_9N_5$ ($M_w=235.25$): C, 66.37; H, 3.86; N, 29.77. Found: C, 66.55; H, 3.67; N, 29.82.

3.3.16. 2,2′,6′,2″-Terpyrazine (16). Method 1—cross-coupling reaction of 2,6-dichloropyrazine (282 mg, 1.89 mmol) with **4** (1.400 g, 3.79 mmol) according to the general procedure B ($t=24$ h) gave after purification by recrystallization in methanol 333 mg (74%) of **16** as a grey solid, mp >265 °C; compound **16** has a very low solubility in the usual organic solvents; ^{1}H NMR (CF_3CD_2OD): δ 8.61 (d, $J=2.8$ Hz, 1H), 8.76 (dd, $J=2.4$, 1.6 Hz, 1H), 9.56 (s, 1H), 9.64 (d, $J=1.2$ Hz, 1H); ^{13}C NMR (CF_3CD_2OD): δ 129.0, 129.2, 131.8, 131.9, 132.4, 133.7. IR (KBr) ν (cm^{-1}) 3053.8, 3012.9, 1530.2, 1473.7, 1431.0, 1382.9, 1108.4, 1030.0, 1015.9, 858.9. MS (EI) *m/z* 236 (M^+, 100).

Method 2—cross-coupling reaction of 2,6-bis(tributyltin)-pyrazine **6** (1.17 mmol) with chloropyrazine (0.446 g, 3 mmol) according to the general procedure C (t = 48 h) gave after purification by recrystallization in methanol 179 mg (65%) of **16**. Anal. Calcd for $C_{12}H_8N_6$ (236.24): C, 61.01; H, 3.41; N, 35.57. Found: C, 61.20; H, 3.42; N, 35.77.

3.3.17. 4,6-Bispyrazinylpyrimidine (17). Cross-coupling reaction of 2,6-dichloropyrimidine (262 mg, 1.76 mmol) with **4** (1.400 g, 3.79 mmol) according to the general procedure B ($t=27$ h) gave after purification by recrystallization in ethanol then in ethyl acetate 254 mg (61%) of **17** as a pale yellow solid, mp 217–218 °C; ^{1}H NMR ($CDCl_3$): δ 8.75 (s, 4H), 9.35 (d, $J=1.2$ Hz, 1H), 9.45 (d, $J=1.2$ Hz, 1H), 9.77 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 114.7, 144.1, 144.5, 146.8, 149.3, 160.0, 163.0. MS (EI) *m/z* 236 (100, M^+). Anal. Calcd for $C_{12}H_8N_6$ (236.24): C, 61.01; H, 3.41; N, 35.57. Found: C, 60.18; H, 3.23; N, 35.61.

3.3.18. 2-Phenyl-4-(pyrazinyl)quinazoline (18). Cross-coupling reaction of 4-chloro-2-phenylquinazoline (240 mg, 1.0 mmol) with **4** (738 mg, 2 mmol) according to the general procedure B ($t=28$ h) gave after purification by column chromatography (silica, eluent: ethyl acetate/dichloromethane (5/5)) 198 mg (70%) of **18** as a colorless solid, mp 206–207 °C; ^{1}H NMR ($CDCl_3$): δ 7.46–7.52 (m, 5H), 7.58 (td, $J=8.29$, 1.13 Hz, 1H), 7.86 (td, $J=8.3$, 1.13 Hz, 1H), 8.12 (d, $J=8.3$ Hz, 1H), 8.65 (dd, $J=7.5$, 1.88 Hz, 2H), 8.86 (dd, $J=8.3$, 1.13 Hz, 1H), 9.62 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 121.9, 127.6, 128.2, 129.0, 129.1, 131.2, 134.4, 138.1, 143.2, 145.5, 147.2, 152.7, 153.1, 160.2, 161.7. IR (KBr) ν (cm^{-1}) 3036.2, 2924.8, 2849.8, 1612.1, 1560.5, 1542.8, 1471.2, 1338.9, 1017.8, 853.8, 767.7, 705.0, 688.8, 649.2. MS (EI) *m/z* 284 (M^+, 100), 205 (M − pyrazine, 24). Anal. Calcd for $C_{18}H_{12}N_4$ ($M_w=$ 284.11): C, 76.04; H, 4.25; N, 19.71. Found: C, 76.01; H, 4.24; N, 19.70.

3.3.19. 2,6-Bis[3′-(6′-methoxypyridazinyl)pyrazine] (19). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 3-chloro-6-methoxypyridazine (0.338 g, 2.34 mmol) according to the general procedure C ($t=48$ h) gave after purification by column chromatography (silica, eluent: petroleum ether/ethyl acetate (5/5)) 280 mg (82%) of **19** as a colorless solid, mp 116–117 °C; ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 6H), 6.94 (d, $J=8.7$ Hz, 2H), 7.02 (t, $J=$ 7.9 Hz, 2H), 7.42 (td, $J=8.7$, 1.5 Hz, 2H), 8.04 (dd, $J=7.9$, 1.5 Hz, 2H), 9.0 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 56.1, 111.9, 120.3, 121.8, 131.9, 133.8, 158.8, 162.5, 166.7. MS (EI) *m/z*

292 (M^+, 100). Anal. Calcd for $C_{18}H_{16}N_2O_2$ (292.12) C, 73.96; H, 5.52; N, 9.58. Found: C, 73.97; H, 5.50; N, 9.57.

3.3.20. 2,6-Bis[1′-(2′-methoxynaphtyl)]pyrazine (20). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 1-bromo-2-methoxynaphthalene (0.702 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by column chromatography (silica, eluent: dichloromethane) 233 mg (51%) of **20** as a colorless solid, mp 190–191 °C; ^{1}H NMR ($CDCl_3$): δ 3.95 (s, 6H), 7.45–7.33 (m, 6H), 7.71 (d, J=8.3 Hz, 2H), 7.83 (d, J=7.9 Hz, 2H), 7.95 (d, J=9.0 Hz, 2H), 8.77 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 55.5, 112.1, 119.4, 122.7, 123.4, 126.1, 127.1, 128.0, 130.0, 132.3, 144.2, 150.3, 153.8. MS (EI) m/z 392 (M^+, 100). Anal. Calcd for $C_{26}H_{20}N_2O_2$ (392.15) C, 79.57; H, 5.14; N, 7.14. Found: C, 79.56; H, 5.14; N, 7.17.

3.3.21. 2,6-Di(2′-thienyl)pyrazine (21). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 2-iodothiophene (0.636 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by column chromatography (silica, eluent: dichloromethane/ethyl acetate (5/5)) 217 mg (76%) of **21** as a yellow solid, mp 187–188 °C; ^{1}H NMR ($CDCl_3$): δ 7.07–7.11 (m, 2H), 7.42 (dd, J=4.89, 1.51 Hz, 2H), 7.66 (dd, J=4.9, 1.51 Hz, 2H), 8.68 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 126.4, 128.7, 129.5, 138.1, 141.7, 147.8. MS (EI) m/z 244 (100, M^+). Anal. Calcd for $C_{12}H_8N_2S_2$ (244.34) C, 58.99; H, 3.30; N, 11.46. Found: C, 59.06; H, 3.29; N, 11.44.

3.3.22. 2,6-Di(2′-pyridyl)pyrazine (22). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 2-bromopyridine (0.294 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by column chromatography (silica, eluent: ethylacetate) 82 mg (30%) of **22** as a colorless solid, mp 167–168 °C; ^{1}H NMR ($CDCl_3$): δ 7.33–7.38 (m, 2H), 7.85 (td, J=7.91, 1.88 Hz, 2H), 8.51 (d, J=7.92 Hz, 2H), 8.72 (dd, J=3.76, 0.75 Hz, 2H), 9.64 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 121.8, 124.8, 137.4, 143.1, 149.8, 154.7. MS (EI) m/z 234 (100, M^+). Anal. Calcd for $C_{14}H_{10}N_4$ (234): C, 71.78; H, 4.30; N, 23.92. Found: C, 71.64; H, 4.42; N, 23.84.

3.3.23. 2,6-Bis[2′-(6′-methoxypyridyl)]pyrazine (23). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 2-chloro-6-methoxypyridine (0.430 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by column chromatography (silica, eluent: dichloromethane/ethanol: (98/2)) 206 mg (60%) of **23** as a colorless solid, mp 178–179 °C; ^{1}H NMR ($CDCl_3$): δ 4.07 (s, 6H), 6.84 (d, J=8.3 Hz, 2H), 7.75 (dd, J=8.3, 7.5 Hz, 2H), 8.14 (d, J=7.5 Hz, 2H), 9.61 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 53.8, 112.5, 114.6, 139.8, 142.8, 149.8, 152.0, 164.0. MS (EI) m/z 294 (M^+, 100), 263 (M−OCH_3, 34). Anal. Calcd for $C_{16}H_{14}N_4O_2$ (M_w=294.31) C, 65.30; H, 4.79; N, 19.04. Found: C, 65.35; H, 4.74; N, 19.01.

3.3.24. 2,6-Bis[3′-(6′-methoxypyridazinyl)]pyrazine (24). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 3-chloro-6-methoxypyridazine (0.433 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by column chromatography (silica, eluent: petroleum ether/ethyl acetate (1/1)) 73 mg (21%) of **24** as a colorless solid, mp 238–239 °C; ^{1}H NMR ($CDCl_3$): δ 4.20 (s, 3H), 7.12 (d, J=9.05 Hz, 1H), 8.44 (d, J=9.05 Hz, 1H), 9.80 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 55.6, 118.4, 128.0, 143.0, 147.8, 153.4, 165.8. MS (EI) m/z 296 (M^+, 100). Anal. Calcd for $C_{14}H_{12}N_6O_2$ (296.10) C, 56.75; H, 4.08; N, 28.36. Found: C, 56.73; H, 4.05; N, 28.19.

3.3.25. 2,6-Bis[4′-(2′-methylsulfanylpyrimidinyl)]pyrazine (25). Cross-coupling reaction of 2,6-bis(tributyltin)-pyrazine **6** (770 mg, 1.17 mmol) with 4-chloro-2-methylsulfanylpyrazine (0.481 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by column chromatography (silica, eluent: n-hexane/dichloromethane/ethyl acetate (4/2/4)) 226 mg (59%) of **25** as a yellow solid, mp 208–209 °C; ^{1}H NMR ($CDCl_3$): δ 2.61 (s, 6H), 8.00 (d, J=4.9 Hz, 2H), 8.66 (d, J=4.9 Hz, 2H), 9.72 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 14.7, 112.9, 134.6, 135.7, 145.3, 148.1, 159.0, 161.2, 173.6. MS (EI) m/z 328 (M^+, 100), 313 (M−CH_3, 10), 299 (M−$2CH_3$, 45), 282 (M−SCH_3, 15), 264 (M−$2SCH_3$, 25). Anal. Calcd for $C_{14}H_{12}N_6S_2$ (328.42): C, 51.20; H, 3.68; N, 25.59; S, 19.53. Found: C, 51.25; H, 3.67; N, 25.82; S, 19.19.

3.3.26. 2,6-Bis(2′-quinolyl)pyrazine (26). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 2-chloro-quinoline (0.490 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by washing with petroleum ether (3×5 mL) 356 mg (95%) of **26** as a colorless solid, mp>260 °C; ^{1}H NMR ($CDCl_3$): δ 7.61 (m, 2H), 7.80 (m, 2H), 7.91 (dd, J=8.3, 1.1 Hz, 2H), 8.25 (d, J=8.3 Hz, 2H), 8.37 (d, J=8.7 Hz, 2H), 8.75 (d, J=8.7 Hz, 2H), 9.97 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 119.3, 127.7, 128.1, 128.8, 130.4, 137.3, 143.9, 144.2, 148.3, 150.0, 154.7. MS (FAB) m/z 335 (10), 154 (100), 136 (70). Anal. Calcd for $C_{22}H_{14}N_4$ (334.38): C, 79.02; H, 4.22; N, 16.76. Found: C, 79.00; H, 4.21; N, 16.79.

3.3.27. 2,6-Bis(2′-quinoxalyl)pyrazine (27). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 2-chloro-quinoxaline (0.494 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by washing with petroleum ether (3×5 mL) 255 mg (65%) of **27** as a colorless solid, mp>260 °C; ^{1}H NMR ($CDCl_3$): δ 8.08–8.11 (m, 4H), 8.43–8.49 (m, 4H), 10.17 (s, 2H), 10.33 (s, 2H). MS (EI) m/z 336 (M^+, 100). Anal. Calcd for $C_{20}H_{12}N_6$ (336.36): C, 71.42; H, 3.60; N, 24.99. Found: C, 71.42; H, 3.59; N, 25.04.

3.3.28. 2,6-Bis[4′-(2′-phenylquinazolyl)]pyrazine (28). Cross-coupling reaction of 2,6-bis(tributyltin)pyrazine **6** (770 mg, 1.17 mmol) with 2-phenyl-4-chloro-quinazoline (0.490 g, 3 mmol) according to the general procedure C (t=48 h) gave after purification by washing with petroleum ether (3×5 mL) 405 mg (71%) of **28** as a colorless solid, mp>260 °C; ^{1}H NMR ($CDCl_3$): δ 7.59 (m, 8H), 7.93 (td, J=7.7 Hz, 1.15 Hz, 4H), 8.22 (d, J=8.3 Hz, 2H), 8.78 (dd, J=7.7 Hz, 1.15 Hz, 4H), 9.01 (d, J=8.3 Hz, 2H), 9.92 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 112.0, 127.5, 128.3, 129.0, 129.1, 129.8, 131.3, 134.4, 138.1, 147.2, 150.7, 153.2, 160.4, 161.3. MS (EI) m/z 488 (100, M^+). Anal. Calcd for $C_{32}H_{20}N_6$ (488.54) C, 78.61; H, 4.13; N, 17.20. Found: C, 78.60; H, 4.20; N, 17.17.

References and notes

1. (a) Thomas, J. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 913. (b) Monflier, E.; Blouet, E.; Barnaux, Y.; Mortreux, A. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2100.
2. (a) Boulas, P. L.; Gomez-Kaifer, M.; Echegoyen, L. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 216. (b) Sleiman, H.; Baxter, P.; Lehn, J.-M.; Rissanen, K. *J. Chem. Soc., Chem. Commun.* **1995**, 715. (c) Hanan, G. S.; Arana, C. R.; Lehn, J.-M.; Fenske, D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1122.
3. (a) Balzani, V.; Scandola, F. *Supramolecular Photochemistry*; Ellis Horwood: Chichester, 1991. (b) Fabbrizzi, L.; Licchelli, M.; Pallavivini, P.; Perotti, A.; Sacchi, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1975.
4. Lehn, J.-M. *Macromol. Symp.* **1993**, *69*, 1.
5. (a) Ohkita, M.; Lehn, J.-M.; Baum, G.; Fenske, D. *Heterocycles* **2000**, *52*, 103. (b) Ohkita, M.; Lehn, J.-M.; Baum, G.; Fenske, D. *Chem. Eur. J.* **1999**, *5*, 3471. (c) Bassani, D. M.; Lehn, J.-M. *Bull. Soc. Chim. Fr.* **1997**, *134*, 897. (d) Bassani, D. M.; Lehn, J.-M.; Baum, G.; Fenske, D. *Angew. Chem. Int. Engl.* **1997**, *36*, 1845. (e) Hanan, G. S.; Schubert, U. S.; Volkmer, D.; Riviere, E.; Lehn, J.-M.; Kyritsakas, N.; Fischer, J. *Can. J. Chem.* **1997**, *75*, 169. (f) Hanan, G. S.; Lehn, J.-M.; Kyritsakas, N.; Fischer, J. *J. Chem. Soc., Chem. Commun.* **1995**, 765. (g) Phillips, I. G.; Steel, P. J. *Inorg. Chim. Acta* **1996**, *244*, 3. (h) Krämer, R.; Fritsky, I. O. *Eur. J. Org. Chem.* **2000**, 3505.
6. (a) Cuccia, L. A.; Lehn, J.-M.; Homo, J.-C.; Schmutz, M. *Angew. Chem. Int. Engl.* **2000**, *39*, 233. (b) Cuccia, L. A.; Ruiz, E.; Lehn, J.-M.; Homo, J.-C.; Schmutz, M. *Chem. Eur. J.* **2002**, *8*, 3448.
7. (a) Hanan, G. S.; Lehn, J.-M.; Kyritsakas, N.; Fischer, J. *J. Chem. Soc., Chem. Commun.* **1995**, 765. (b) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304. (c) Lehn, J.-M. *Supramolecular Chemistry—Concepts and Perspectives*; VCH: Weinheim, Germany, 1995. (d) Bejan, E.; Aït-Haddou, H.; Daran, J.-C.; Balavoine, G. G. A. *Eur. J. Org. Chem.* **1998**, 2907. (e) Pezet, F.; Routaboul, L.; Daran, J.-C.; Sasaki, I.; Aït-Haddou, H.; Balavoine, G. G. A. *Tetrahedron* **2000**, 8489. (f) Garcia, A. M.; Bassani, D. M.; Lehn, J.-M.; Baum, G.; Fenske, D. *Chem. Eur. J.* **1999**, *5*, 1234.
8. (a) Ishikura, M.; Kamada, M.; Terashima, M. *Synthesis* **1984**, 936. (b) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457. (c) Lehmann, U.; Henze, O.; Schlüter, D. *Chem. Eur. J.* **1999**, *5*, 854.
9. (a) Negishi, E. *Current Trends in Organic Synthesis*; Pergamon: New York, 1983. (b) Savage, S. A.; Smith, A. P.; Fraser, C. L. *J. Org. Chem.* **1999**, *5*, 10048.
10. (a) Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508. (b) Labadie, J. W.; Stille, J. K. *J. Am. Chem. Soc.* **1983**, *105*, 6129.
11. (a) Bouillon, A.; Lancelot, J. C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 2885. (b) Bouillon, A.; Lancelot, J. C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 3323. (c) Bouillon, A.; Lancelot, J. C.; de Oliviera Santos, J. S.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2002**, *58*, 4369. (d) Bouillon, A.; Lancelot, J. C.; Collot, V.; Bovy, P. R.; Rault, S. *Tetrahedron* **2003**, *59*, 10043.
12. Tyrrell, E.; Brookes, P. *Synthesis* **2003**, *4*, 469 and citations therein.
13. Turck, A.; Plé, N.; Leprêtre-Gaquère, A.; Quéguiner, G. *Heterocycles* **1998**, *49*, 205.
14. (a) Schubert, U. S.; Eschbaumer, C.; Heller, M. *Org. Lett.* **2000**, *2*, 3373. (b) Heller, M.; Schubert, U. S. *J. Org. Chem.* **2002**, *67*, 8269.
15. (a) Turck, A.; Trohay, D.; Mojovic, L.; Plé, N.; Quéguiner, G. *J. Organomet. Chem.* **1991**, *412*, 301. (b) Toudic, F.; Heynderickx, A.; Plé, N.; Turck, A.; Quéguiner, G. *Tetrahedron* **2003**, *59*, 6375.
16. Sandosham, J.; Undheim, K. *Tetrahedron* **1994**, *50*, 275.
17. Lardenois, P.; Sélim, M.; Sélim, M. *Bull. Soc. Chim. Fr.* **1971**, 1858.
18. Fürstner, A.; Leitner, A.; Mendez, M.; Krause, H. *J. Am. Chem. Soc.* **2002**, *124*, 13856.
19. Hinsberg, O.; König, O. *Ber* **1891**, *24*, 719.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 61 (2005) 2907–2912
Tetrahedron

Convenient synthetic route of versatile 21-monothiatetraphenylporphyrins of the A_4 and AB_3 type

Silvio Stute,* Kerstin Gloe and Karsten Gloe

Institut für Anorganische Chemie, Technische Universität Dresden, 01062 Dresden, Germany

Received 2 December 2004; revised 11 January 2005; accepted 12 January 2005

Abstract—A novel convenient synthetic route for poly-functional 21-monothiatetraphenylporphyrins of the type A_4 und AB_3 having base labile substituents in meso position was developed. Using this method a series of symmetric and asymmetric 21-thiaporphyrins containing different functional groups at the meso position is reported. The new products were characterized by NMR, UV–Vis and mass spectroscopy.

1. Introduction

Core-modified porphyrins show new interesting properties in metal-complexation, acid–base behaviour, redox-potentials, photochemistry and medicine in comparison with the well-known N_4 porphyrin system that may lead to useful applications.[1–6] 21-Monothiatetraphenylporphyrins represent such a promising type; asymmetric substituted AB_2C and AB_3 monothiatetraphenylporphyrins were previously reported, although the synthesis of reactive functional groups at the phenyl substituents has been difficult to achieve.[7,8] Groups such as carboxylic, amino, bromo, and hydroxy are needed as building blocks for the construction of large porphyrin arrays or for the dendritic encapsulation of the units. Such large and defined supramolecular structures and their metal complexes open up new application possibilities in medicine, optoelectronics and catalysis.[9–12]

2. Results and discussion

2.1. Modified synthesis of symmetric thiophene diols and corresponding porphyrins

The preparation of 2,5-bis-phenylhydroxymethyl substituted thiophene occurs with the classical deprotonation of the α-hydrogens on the thiophene with *n*-butyl lithium and subsequent addition of the benzaldehyde-derivative to the bislithiated dianion.[13] For base-insensitive phenyl substituents is the established reaction sequence (addition the aldehyde to the dianion) the favoured method. However, the procedure does not work when base-labile substituents on the benzaldehyde, for example, phenylcarboxylates, are used due to side reactions between the carboxylic substituents and the dianion. In order to avoid more complex protective groups or longer reaction sequences, we changed the reaction order at the addition step. The addition of the thiophene dilithiate to the base-labile benzaldehyde leads to the wanted substituted diols in good yields (Table 1). The synthesis of the desired 21-monothiatetraphenylporphyrins from the prepared diols occurred with the well established Lindsey-conditions,[14,15] which obviously gives higher yields in comparison with the method described by Adler et al.[16,17] The porphyrin condensation resulted in the mixture of three porhyrins with different core patterns: N_4, N_3S and N_2S_2. The porphyrins were separated by column chromatography, in which the desired monothiaporphyrins eluted as the second porphyrin fraction. The ether and ester cleavage was accomplished by common methods (Table 2) (Scheme 1).

Table 1. Yields of thiophene diols

Compound	**1**	**2**
Substituent R	4-COOMe	3,5-OMe
Yield [%]	40	95

2.2. Modified synthesis of asymmetric thiophene diols and corresponding porphyrins

We are also interested in synthesizing asymmetric monothiatetraphenylporphyrins of the type AB_3. The direct condensation of thiophene mono-ols to thiaporphyrins

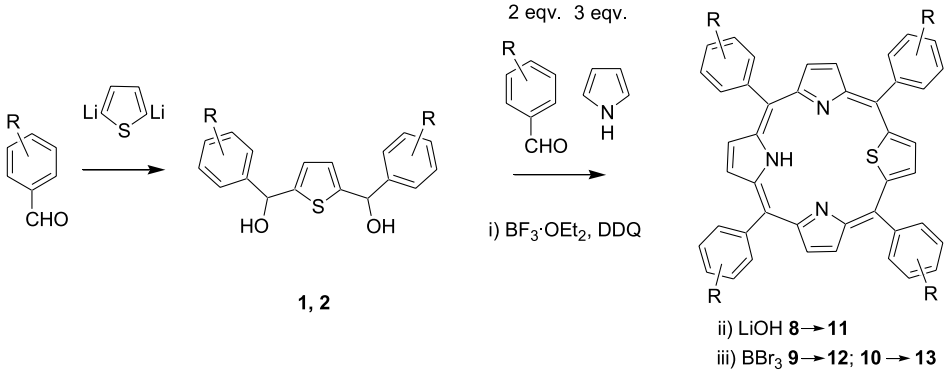
Keywords: Porphyrins; Synthesis; Sulphur; Asymmetry.
* Corresponding author. Tel.: +49 351 463 31479; fax: +49 351 463 31478; e-mail: silvio.stute@chemie.tu-dresden.de

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.037

Table 2. Yields of symmetric thiaporphyrins

Compound	**8**	**9**	**10**	**11**	**12**	**13**
Substituent R	4-COOMe	3,5-OMe	4-OMe	4-COOH	3,5-OH	4-OH
Yield [%]	22	18	19	96[a]	91[a]	92[a]

[a] These yields are related to the deprotecting reaction.



Scheme 1. General synthetic scheme of symmetric thiaporphyrins.

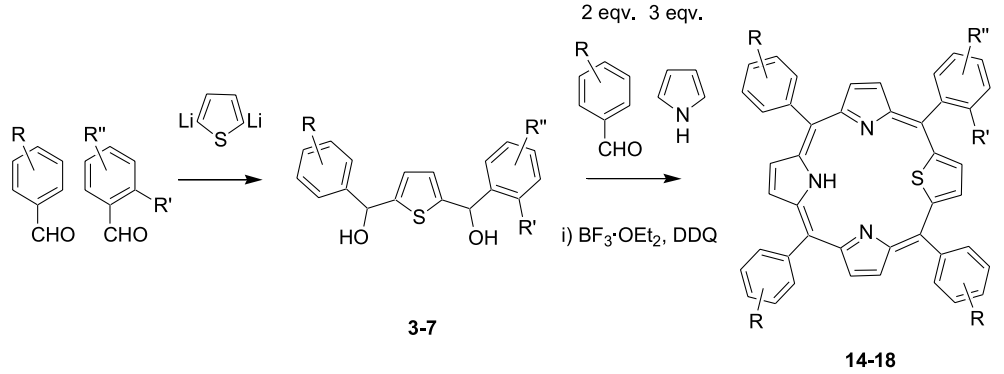
Table 3. Yields of asymmetric thiophene diols

Compound	**3**	**4**	**5**	**6**	**7**
Substituent R	4-Br	4-Br	4-Br	3,5-OMe	4-COOMe
Substituent R′	OMe	OAllyl	OMe	OAllyl	OAllyl
Substituent R″	H	5-Br	5-Br	3-OMe	H
Yield [%]	36	21	38	18	11

appears due to the lower yields rather unfavourable, thus the preparation of unsymmetrical thiophene diols is necessary. This can be effected, such as already described, with a stepwise procedure[8] or like in our case in one concerted step as described above, with the main difference of using two differently substituted aldehydes (Table 3). This reaction yields more side-products, but saves one reaction step, and is necessary for substituents that are unstable under the drastic lithiation condition. The chromatographic separation of the desired diol was achieved without any problems. Standard porphyrin synthesis resulted in asymmetric monothiaporphyrins with different core patterns: N_4, N_3S and N_2S_2. The porphyrins were separated by column chromatography, in which the desired

Table 4. Yields of asymmetric thiaporphyrins

Compound	**14**	**15**	**16**	**17**	**18**
Substituent R	4-Br	4-Br	4-Br	3,5-OMe	4-COOMe
Substituent R′	OMe	OAllyl	OMe	OAllyl	OAllyl
Substituent R″	H	5-Br	5-Br	3-OMe	H
Yield [%]	3	15	9	4	7



Scheme 2. General synthetic scheme of asymmetric thiaporphyrins.

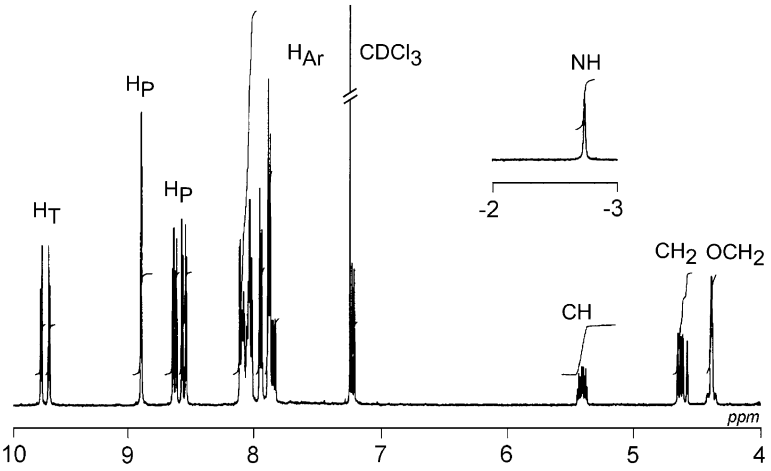


Figure 1. ^{1}H NMR spectrum of **15** recorded in $CDCl_3$. Resonance assignments: H_T, thiophene H; H_P, pyrrole H; H_{Ar}, H of the phenyl rings. The showed spectrum corresponds with the accomplished 2D NMR measurements.

monothiaporphyrins eluted as the second porphyrin fraction (Table 4) (Scheme 2).

The displayed NMR (Fig. 1) and absorption (Fig. 2) spectras of the monothiatetraphenylporhyrin **15** indicate the asymmetric structure and the typical absorption behaviour.

3. Conclusion

We report a modified synthetic method to generate novel monothiatetraphenylporphyrins having carboxyphenylic substituents at the meso position. The marginal additional preparative expense of our method results in better yields and smaller amounts of side products. We also describe an alternative way to obtain AB_3-type 21-thiaporphyrins with acceptable yields based on the one step synthesis of the asymmetrical thiophene diol precursor. The preparation and characterization of transition metal complexes with these heteroporphyrins are now underway in our group.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were obtained on a Bruker DRX-500 at 500 and 126 MHz, respectively. UV/Vis spectra were recorded on a Perkin Elmer Lambda 2. The mass spectra were measured on a Esquire Hewlett & Packard and a Shimadzu Kratos Kompact MALDI II mass spectrometer. Column chromatography was carried out with silica gel 60 (0.040–0.063 mm Merck). Fine chemicals were supplied by Fluka and solvents were dried by standard procedures before use.

4.2. Data for compounds

The preparation of 5,10,15,20-(4-methoxyphenyl)-21-thiaporphyrin **10** and 5,10,15,20-(4-bromophenyl)-21-thiaporphyrin were described earlier but we used our modified approach.[18]

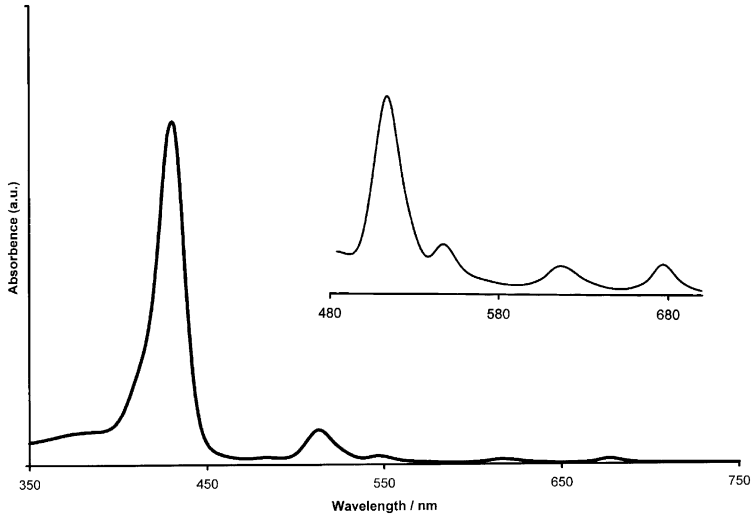


Figure 2. Absorption spectrum of **15** recorded in toluene. The concentrations used were 5×10^{-6} and 5×10^{-5} M, respectively. Enlarged Q-Bands are shown in the inset.

4.2.1. 2,5-Bis[(4-carboxymethyl)phenyl(hydroxy)-methyl]-thiophene 1. In a three neck flask equipped with rubber septum, gas inlet tube and reflux condenser dry *n*-hexane (25 ml) was taken. TMEDA (16 mmol, 1.86 ml) and *n*-butyl lithium (20 ml of a 1.6 M solution) were added into the stirred solution. Destilled thiophene (8 mmol, 317 μl) was added and the reaction mixture was refluxed for 1 h under slow argon purging.

After this time, the dilithiated thiophene mixture was cooled to 0 °C and transferred into the ice cold solution of methyl 4-formylbenzoate (16 mmol, 2.63 g) in dry THF (30 ml). The mixture was stirred for 10 min and saturated NH_4Cl added to quench the reaction. The organic layer was extracted with ethyl acetate and dried over Na_2SO_4. The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-heptane 1:1) and the desired diol **1** was obtained besides small amounts of unreacted aldehyde and mono-ol as the third fraction (1.32 g, 40%).

^{1}H NMR ($CDCl_3$, δ in ppm) 2.65 (bs, 2H, OH), 3.9 (s, 6H, OMe), 6.0 (s, 2H, CH), 6.7 (s, 2H, β-thiophene), 7.5 (d, 4H, meta-H), 8.0 (d, 4H, ortho-H).

4.2.2. 2,5-Bis[(3,5-methoxy)phenyl(hydroxy)methyl]-thiophene 2. Compound **2** was prepared following the same method given for **1** by using dry *n*-hexane (10 ml), TMEDA (10 mmol, 1.5 ml), *n*-butyl lithium (6.25 ml of 1.6 M solution) and thiophene (5 mmol, 397 μl) for the dilithiated thiophene, which was added at 0 °C to a precooled solution of 3,5-methoxybenzaldehyde (10 mmol, 1.66 g) in dry THF (10 ml). The crude product was purified by silica gel column chromatography in ethyl acetate/*n*-heptane (4:6). Compound **2** was obtained as a white solid (1.98 g, 95%).

^{1}H NMR ($CDCl_3$, δ in ppm) 2.9 (bs, 2H, OH); 3.75 (s, 12H, OMe); 5.85 (d, 2H, CH); 6.45 (t, 2H, para-H); 6.6 (d, 4H, ortho-H); 6.7 (s, 2H, β-thiophene).

4.2.3. 2-[(4-Bromo)phenylhydroxymethyl]-5-[(2-methoxy-phenyl)hydroxymethyl]-thiophene 3. Compound **3** was synthesized in the same way as **1** by using dry *n*-hexane (25 ml), TMEDA (19 mmol, 2.87 ml), *n*-butyl lithium (23.9 ml of 1.6 M solution) and thiophene (9.6 mmol, 766 μl) for the dilithiated thiophene. This mixture was added at 0 °C to a precooled 1:1 mixture of 4-bromobenz-aldehyde (9.6 mmol, 1.77 g) and 2-methoxybenzaldehyde (9.6 mmol, 1.30 g) in dry THF (30 ml). The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-heptane 1:1). **2** was obtained as a yellow solid (1.39 g, 36%).

^{1}H NMR ($CDCl_3$, δ in ppm): 2.4 (bs, 1H, OH); 3.25 (bs, 1H, OH); 3.8 (s, 3H, OMe); 5.9 (s, 1H, CH); 6.1 (s, 1H, CH); 6.7 (m, 2H, thiophene); 6.85 (d, 1H, H-Ar); 6.95 (t, 1H, H-Ar); 7.3 (t, 2H, ortho-H); 7.35 (d, 1H, H-Ar) 7.4 (d, 2H, metha-H).

4.2.4. 2-[(4-Bromo)phenylhydroxymethyl]-5-[(2-allyl-oxy-5-bromophenyl)hydroxymethyl]-thiophene 4. Preparation of **4** was done following the same method given for **1** by using dry *n*-hexane (30 ml), TMEDA (19 mmol, 2.97 ml), *n*-butyl lithium (38 ml of 1.6 M solution) and thiophene (9.5 mmol, 750 μl) for the dilithiated thiophene, which was added at 0 °C to a precooled 1:1 mixture of 4-bromobenzaldehyde (9.5 mmol, 1.76 g) and 2-allyloxy-5-bromobenzaldeyhde (9.5 mmol, 2.285 g) in dry THF (30 ml). The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-heptane 3:7) and obtained as yellow solid (1.0 g, 21%).

^{1}H NMR ($CDCl_3$, δ in ppm) 2.3 (bs, 1H, OH); 3.0 (m, 1H, OH); 4.5 (m, 2H, OCH_2); 5.25 (dd, 2H, CH_2); 5.9 (dq, 1H, CH); 5.95 (d, 1H, CH); 6.15 (d, 1H, CH); 6.65 (d, 2H, β-thiophene); 6.7 (d, 1H, meta-H); 7.3 (d, 2H, ortho-H); 7.35 (dd, 1H, para-H); 7.45 (d, 2H, meta-H); 7.5 (d, 1H, ortho-H).

4.2.5. 2-[(4-Bromo)phenylhydroxymethyl]-5-[(2-meth-oxy-5-bromophenyl)hydroxymethyl]-thiophene 5. Compound **5** was prepared following the same method given for **1** by using dry *n*-hexane (20 ml), TMEDA (10.8 mmol, 1.62 ml), *n*-butyl lithium (13.5 ml of 1.6 M solution) and thiophene (5.4 mmol, 430 μl) for the dilithiated thiophene. The mixture was cooled to 0 °C and transferred into a precooled 1:1 mixture of 4-bromobenzaldehyde (5.4 mmol, 1.0 g) and 2-methoxy-5-bromobenzaldehyde (5.4 mmol, 1.16 g) in dry THF (30 ml). The crude product was purified by silica gel column chromatography (ethyl acetate/*n*-heptane 3:7). Compound **5** was obtained as a colorless solid (0.992 g, 38%).

^{1}H NMR ($CDCl_3$, δ in ppm) 2.45 (m, 1H, OH); 3.05 (m, 1H, OH); 3.75 (d, 3H, OMe); 5.9 (d, 1H, CH); 6.1 (d, 1H, CH); 6.15 (2d, 2H, β-thiophene); 6.25 (dd, 1H, meta-H); 7.3 (d, 2H, ortho-H); 7.35 (dd, 1H, para-H); 7.45 (d, 2H, meta-H); 7.5 (d, 1H, ortho-H).

4.2.6. 2-[(3,5-Methoxy)phenylhydroxymethyl]-5-[(2-allyl-oxy-3-methoxyphenyl)hydroxymethyl]-thiophene 6. Compound **6** was synthesized in the same way as **1** by using dry *n*-hexane (25 ml), TMEDA (15.6 mmol, 2.34 ml), *n*-butyl lithium (19.5 ml of 1.6 M solution) and thiophene (7.8 mmol, 660 μl). The mixture of the dilithiated thiophene was cooled to 0 °C and transferred into a cold solution of 3,5-dimethoxybenzaldehyde (7.8 mmol, 1.29 g) and 2-allyloxy-3-methoxybenzaldehyde (7.8 mmol, 1.5 g) in dry THF (30 ml). Purification of the product was done by silica gel column chromatography (ethyl acetate/*n*-heptane 1:1). Compound **6** was isolated as a yellow solid (0.61 g, 18%).

^{1}H NMR ($CDCl_3$, δ in ppm) 2.3 (bs, 1H, OH); 3.2 (dd, 1H, OH); 3.75 (s, 6H, OMe); 3.85 (s, 3H, OMe); 4.35 (m, 2H, OCH_2); 5.2 (m, 2H, CH_2); 5.85 (m, 1H, CH); 5.9 (d, 1H, CH); 6.1 (t, 1H, CH); 6.35 (t, 2H, β-thiophene); 6.55 (s, 2H, ortho-H); 6.65 (t, 1H, para-H); 6.85 (dd, 1H, H-Ar); 7.0 (dt, 1H, H-Ar); 7.05 (dd, 1H, H-Ar).

4.2.7. 2-[(4-Carboxymethyl)phenylhydroxymethyl]-5-[(2-allyloxyphenyl)hydroxymethyl]-thiophene 7. The synthesis of **7** followed the same manner as given for **1** by using dry *n*-hexane (10 ml), TMEDA (26 mmol, 3.9 ml), *n*-butyl lithium (32.5 ml of 1.6 M solution) and thiophene (13 mmol, 1.03 ml). After cooling to 0 °C, the mixture of the

dilithiated thiophene was cooled added to a cold solution of methyl 4-formylbenzoate (13 mmol, 2.17 g) and 2-allyloxybenzaldehyde (13 mmol, 2.14 g) in dry THF (15 ml). After separating by silica gel column chromatography (ethyl acetate/*n*-heptane 1:1), **7** was isolated as a yellow solid (0.59 g, 11%).

^{1}H NMR ($CDCl_3$, δ in ppm) 2.4 (bs, 1H, OH); 3.3 (m, 1H, OH); 3.9 (s, 3H, OMe); 4.5 (bs, 2H, OCH_2); 5.2 (m, 2H, CH_2); 5.9 (dqi, 1H, CH); 6.0 (d, 1H, CH); 6.15 (t, 1H, CH); 6.7 (m, 2H, β-thiophene); 6.85 (d, 1H, H-Ar); 6.95 (t, 1H, H-Ar); 7.3 (t, 1H, H-Ar); 7.4 (d, 1H, H-Ar); 7.5 (d, 2H, ortho-H); 8.0 (d, 2H, metha-H).

4.2.8. 5,10,15,20-(4-Carboxymethyl)phenyl-21-thiaporphyrin 8. Diol **1** (3.2 mmol, 1.32 g), methyl 4-formylbenzoate (6.4 mmol, 1.05 g) and pyrrole (9.6 mmol, 665 µl) were dissolved in dry CH_2Cl_2 (350 ml) under argon purging (degassing) in a one-neck flask. $BF_3 \cdot OEt_2$ (0.32 mmol, 40 µl) was added to start the cyclocondensation and the reaction mixture was stirred for 2 h under argon atmosphere in the dark at room temperature. DDQ (96 mmol, 2.18 g) was added and the solution was stirred on air for additional 3 h. The solvent was removed under reduced pressure and the compound was isolated by silica gel column chromatography (chloroform/methanol 99:1) as eluent to acquire the purple solid in the second yellow-brown fraction (0.61 g, 22%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (s, 1H, NH), 4.1 (s, 9H, COOMe), 8.28 (d, 4H, H_{aryl}), 8.33 (d, 4H, H_{aryl}), 8.45 (d, 4H, H_{aryl}), 8.5 (d, 4H, H_{aryl}), 8.6 (d, 4H, β-pyrrole), 8.9 (s, 2H, β-pyrrole), 9.7 (s, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 864, calcd mass 863.95.

4.2.9. 5,10,15,20-(3,5-Methoxy)phenyl-21-thiaporphyrin 9. Compound **9** was synthesized in the same procedure like **8**, using dry CH_2Cl_2 (200 ml), diol **2** (2.6 mmol, 1.1 g), 3,5-methoxybenzaldehyde (5.3 mmol, 0.88 g) and pyrrole (7.9 mmol, 547 µl). $BF_3 \cdot OEt_2$ (0.3 mmol, 37.5 µl) was added to start the reaction. After stirring for 1 h, DDQ (5.3 mmol, 1.2 g) was added. The crude product was purified by silica gel column chromatography (chloroform) and isolated as a purple, crystalline solid (0.40 g, 18%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (bs, 1H, NH); 3.9 (d, 24H, OMe); 6.9 (t, 4H, para-H); 7.4 (dd, 8H, ortho-H); 8.7 (dd, 4H, β-pyrrole); 9.0 (d, 2H, β-pyrrole); 9.8 (s, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 872, calcd mass 872.02.

4.2.10. 5,10,15,20-(4-Methoxy)phenyl-21-thiaporphyrin 10. Compound **10** was synthesized in the same manner like **8**, using dry CH_2Cl_2 (250 ml), 2,5-Bis[(4-methoxy)-phenyl(hydroxy)methyl]-thiophene (3.9 mmol, 1.4 g), 4-methoxybenzaldehyde (7.8 mmol, 0.95 ml) and pyrrole (11.8 mmol, 814 µl). $BF_3 \cdot OEt_2$ (0.4 mmol, 49 µl) was added to start the reaction. After stirring for 3 h, DDQ (5.3 mmol, 1.2 g) was added. The crude product was purified by silica gel column chromatography (chloroform/methanol 99:1) and isolated as a purple, crystalline solid (0.56 g, 19%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.7 (bs, 1H, NH); 4.1 (s, 12H, OMe); 7.3 (dd, 8H, meta-H); 8.1 (dd, 8H, ortho-H); 8.65 (dd, 4H, β-pyrrole); 8.95 (d, 2H, β-pyrrole); 9.75 (s, 2H, β-thiophene). MALDI-MS obsd mass 753, calcd mass 751.9.

4.2.11. 5,10,15,20-(4-Carboxy)phenyl-21-thiaporphyrin 11. Compound **8** (0.17 mmol, 150 mg) was dissolved in THF (20 ml) and water (10 ml) and LiOH (30 mmol, 750 mg) was added. The mixture was refluxed for 5 h, THF was removed and the pH value was set to 4 (1 N HCl), so the porphyrin **10** precipitated. After centrifugation the water was removed and the product was washed with cold ethyl acetate and water (0.134 g, 96%).

^{1}H NMR (DMSO-d_6, δ in ppm) −2.8 (s, 1H, NH); 8.37 (d, 8H, ortho-H); 8.39 (d, 8H, metha-H); 8.6 (dd, 4H, β-pyrrole); 9.0 (d, 2H, β-pyrrole); 9.8 (s, 2H, β-thiophene); 11.1 (bs, 4H, COOH). ESI-MS (+75 V) obsd mass 808, calcd mass 807.95.

4.2.12. 5,10,15,20-(3,5-Hydroxy)phenyl-21-thiaporphyrin 12. BBr_3 (6.02 mmol, 580 µl) was transferred into a solution of **9** (0.40 g, 0.46 mmol) in 25 ml of dry CH_2Cl_2 and stirred for 24 h at room temperature under dry conditions. The reaction was quenched with water, the pH value was set to 7 (1 N NaOH) and the phases were separated. The aqueous phase was extracted with ethyl acetate. The organic layers were dried over Na_2SO_4 and the solvent was evaporated. Purification was done by silica gel column chromatography (ethyl acetate). After removing solvent the product affords as dark purple solid (0.32 g, 91%).

^{1}H NMR (acetone-d_6, δ in ppm) −2.7 (s, 1H, NH); 6.9 (t, 4H, para-H); 7.3 (d, 8H, ortho-H); 8.75 (2 s, 8H, OH); 8.8 (d, 4H, β-pyrrole); 9.2 (d, 2H, β-pyrrole); 9.95 (s, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 760, 821 [M+HAc], calcd mass 759.8.

4.2.13. 5,10,15,20-(4-Hydroxy)phenyl-21-thiaporphyrin 13. Compound **13** was synthesized like porphyrin **11**, dissolving **12a** (0.114 mmol, 0.086 g) in CH_2Cl_2 (8 ml) and adding BBr_3 (1.14 mmol, 110 µl). The product (73 mg, 92%) was isolated by silica gel column chromatography (ethyl acetate).

^{1}H NMR (acetone-d_6, δ in ppm) −2.5 (s, 1H, NH); 7.4 (dd, 8H, meta-H); 8.1 (dd, 8H, ortho-H); 8.7 (d, 2H, β-pyrrole); 8.9 (2 s, 4H, OH); 9.1 (s, 2H, β-pyrrole); 9.9 (s, 2H, β-thiophene). MALDI-MS obsd mass 697, calcd mass 695.8.

4.2.14. 5-(2-Methoxy)phenyl-10,15,20-(4-bromo)phenyl-21-thiaporphyrin 14. Compound **14** was prepared following the method given for **8**, using dry CH_2Cl_2 (250 ml), **3** (3.5 mmol, 1.35 g), 4-bromobenzaldehyde (7 mmol, 1.29 g) and pyrrole (10.5 mmol, 724 µl). $BF_3 \cdot OEt_2$ (0.35 mmol, 44 µl) was added and the mixture was stirred for 3 h before DDQ (5.2 mmol, 1.19 g) was added. The crude product was purified by silica gel column chromatography (chloroform) and isolated as a purple, crystalline solid (100 mg, 3%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (bs, 1H, NH); 3.6 (s, 3H,

OMe); 7.4 (m, 2H, ortho-H); 7.8–8.1 (m, 14H, H-Ar); 8.6 (2d, 4H, β-pyrrole); 8.9 (s, 2H, β-pyrrole); 9.5 (dd, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 898, calcd mass 898.52.

4.2.15. 5-(2-Allyloxy-5-bromo)phenyl-10,15,20-(4-bromo)phenyl-21-thiaporphyrin 15. Preparation of **15** followed the same steps like the preparation of **8**, using dry CH_2Cl_2 (250 ml), **4** (1.96 mmol, 1 g), 4-bromobenzaldehyde (3.92 mmol, 0.72 g) and pyrrole (5.88 mmol, 410 µl). After $BF_3 \cdot OEt_2$ (0.2 mmol, 25 µl) was added, the mixture was stirred for 3 h before DDQ (3.92 mmol, 0.89 g) was added. The crude product was purified by silica gel column chromatography (chloroform). After another silica gel column chromatography (*n*-heptane/chloroform 1:2) **15** was isolated as a purple, crystalline solid (300 mg, 15%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (bs, 1H, NH); 4.4 (m, 2H, OCH_2); 4.6 (dd, 2H, CH_2); 5.4 (dq, 1H, CH); 7.2 (dd, 1H, H-Ar); 7.8 (dd, 1H, H-Ar); 7.85–8.1 (12H, H-Ar); 8.15 (d, 1H, H-Ar); 8.6 (2 d, 4H, β-pyrrole); 8.9 (s, 2H, β-pyrrole); 9.65 (dd, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 1003, 962 [M−allyl], calcd mass 1003.45.

4.2.16. 5-(2-Methoxy-5-bromo)phenyl-10,15,20-(4-bromo)phenyl-21-thiaporphyrin 16. Compound **16** was synthesized using the general procedure (s. **8**), with dry CH_2Cl_2 (450 ml), diol **5** (3.1 mmol, 1.49 g), 4-bromobenzaldehyde (6.2 mmol, 1.14 g), pyrrole (9.2 mmol, 640 µl), $BF_3 \cdot OEt_2$ (0.3 mmol, 37 µl) and DDQ (6.2 mmol, 1.396 g). The purification of the product was done by silica gel column chromatography (chloroform). After another silica gel column chromatography (*n*-heptane/chloroform 1:2) **16** was obtained as a purple, crystalline solid (270 mg, 9%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (bs, 1H, NH); 3.55 (s, 3H, OMe); 7.2 (d, 1H, H-Ar); 7.8–8.1 (m, 14H, H-Ar); 8.55 (dd, 2H, β-pyrrole); 8,6 (dd, 2H, β-pyrrole); 8,9 (d, 2H, β-pyrrole); 9,6 (dd, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 977, calcd mass 977.42.

4.2.17. 5-(2-Allyloxy-3-methoxy)phenyl-10,15,20-(3,5-methoxy)phenyl-21-thiaporphyrin 17. Compound **17** was prepared in the general procedure (s. **8**), with dry CH_2Cl_2 (100 ml), **6** (1.4 mmol, 0.61 g), 4-bromobenzaldehyde (2.8 mmol, 0.456 g) and pyrrole (4.1 mmol, 290 µl), $BF_3 \cdot OEt_2$ (0.14 mmol, 17 µl) and DDQ (2.8 mmol, 0.635 g). After two silica gel column chromatographies (chloroform) the product was isolated as a orange-purple, crystalline solid (50 mg, 4%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (bs, 1H, NH); 4.0 (s, 18H, OMe); 4.1 (s, 3H, OMe); 4.1 (s, 2H, OCH_2); 4.3 (dd, 2H, CH_2); 4.9 (dq, 1H, CH); 6.9 (d, 6H, ortho-H); 7.4 (m, 8H, H-Ar); 7.65 (dd, 1H, H-Ar); 8.65 (s, 2H, β-pyrrole); 8.7 (d, 1H, β-pyrrole); 8.75 (d, 1H, β-pyrrole); 9.02 (s, 2H, β-pyrrole); 9.7 (d, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 898, 857 [M−allyl], calcd mass 898.06.

4.2.18. 5-(2-Allyloxy)phenyl-10,15,20-(4-carboxymethyl)phenyl-21-thiaporphyrin 18. Preparation of **18** was prepared in analogue to **8**, using dry CH_2Cl_2 (100 ml), **7** (1.3 mmol, 0.534 g), methyl 4-formylbenzoate (2.6 mmol, 0.426 g) and pyrrole (3.9 mmol, 270 µl), $BF_3 \cdot OEt_2$ (0.13 mmol, 16 µl) and DDQ (2.6 mmol, 0.59 g). The crude product was purified by two silica gel column chromatographies (chloroform) and the product afforded as a orange-purple, crystalline solid (82 mg, 7%).

^{1}H NMR ($CDCl_3$, δ in ppm) −2.75 (bs, 1H, NH); 4.0 (s, 9H, OMe); 4.4 (s, 2H, OCH_2); 4.6 (dd, 2H, CH_2); 5.45 (dq, 1H, CH); 7.35 (d, 1H, H-Ar); 7.4 (t, 1H, H-Ar); 7.75 (dt, 1H, H-Ar); 8.0 (d, 1H, H-Ar); 8.3–8.7 (m, 16H, H-Ar, β-pyrrole); 8.9 (s, 2H, β-pyrrole); 9.5 (s, 2H, β-thiophene). ESI-MS (+75 V) obsd mass 862, 841 [M−allyl], calcd mass 861.98.

References and notes

1. Ulman, A.; Manassen, J. *J. Am. Chem. Soc.* **1975**, *97*, 6540–6544.
2. Latos-Grazynski, L. In Kadish, K. M., Smith, K. M., Guilard, R., Eds.; The Porphyrin Handbook; Academic Press: New York, 2000; Vol. 2, pp 361–416.
3. Broadhurst, M. J.; Grigg, R.; Johnson, A. W. *J. Chem. Soc. C* **1971**, 3681–3690.
4. (a) Ulman, A.; Manassen, J.; Frolow, F.; Rabinovich, D. *Inorg. Chem.* **1981**, *20*, 1987–1990. (b) Pandian, R. P.; Chandrashekar, T. K. *Inorg. Chem.* **1994**, *33*, 3317–3324.
5. (a) Gopinath, C. S.; Pandian, R. P.; Manoharan, P. T. *J. Chem. Soc., Dalton Trans.* **1996**, 1255–1259. (b) Ulman, A.; Manassen, J.; Frolow, F.; Rabinovich, D. *Tetrahedron Lett.* **1978**, 167–170. (c) Ulman, A.; Manassen, J.; Frolow, F.; Rabinovich, D. *Tetrahedron Lett.* **1978**, 1885–1886.
6. Hilmey, D. G.; Abe, M.; Nelen, M. I.; Stilts, C. E.; Baker, G. A.; Baker, S. N.; Bright, F. V.; Davies, S. R.; Gollnick, S. O.; Oseroff, A. R.; Gibson, S. L.; Hilf, R.; Detty, M. R. *J. Med. Chem.* **2002**, *45*, 449–461.
7. Cho, W.-S.; Kim;, H.-J.; Littler, B. J.; Miller, M. A.; Lee, C.-H.; Lindsey, J. S. *J. Org. Chem.* **1999**, *64*, 7890–7901.
8. (a) Agarwal, N.; Hung, C.-H.; Ravikanth, M. *Eur. J. Org. Chem.* **2003**, *19*, 3730–3734. (b) Gupta, I.; Agarwal, N.; Ravikanth, M. *Eur. J. Org. Chem.* **2004**, 1693–1697.
9. Felber, B.; Calle, C.; Schweiger, A.; Diederich, F. *Org. Biomol. Chem.* **2003**, *1*, 1090–1093.
10. Finikova, O.; Galkin, A.; Rozhkov, V.; Cordero, M.; Hägerhäll, C.; Vinogradov, S. *J. Am. Chem. Soc.* **2003**, *125*, 4882–4893.
11. Matos, M. S.; Hofkens, J.; Verheijen, W.; De Schryver, F. C.; Hecht, S.; Pollak, K. W.; Frechet, J. M. J.; Forier, B.; Dehaen, W. *Macromolecules* **2000**, *33*, 2967–2973.
12. Osuka, A.; Kim, D. *Acc. Chem. Res.* **2004**, *37*, 735–745.
13. Ulman, A.; Manassen, J. *J. Chem. Soc., Perkin Trans. 1* **1979**, *4*, 1066–1069.
14. Lindsey, J. S.; Schreimann, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827–836.
15. Chmielewski, P.; Grzeszczcuk, M.; Latos-Grazynski, L.; Lisowski, J. *Inorg. Chem.* **1989**, *28*, 3546–3552.
16. Adler, A. D.; Longo, F. R.; Finarelli, J. D.; Goldmacher, J.; Assour, J.; Korsakoff, L. *J. Org. Chem.* **1967**, *32*, 476.
17. Latos-Grazynski, L.; Lisowski, J. *J. Am. Chem. Soc.* **1987**, *109*, 4428–4429.
18. Srinivasan, A.; Srivedi, B.; Reddy, M. V. R.; Narayanan, S. J.; Chandrashekar, T. K. *Tetrahedron Lett.* **1997**, *38*, 4149–4152.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
ELSEVIER
Tetrahedron 61 (2005) 2913–2919

Aspartame analogues containing 1-amino-2-phenylcyclohexanecarboxylic acids (c_6Phe). Part 2

Miriam Alías, Marta Lasa, Pilar López, José I. García and Carlos Cativiela*

Departamento de Química Orgánica, ICMA, Facultad de Ciencias, Universidad de Zaragoza-CSIC, 50009 Zaragoza, Spain

Received 22 November 2004; revised 7 January 2005; accepted 12 January 2005

Abstract—This report describes the synthesis and conformational analysis of optically pure dipeptide analogues of aspartame, namely H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe, in which the Phe residue of aspartame has been replaced by a restricted Phe with a cyclohexane skeleton: 1-amino-2-phenylcyclohexanecarboxylic acid (c_6Phe). The dipeptide that incorporates (1*R*,2*S*)-c_6Phe is sweet whereas the compound that incorporates (1*S*,2*R*)-c_6Phe is bitter. This relationship between the absolute configuration of the dipeptides and the properties is explained in terms of the different conformational behaviour displayed by each molecule, as determined by molecular mechanics and molecular dynamics calculations that take into account solvent effects.

1. Introduction

The discovery of the dipeptide sweetener Aspartame[1] [H-(*S*)-Asp-(*S*)-Phe-OMe] has led to the synthesis of a large number of dipeptide analogues in an attempt to find a better taste profile and to elucidate the mechanism for the sweet response.

Analysis of the preferred conformations of aspartame using a combination of X-ray crystallography, ^{1}H NMR spectroscopy and molecular mechanics calculations has led several authors to propose different models for the tastant–receptor interaction. Two such models have emerged as the most appropriate: Temussi[2] and Goodman.[3] The difference between the two models concerns the conformational flexibility of aspartame dipeptides. As a result, the availability of conformationally restricted analogues is very important to establish the molecular arrays required for sweet and bitter tastes. The incorporation of side-chain constrained amino acids constitutes a powerful tool to explore this aspect. Restrictions on the χ^1 torsion angles will affect the conformational preferences of the side substituents and have an impact on the rotameric distribution with respect to unmodified residues. Thus, the incorporation of amino acids with well-defined χ^1 tendencies into bioactive peptides may provide fundamental insights into the precise conformational requirements of the side-chain groups to fit the receptor binding site.

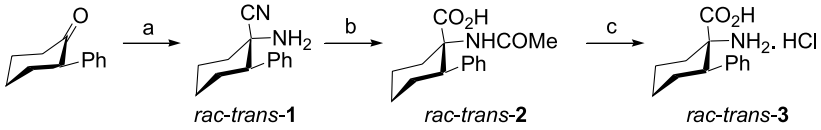
Several conformationally restricted analogues of aspartame in which the Phe residue has been replaced by different conformationally restricted amino acids have been synthesised and studied. Particular examples have incorporated different 1-aminocycloalkanecarboxylic acids[4,5] and 1-amino 2-phenylcyclopropanecarboxylic acids (c_3Phe).[6] Some of the resulting compounds were found to be sweet whereas others are bitter or tasteless.

The presence in the phenylalanine analogue of a cyclohexane structure, which tethers C_α to C_β, (c_6Phe) appears to be very efficient for the restriction of the side-chain flexibility and is far superior to other restrictions such as α- and/or β-methylation. For each enantiomer only two χ^1 arrangements are accessible, with one of these being strongly discriminated by the propensity of the bulky phenyl group to occupy equatorial positions in a chair conformation. As a result, (1*S*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe can be viewed as frozen *gauche* (+) side-chain analogues of (*S*)-Phe and, conversely, (1*R*,2*S*)-c_6Phe as *gauche* (−) analogues of (*R*)-Phe.[7]

Due to this interesting restriction, and as an extension of our studies on constrained analogues of aspartame, we previously described the replacement of the Phe residue with

Keywords: Strecker; Cyclohexanes; Constrained phenylalanines; Aspartame analogues; Computer-assisted methods; Chiral stationary phase; HPLC resolution.
Abbreviations: TEA, triethylamine; Cbz, benzyloxycarbonyl; EtOAc, ethyl acetate; MeOH, methanol; NMM, *N*-Methylmorpholine; O^{t}Bu, *tert*-butoxy; OMe, methoxy; TFA, trifluoroacetic acid; Bz, benzoyl.
* Corresponding author. Tel./fax: +34 976 761 210;
e-mail: cativiela@unizar.es

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.033



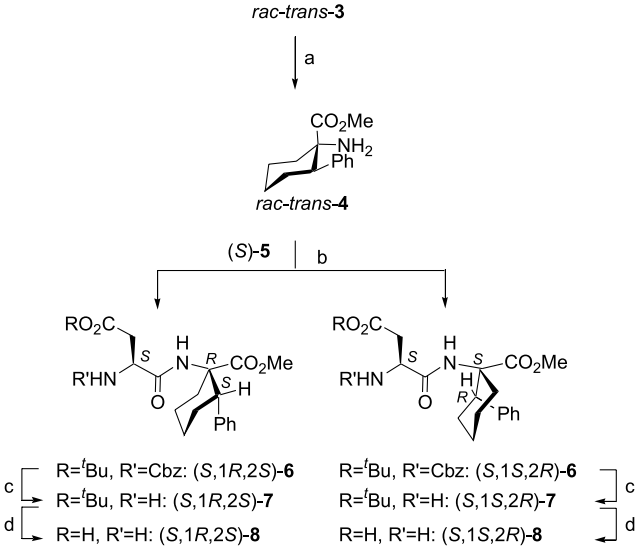
Scheme 1. Synthetic route to racemic *trans*-c_6Phe. Reagents and conditions: (a) NaCN, NH_4Cl, ^{i}PrOH, NH_4OH. (b) CH_3COCl, TEA, CH_2Cl_2. (c) 12 N HCl, reflux.

two enantiomers of *cis*-c_6Phe, namely (1*R*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe, which resulted in a sweet and a bitter compound, respectively.[8] The different organoleptical properties of the two molecules are related to the different conformational behaviour of each dipeptide, as exemplified by molecular calculations. These conformational preferences depend on the absolute configuration of each enantiomer of the Phe analogue.

cis-c_6Phe *trans*-c_6Phe

In the course of our work on the synthesis of conformationally restricted phenylalanines, we recently developed[9] a convenient route for the preparation of *trans*-c_6Phe in racemic form by a Strecker reaction on 2-phenylcyclohexanone. The importance of the absolute configuration of the Phe analogue in the sweet properties of the aspartame dipeptide makes the incorporation of the two enantiomers of *trans*-c_6Phe, i.e. (1*S*,2*R*)-c_6Phe and (1*R*,2*S*)-c_6Phe, indispensable to complete the synthesis and conformational studies of aspartame analogues incorporating all four isomers of restricted phenylalanine c_6Phe.

We report here the synthesis and conformational behaviour of H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe as conformationally restricted aspartame analogues.



Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH. (b) (i) ^{t}BuOCOCl, NMM, CH_2Cl_2; (ii) HPLC. (c) TFA/CH_2Cl_2. (d) H_2, Pd/C.

2. Results and discussion

2.1. Peptide synthesis

The first step in the synthesis of the aspartame analogues involved the preparation of *rac-trans*-**3**. As previously described by our group,[9] a convenient route for the preparation of *trans*-c_6Phe in its racemic form involves a Strecker reaction of 2-phenylcyclohexanone and subsequent hydrolysis of the product (Scheme 1). The synthesis of amino ester *rac-trans*-**4** was achieved in moderate yield from *rac-trans*-**3** by formation of the methyl ester with thionyl chloride/methanol (Scheme 2). Once this starting material had been obtained, we attempted to couple these amino esters, *rac-trans*-**4**, with conveniently protected aspartic acid Cbz-(*S*)-Asp(O^{t}Bu)-OH, (*S*)-**5**. Coupling by the mixed-anhydride procedure,[10,11] using ^{i}BuOCOCl in the presence of NMM in CH_2Cl_2 at −15 °C, afforded a mixture of the desired diastereoisomers (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** in very good yield.

However, all attempts to separate the diastereoisomers by flash chromatography were unsuccessful. We therefore decided to explore the use of semi-preparative HPLC for the resolution of (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6**. Analytical separation was initially examined in normal phase using silica as the stationary phase, but separation was not observed under any of the conditions tested. Better results were obtained on an amylose-derived chiral stationary phase, which has proved to be very efficient in other semi-preparative resolutions of phenylalanine surrogates.[8,12–16] Elution and detection conditions for the best separations are given in Table 1 and the analytical resolution is shown in Figure 1.

Table 1. Selected chromatographic data for the HPLC resolution of (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** on the amylose-derived chiral stationary phase

Column	Eluent[a] A/B/C	Flow (mL/min)	λ (nm)	k_1'	α	R_s
Analytical[b]	97/3/0	0.8	210	1.97	1.25	1.67
Semi-preparative[c]	96/3/1	15	254	1.79	1.22	0.90

For the definition of k', α and R_s see Section 4.
[a] A: *n*-hexane, B: 2-propanol, C: chloroform.
[b] Steel column, 150 mm×4.6 mm ID, t_o=2.64 min. c=5 mg/mL. Temperature: 25 °C.
[c] Steel column, 150 mm×20 mm ID, t_o=2.52 min. c=200 mg/mL. Temperature: 25 °C.

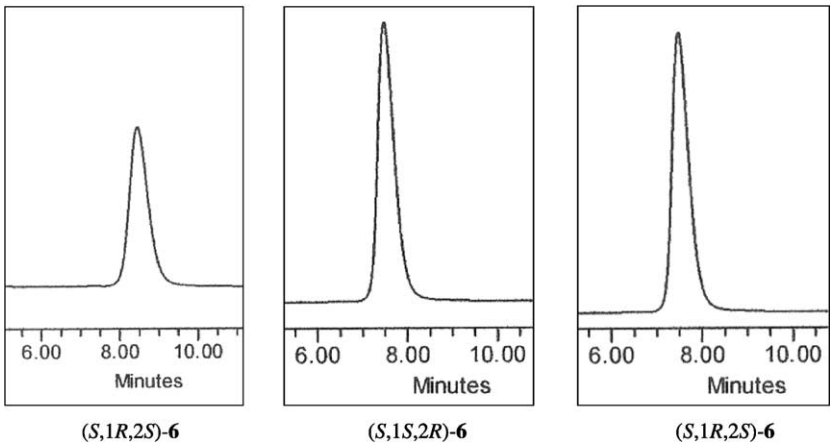


Figure 1. HPLC analytical resolution of Cbz-Asp(O^{t}Bu)-*rac*-*trans*-c_6Phe-OMe and resolved diastereoisomers (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6**.

For the extension of the analytical conditions to the semi-preparative scale we selected the mixture *n*-hexane/2-propanol/chloroform indicated in Table 1. This solvent system was found to be the most appropriate eluent to optimise the resolution in relation to the column loadability.

Direct assignment of the stereochemistry of the dipeptides (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** was made by comparing their spectral data with those corresponding to the dipeptides synthesised by coupling the two optically pure enantiomers (1*R*,2*S*)-**4** and (1*S*,2*R*)-**4**, prepared from optically pure amino acids (1*R*,2*S*)-**3** and (1*S*,2*R*)-**3**,[9] with protected aspartic acid (*S*)-**5**.

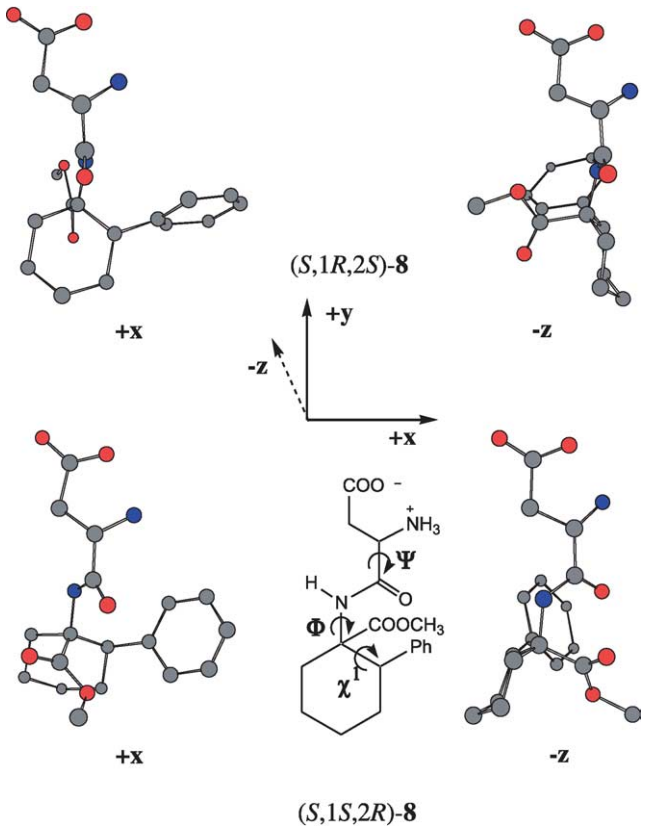


Figure 2. Calculated minimum energy conformations of (*S*,1*R*,2*S*)-**8** and (*S*,1*S*,2*R*)-**8** (hydrogen atoms have been omitted for clarity).

The final steps leading to deprotected dipeptides were carried out with each diastereoisomer as follows. Firstly, treatment with TFA in CH_2Cl_2 afforded (*S*,1*R*,2*S*)-**7** and (*S*,1*S*,2*R*)-**7**. Secondly, hydrogenolysis using palladium on carbon as a catalyst and further purification by RP-HPLC gave the optically pure analogues of aspartame: H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe and H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe, (*S*,1*R*,2*S*)-**8** and (*S*, 1*S*,2*R*)-**8**.

2.2. Peptide taste determination

Taste tests were carried out by a 'sip and spit' qualitative assessment of solutions of the compounds using a three-volunteer taste panel. The analogues were tasted in water at room temperature without any pH adjustment at 0.5% (w/v) concentration. These taste determinations show that compound (*S*,1*R*,2*S*)-**8** is sweet, albeit less potent than aspartame, whereas compound (*S*,1*S*,2*R*)-**8** is bitter.

2.3. Molecular modelling studies

The research carried out on the elucidation of detailed structure-taste relationships of aspartame and its analogues has led to a widely accepted model that has considerable predictive power for related molecules.[3] According to this model, the sweet taste is associated with an L-shaped conformation, in which the hydrophobic group projects along the +x axis (Fig. 2). A reverse L-shaped structure, in which the hydrophobic group points to the −z axis, is associated with a bitter taste. Other possible topochemical arrays would lead to tasteless compounds.

In order to account for solvation effects, we decided to carry out the same mixed scheme as reported previously,[8] combining molecular mechanics and quantum mechanics calculations. Thus, single point energy semi-empirical AM1 calculations on the MM2 optimised structures (see Section 4 for details) were carried out using the COSMO continuum model with the dielectric constant of water. The solvation energy was obtained as the energy difference between the solvated and the isolated structures, and this energy was then added to the MM2 steric energy to obtain the relative energies of the different conformers. Although this procedure does not take into account solvent effects during the geometry optimisation, it has the advantage of combining

Table 2. Calculated energies (kcal mol^{-1}) and some selected dihedral angles for the most stable conformations of (*S*,1*R*,2*S*)-**8** and (*S*,1*S*,2*R*)-**8**

Compound	Conformer	Dihedral angles[a]			E_{MM2}	ΔG_{solv}	E_{Tot}	ΔE_{Tot}
		Ψ	Φ	χ^1				
(*S*,1*R*,2*S*)-**8**	+x	−56.3	175.5	−58.2	−58.9	−77.7	−136.6	0
	−z	−55.7	90.4	−60.3	−54.3	−81.9	−136.2	0.4
(*S*,1*S*,2*R*)-**8**	+x	−89.5	39.2	57.2	−51.2	−80.0	−131.2	3.2
	−z	−166.6	45.7	60.8	−55.4	−79.0	−134.4	0

[a] See Figure 2 for the definition of the dihedral angles.

the strength of the MM2 force field for conformational analysis with that of a successful quantum mechanical method for calculating the solvation energy.

The minimum energy values obtained for conformations +x and −z for compounds (*S*,1*R*,2*S*)-**8** are gathered in Table 2 and the corresponding structures are shown in Figure 2. As can be seen, based on total energies, compound (*S*,1*R*,2*S*)-**8** should be sweet (since conformer +x is more stable than conformer −z), whereas compound (*S*,1*S*,2*R*)-**8** should be bitter (since the reverse relative stability is obtained).

Analysis of the results obtained for the four new analogues of aspartame reveals that the conformational preferences of the c_6Phe residues have been shifted with respect to their (*S*)-Phe and (*R*)-Phe counterparts.[17] Thus, in (1*S*,2*R*)-c_6Phe and (1*S*,2*S*)-c_6Phe, analogues of (*S*)-Phe, a *gauche* (+) side-chain conformation is shown, while this rotamer proves to be the least favoured for the (*S*)-Phe residue. As for (1*R*,2*R*)-c_6Phe and (1*R*,2*S*)-c_6Phe, analogues of (*R*)-Phe, a *gauche* (−) disposition is exhibited, whereas this rotamer is the least favoured for the (*R*)-Phe residue.

It has been reported[3,18] that in the minimum energy conformations calculated for sweet H-(*S*)-Asp-(*S*)-Phe-OMe, the side chain in (*S*)-Phe can assume any of the three possible staggered C_α-C_β rotamers: *gauche* (+), *gauche* (−) and *anti*; but the conformation responsible for sweet taste demands a value of $\chi^1 = -60°$ in the C-terminal residue.

In addition, it should be noted that the incorporation into aspartame of c_6Phe residues with a *gauche* (−) preference, i.e. (1*R*,2*R*)-c_6Phe and (1*R*,2*S*)-c_6Phe, leads to sweet derivatives whereas replacement by c_6Phe residues where a gauche (+) preference is exhibited, i.e. (1*S*,2*S*)-c_6Phe and (1*S*,2*R*)-c_6Phe, leads to bitter analogues. This fact suggests that, for a sweet response, the stereochemistry at C_α in the Phe residue is not as crucial as the value of the dihedral angle χ^1, with the latter being necessary but not sufficient on its own.

It can therefore be concluded that the results of molecular mechanics calculations are in good agreement with the taste experimentally found. Once again, the procedure followed to describe the solvation effects has proved to be both convenient and very useful, as the results obtained in vacuo would have led to incorrect taste predictions for some of the new analogues; e.g. the case of (*S*,1*R*,2*R*)-**8**.[8]

These results support the model reported by Goodman et al.[3] which has been assumed to explain the structure-taste relationship in all four aspartame analogues that incorporate c_6Phe.

3. Conclusion

Two new aspartame derivatives that incorporate the constrained phenylalanines *trans*-c_6Phe have been synthesised. The isomer H-(*S*)-Asp-(1*R*,2*S*)-c_6Phe-OMe is sweet whereas isomer H-(*S*)-Asp-(1*S*,2*R*)-c_6Phe-OMe is bitter. The relationship between the absolute configurations of the dipeptides and the taste response can be explained in terms of the different conformational behaviour displayed by each molecule.

4. Experimental

4.1. General

4.1.1. Instrumentation. Solvents were purified according to standard procedures. Analytical TLC was performed using Merck 60 SI F_{254} precoated silica gel polyester plates using the following solvent systems: 1 (hexane/EtOAc, 5:2); 2 (CH_2Cl_2/MeOH, 8:2). The products were examined by UV fluorescence or developed by iodine or ninhydrin chromatic reaction as appropriate. Column chromatography was performed using silica gel 60 (230–400 mesh). Melting points were determined on a Büchi SMP-20 apparatus and were not corrected. IR spectra were registered on a Mattson Genesis FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity-300 or Bruker ARX-300 instruments, using TMS as the internal standard; chemical shifts are reported in ppm on the δ scale, coupling constants in Hz. Optical rotations were measured on a Perkin–Elmer 241 polarimeter-C in a 1 dm cell of 1 mL capacity. Microanalyses were carried out on a Perkin–Elmer 200 C, H, N, S analyser and are in good agreement with the calculated values.

4.1.2. High performance liquid chromatography. HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 991 photodiode array detector. The chiral stationary phase, which consisted of mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to allylsilica, was prepared according to a previously described procedure.[19,20] The analytical assays were carried out on a 150 mm×4.6 mm ID column and the semi-preparative resolution was achieved on a 150 mm× 20 mm ID column. All analytical assays and semi-preparative chromatography were performed under the conditions given in Table 1. Final products were purified

on a Xterra™ MS C_8 250 mm×4.6 mm ID column. The solvents used as mobile phases were of spectral grade.

The capacity (k'), selectivity (α) and resolution (R_s) values were calculated according to the equations $k_R{}' = (t_R - t_0)/t_0$, $\alpha = k_2{}'/k_1{}'$, $R_s = 1.18(t_2 - t_1)/(w_2 + w_1)$. Subscripts 1 and 2 refer to the first and second eluted diastereoisomers, respectively; t_R ($R = 1, 2$) are their retention times, and w_2 and w_1 denote their bandwidths at half height; t_0 is the dead time.

4.1.3. Molecular modelling methodology. All computer simulations were carried out using the Chem3D program.[21] Molecular Dynamics were obtained by means of the MM2 force field,[22] as implemented in Chem3D. Molecular dynamics trajectories were collected in periods of 1 ns, using a step interval of 1 fs and a target temperature of 473 K. It is well known that the dihedral angles of the Asp residue remain essentially unchanged for all minimum energy conformations of aspartame, and that this disposition is very similar to the X-ray crystal structure.[3] Consequently, the geometry of this residue was kept fixed in the position of the X-ray crystal structure of aspartame throughout all the simulations.

Solvation effects were taken into account by means of single point energy semi-empirical AM1[23] calculations on the MM2 optimised structures, using the COSMO continuum model[24,25] with the dielectric constant of water.

4.2. Synthesis of *rac-trans*-c_6Phe-OMe, *rac-trans*-4

The synthesis of 1-amino-2-phenylcyclohexane-1-carboxylic acid hydrochloride, *rac-trans*-**3**, was performed as described previously.[9] The corresponding methyl ester, *rac-trans*-**4**, was prepared using the following procedure.

$SOCl_2$ (1.8 mL, 24.67 mmol) was added dropwise to a stirred solution of MeOH (20 mL) cooled in an ice-bath. After 15 min stirring at that temperature, *rac-trans*-**3** (316 mg, 1.24 mmol) was added to the reaction mixture and this was heated under reflux for 8 h. A further 3 portions of $SOCl_2$/MeOH, prepared as described above, were added and the solvents were removed in vacuo. The residue was partitioned between 5% $NaHCO_3$/EtOAc. The organic layer was washed with an additional portion of 5% $NaHCO_3$, dried and concentrated in vacuo to afford the racemic α-amino ester (yield 42%).

4.3. General procedure for the synthesis of protected dipeptides (*S*,1*R*,2*S*)-6 and (*S*,1*S*,2*R*)-6

Cbz-(*S*)-Asp(O^{t}Bu)-OH, (*S*)-**5**, (198 mg, 0.61 mmol) and NMM (62 mg, 0.61 mmol) were dissolved in dry CH_2Cl_2 (15 mL) under an inert atmosphere. The mixture was cooled to −15 °C and a precooled solution of ^{t}BuOCOCl (84 mg, 0.61 mmol) in dry CH_2Cl_2 (3 mL) was added. The mixture was stirred for 20 min and a precooled solution of H-*rac-trans*-c_6Phe-OMe, *rac-trans*-**4** (137 g, 0.51 mmol)—obtained as described in Section 4.2 and used without further purification—in CH_2Cl_2 (3 mL) was added dropwise. The reaction mixture was stirred at −15 °C for 1 h and the solution was allowed to warm up to room temperature. The solution was diluted with CH_2Cl_2 and washed with 5% $KHSO_4$, 5% $NaHCO_3$, brine, dried and evaporated to dryness. The product was purified by silica gel column chromatography using hexane/ethyl acetate (5:2) as eluent to give the protected dipeptides in high yield (>99%). The diastereomeric mixture of protected dipeptides was purified by silica gel column chromatography and then resolved by semi-preparative HPLC as described below.

4.3.1. Resolution of two stereoisomers of Cbz-(*S*)-Asp(O^{t}Bu)-*rac-trans*-c_6Phe-OMe: isolation of (*S*,1*R*,2*S*)-6 and (*S*,1*S*,2*R*)-6. HPLC resolution of a mixture of (*S*,1*R*,2*S*)-**6** and (*S*,1*S*,2*R*)-**6** (235 mg) dissolved in chloroform (1.2 mL) was carried out by successive injections of 0.10 mL onto a 150 mm×20 mm column filled with mixed 10-undecenoate-3,5-dimethyl phenylcarbamate of amylose bonded to allylsilica. A mixture of *n*-hexane/2-propanol/chloroform (96/3/1) was used as the eluent (flow rate 15 mL/min). Each run was collected into three separate fractions: 6.5–7.2, 7.2–8.2 and 8.2–9.6 min. In this way 85 mg and 53 mg of the less and more strongly retained diastereoisomers were obtained, respectively. The combined second fractions, containing 95 mg of diastereomeric mixture, were reinjected to afford 23 mg and 32 mg of the less and more strongly retained diastereoisomers, respectively. As a result, 108 mg of optically pure first eluted diastereoisomer and 85 mg of optically pure last eluted diastereoisomer were obtained.

4.3.2. CBz-(*S*)-Asp(O^{t}Bu)-(1*R*,2*S*)-c_6Phe-OMe, (*S*,1*R*,2*S*)-6. Mp: oil. $Rf_1 = 0.40$. $[\alpha]_D^{20} = -5.45$ ($c = 1$, $CHCl_3$). IR: 3336.94; 1727.73; 1711.31; 1693.51; 1678.15 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.2–2.0 (m, 5H); 1.38 (m, 9H); 2.1–2.5 (m, 3H); 2.54 (dd, 1H, $J = 6$ Hz, $J = 16.5$ Hz); 2.9 (m, 1H); 3.56 (m, 1H); 3.62 (s, 3H); 4.49 (m, 1H); 5.09 (m, 2H); 5.84 (d, 1H, $J = 7.8$ Hz); 7.0–7.4 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.34; 170.96 169.38; 155.79; 140.97; 136.30; 128.54; 128.33; 128.23; 128.18; 128.06; 127.20; 81.67; 67.01; 64.26; 51.95; 51.59; 47.07; 37.54; 32.35; 28.39; 27.97; 25.02; 22.54. Anal. Calcd for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 66.99; H, 7.11; N, 5.17.

4.3.3. CBz-(*S*)-Asp(O^{t}Bu)-(1*S*,2*R*)-c_6Phe-OMe, (*S*,1*S*,2*R*)-6. Mp: oil. $Rf_1 = 0.40$. $[\alpha]_D^{20} = +30.34$ ($c = 0.92$, $CHCl_3$). IR: 3336.58; 1729.23; 1678.47 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.2–2.5 (m, 8H); 1.45 (s, 9H); 2.58 (dd, 1H, $J = 6$ Hz, $J = 18.3$ Hz); 2.87 (dd, 1H, $J = 17.1$ Hz, $J = 3$ Hz); 3.57 (m, 1H); 3.63 (s, 3H); 4.45 (m, 1H); 5.07 (s, 2H); 5.93 (d, 1H, $J = 8.4$ Hz); 7.1–7.4 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 172.93; 171.49; 169.85; 156.51; 141.35; 136.68; 129.01; 128.91; 128.88; 128.60; 128.46; 127.59; 82.09; 67.56; 64.88; 52.47; 47.28; 37.33; 32.39; 29.82; 28.77; 28.50; 25.32; 22.97. Anal. Calcd for $C_{30}H_{38}N_2O_7$: C, 66.89; H, 7.11; N, 5.20. Found: C, 66.75; H, 7.15; N, 5.27.

4.4. General procedure for the synthesis of (*S*,1*R*,2*S*)-7 and (*S*,1*S*,2*R*)-7

The corresponding protected dipeptide (1 mmol) was dissolved in CH_2Cl_2 (15 mL) and TFA (7.5 mL) was added. The solution was stirred at room temperature for

1.5 h. The TFA and CH_2Cl_2 were evaporated under reduced pressure to afford the final product.

4.4.1. CBz-(*S*)-Asp-(1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-7. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp(O^{t}Bu)-(1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-**6**, (100 mg, 0.19 mmol); CH_2Cl_2 (3 mL); TFA (1.55 mL). Yield: quantitative. Mp: oil. $Rf_1=0.04$, $Rf_2=0.79$. $[\alpha]_D^{20}=-19.76$ ($c=0.47$, $CHCl_3$). IR (nujol): 2500–3600; 1725.02 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.2–2.0 (m, 5H); 2.0–2.4 (m, 3H); 2.72 (dd, 1H, $J=6.1$ Hz $J=17.3$ Hz); 2.92 (m, 1H); 3.55 (m, 1H); 3.64 (s, 3H); 4.54 (m, 1H); 5.09 (s, 2H); 5.92 (d, 1H, $J=8$ Hz); 6.8–7.4 (m, 11H); 8.50 (s, 1H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 175.58; 172.49; 169.68; 159.76; 156.13; 140.60; 135.78; 128.58; 128.32; 128.15; 127.29; 67.50; 64.61; 52.24; 51.41; 47.26; 35.87; 31.93; 29.37; 24.93; 22.68. Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72; H, 6.27; N, 5.81. Found: C, 64.77; H, 6.20; N, 5.74.

4.4.2. CBz-(*S*)-Asp-(1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-7. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp(O^{t}Bu)-(1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-**6**, (65 mg, 0.12 mmol); CH_2Cl_2 (2 mL); TFA (1 mL). Yield: quantitative. Mp: oil. $Rf_1=0.04$, $Rf_2=0.79$. $[\alpha]_D^{20}=+16.63$ ($c=0.19$, $CHCl_3$). IR (nujol): 2500–3600; 1723.26; 1686.86; 1671.37; 1660.74 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.1–2.0 (m, 5H); 2.05 (m, 1H); 2.15 (m, 1H); 2.30 (m, 1H); 2.63 (dd, 1H, $J=4.2$ Hz, $J=12.9$ Hz); 2.84 (dd, 1H, $J=3$ Hz, $J=12.6$ Hz); 3.47 (m, 1H); 3.56 (s, 3H); 4.46 (m, 1H); 4.98 (m, 2H); 5.93 (d, 1H, $J=6.6$ Hz); 6.87 (s, 1H); 6.9–7.4 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 175.74; 172.47; 169.42; 156.17; 140.72; 135.98; 128.54; 128.30; 128.24; 128.15; 128.02; 127.19; 67.32; 64.48; 52.11; 51.54; 46.95; 35.44; 31.92; 28.30; 24.87; 22.45. Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.72; H, 6.27; N, 5.81. Found: C, 64.79; H, 6.30; N, 5.77.

4.5. General procedure for the synthesis of (*S*,1*R*,2*S*)-8 and (*S*,1*S*,2*R*)-8

The corresponding semi-protected dipeptide (1 mmol) was dissolved in EtOAc/MeOH (1:1) (30 mL) and hydrogenated at room temperature in the presence of 10% palladium/carbon (45 mg) for 12 h. The solution was filtered, evaporated under reduced pressure and further lyophilised to afford a white solid. Both deprotected dipeptides were purified by reversed phase HPLC (column: 5 μm Xterra™ MS C_8, 150×4.6 mm ID). The elutions were performed isocratically with 20% CH_3CN/80% H_2O (v/v) at a flow rate of 2 mL(min, with UV detection at 220 nm.

4.5.1. H-(*S*)-Asp-(1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-8. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp-(*S*,1*R*,2*S*)-c$_6$Phe-OMe, (*S*,1*R*,2*S*)-**7**, (78 mg, 0.16 mmol); EtOAc/MeOH (6 mL); 10% Pd/C (15 mg). Yield: 97%. Mp: 144 °C. $Rf_2=0.51$. $[\alpha]_D^{20}=-14.76$ ($c=0.49$, MeOH). IR (nujol): 2400–3600; 1720.20; 1690.31 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.5–1.7 (m, 2H); 1.8–2.2 (m, 5H); 2.37 (m, 1H); 2.90 (m, 2H); 3.19 (dd, 1H, $J=3$ Hz, $J=6$ Hz); 3.58 (s, 3H); 4.31 (t, 1H, $J=6$ Hz); 7.3–7.5 (m, 5H). ^{13}C NMR (D_2O/MeOH-d_4 75 MHz) δ 174.13; 173.74; 167.47; 140.69; 128.91; 128.26; 127.35; 63.55; 52.13; 49.78; 36.30; 30.59; 27.63; 22.95; 21.25. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.12; H, 6.95; N, 8.07.

4.5.2. H-(*S*)-Asp-(1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-8. This compound was prepared according to the procedure described above. Cbz-(*S*)-Asp-(*S*,1*S*,2*R*)-c$_6$Phe-OMe, (*S*,1*S*,2*R*)-**7**, (47 mg, 0.1 mmol); EtOAc/MeOH (4 mL); 10% Pd/C (5 mg). Yield: 84%. Mp: 165 °C. $Rf_2=0.51$. $[\alpha]_D^{20}=+36.94$ ($c=0.71$, MeOH). IR (nujol): 2400–3600; 1730.81; 1684.52 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.4–1.6 (m, 1H); 1.6–1.8 (m, 2H); 1.8–2.0 (m, 3H); 2.15 (m, 1H); 2.45 (m, 1H); 2.53 (dd, 1H, $J=6$ Hz, $J=13.2$ Hz); 2.71 (dd, 1H, $J=4.2$ Hz, $J=12.9$ Hz); 3.03 (dd, 1H, $J=3$ Hz, $J=6.9$ Hz); 3.53 (s, 3H); 4.22 (t, 1H, $J=4.2$ Hz); 7.2–7.4 (m, 5H). ^{13}C NMR (D_2O/MeOH-d_4 75 MHz) δ 175.79; 173.61; 167.86; 140.98; 129.13; 128.18; 127.25; 63.28; 52.06; 50.44; 49.52; 37.14; 32.35; 27.78; 23.38; 21.35. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 62.25; H, 6.91; N, 8.09.

Acknowledgements

This work was supported by FEDER, Ministerio de Ciencia y Tecnología (PPQ2001-1834) and Productos Aditivos.

References and notes

1. Mazur, R. H.; Schlatter, J. M.; Goldkamp, A. H. *J. Am. Chem. Soc.* **1969**, *91*, 2684–2691.
2. Kamphuis, J.; Lelj, F.; Tancredi, T.; Toniolo, C.; Temussi, P. A. *Quant. Struct. -Act. Relat.* **1992**, *11*, 486–491.
3. Yamazaki, T.; Benedetti, E.; Kent, D.; Goodman, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1437–1451 and references cited therein.
4. Tsang, J. W.; Schmied, B.; Nyfeler, R.; Goodman, M. *J. Med. Chem.* **1984**, *27*, 1663–1668.
5. Mapelli, C.; Newton, M. G.; Ringold, C. E.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1987**, *30*, 498–510.
6. Mapelli, C.; Stammer, C. H.; Lok, S.; Mierke, D. F.; Goodman, M. *Int. J. Prot. Res.* **1988**, *32*, 484–495.
7. Gómez-Catalán, J.; Jiménez, A. I.; Cativiela, C.; Pérez, J. J. *J. Pept. Res.* **2001**, *57*, 435–446.
8. Avenoza, A.; París, M.; Peregrina, M. A.; Alías, M.; López, M. P.; García, J. I.; Cativiela, C. *Tetrahedron* **2002**, *58*, 4899–4905.
9. Cativiela, C.; López, P.; Lasa, M. *Eur. J. Org. Chem.* **2004**, 3898–3908.
10. Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*; Springer: Berlin, 1994.
11. Paglialunga, M.; Torrini, I.; Pagani, G.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G. *Tetrahedron* **1995**, *51*, 2379–2386.
12. Cativiela, C.; Díaz-de-Villegas, M. D.; Jiménez, A. I.; López, P.; Marraud, M.; Oliveros, L. *Chirality* **1999**, *11*, 583–590.
13. Alías, M.; Cativiela, C.; Jiménez, A. I.; López, P.; Oliveros, L.; Marraud, M. *Chirality* **2001**, *13*, 48–55.
14. Jiménez, A. I.; López, P.; Oliveros, L.; Cativiela, C. *Tetrahedron* **2001**, *57*, 6019–6026.
15. Royo, S.; López, P.; Jiménez, A. I.; Oliveros, L.; Cativiela, C. *Chirality* **2002**, *14*, 39–46.

16. Alías, M.; López, M. P.; Cativiela, C. *Tetrahedron* **2004**, *60*, 885–891.
17. Jiménez, A. I.; Cativiela, C.; Gómez-Catalán, J.; Pérez, J. J.; Aubry, A.; París, M.; Marraud, M. *J. Am. Chem. Soc.* **2000**, *122*, 5811–5821.
18. Mattern, R. H.; Amino, Y.; Benedetti, E.; Goodman, M. *J. Pept. Res.* **1997**, *50*, 286–299.
19. Oliveros, L.; López, P.; Minguillón, C.; Franco, P. *J. Liq. Chromatogr.* **1995**, *18*, 1521–1532.
20. Franco, P.; Senso, A.; Minguillón, C.; Oliveros, L. *J. Chromatogr. A* **1998**, *796*, 265–272.
21. CS Chem3D 6.0. CambridgeSoft Corporation, 2001.
22. Burkert, U.; Allinger, N. L. Molecular Mechanics In *ACS Monograph 177*; American Chemical Society: Washington, DC, 1982.
23. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
24. Klamt, A. *J. Phys. Chem.* **1995**, *99*, 2224–2235.
25. Nielsen, P. A.; Jaroszewski, J. W. J.; Norrby, P.-O.; Liljefors, T. *J. Am. Chem. Soc.* **2001**, *123*, 2003–2006.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2921–2929

Bellisosides A–F, six novel acylated triterpenoid saponins from *Bellis perennis* (compositae)

Wei Li,[a] Yoshihisa Asada,[b,*] Kazuo Koike,[a] Tamotsu Nikaido,[a] Tsutomu Furuya[b] and Takafumi Yoshikawa[b]

[a]*Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan*
[b]*School of Pharmaceutical Sciences, Kitasato University, Shirokane 5-9-1, Minato-ku, Tokyo 108-8641, Japan*

Received 22 December 2004; revised 11 January 2005; accepted 11 January 2005

Abstract—Six new acylated triterpenoid saponins, bellisosides A–F (**1**–**6**), were isolated from the roots of *Bellis perennis* (Compositae) together with a known saponin, bellissaponin BS2 (**7**). These saponins which bear polygalacic acid as sapogenin, are bisdesmosidic glycosides. The structures were elucidated on the basis of chemical and physicochemical evidence. The cytotoxic activities of **1**–**7** against HL-60 human promyelocytic leukemia cells are also reported.

1. Introduction

Bellis perennis L. (Compositae), the common daisy, is widely distributed in Europe and North Africa. It has been used in traditional medicine for the treatment of rheumatism, and as an expectorant.[1] The existence of triterpenoid saponins in *B. perennis* has been known since 1931.[2] To our best knowledge, to date, 12 triterpenoid saponins were reported.[3] But in all these reports, partial deacylation by alkaline hydrolysis was carried out during the isolation. The potential medicinal importance and our interest in the chemistry of saponins prompted us to investigate the genuine acylated saponins of this plant. This paper deals with the isolation and structure elucidation of six novel acylated saponins, bellisosides A–F (**1**–**6**), from the root of *B. perennis*. The cytotoxic activities of these saponins, along with a known saponin, bellissaponin BS2 (**7**), against HL-60 human promyelocytic leukemia cells are also reported.

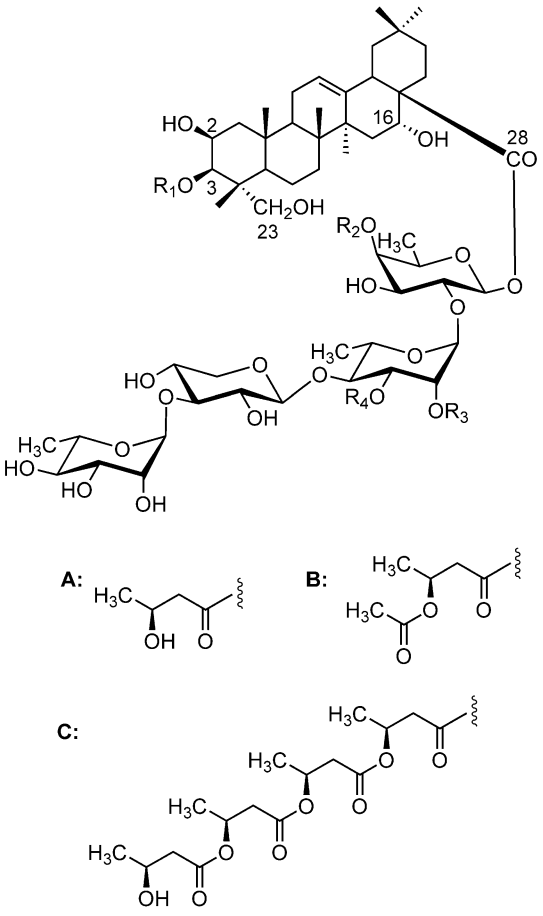
2. Results and discussion

A methanolic extract of the roots of *B. perennis* was suspended in H_2O and then partitioned successively with EtOAc. The H_2O layer was then passed through a Diaion HP-20 column, followed by washing of MeOH. The MeOH eluate was chromatographed on an ODS column with 50% MeOH to remove flavonoids, and then with MeOH to result in a crude saponin fraction. Further purification of the fraction by combination of reversed- and normal-phase HPLC successively gave six novel acylated triterpenoid saponins, termed bellisosides A–F (**1**–**6**), and a known saponin, bellissaponin BS2 (**7**) (Fig. 1).[2a]

Bellisoside A (**1**) was obtained as an amorphous powder. It revealed an $[M+Na]^+$ ion peak at *m/z* 1343 in positive FABMS. The molecular formula of $C_{63}H_{100}O_{29}$ was confirmed by HRFABMS. The compound displayed 63 carbon signals in its ^{13}C NMR spectrum, of which 30 could be assigned to the signals of aglycon. The six sp^3 carbons at δ 15.3, 17.7, 17.8, 24.7, 27.3 and 33.3, and the two sp^2 carbons at 122.9 (d) and 144.4 (s), coupled with information from the ^1H NMR (six methyl proton singlets at δ 0.93, 1.04, 1.18, 1.39, 1.58 and 1.78, and a broad triplet-like vinyl proton signal at δ 5.63), indicated that the aglycon had an olean-12-ene skeleton. Also, in the ^1H NMR spectrum, signals assignable to carbinylic protons of the aglycon were observed at δ 4.81 (ddd, $J=3.5$, 3.5, 3.5 Hz), 4.35 (d, $J=$ 3.5 Hz) and 5.15 (br.s), suggesting the carbinylic protons could be placed at 2α, 3α and 16β, respectively. Furthermore, the hydroxymethyl (δ_H 4.35 and 3.74, and δ_C 66.2) and ester carbonyl (δ_C 176.3) groups were assigned to C-23 and C-28 after an extensive 2D NMR study. Thus, the aglycon was identified as 2β,3β,16α,23-tetrahydroxy-olean-12-en-28-oic acid (polygalacic acid).[4] The aglycon was isolated after acid hydrolysis of **1** and confirmed by comparison of the spectral and physical data with those of

Keywords: Saponin; *Bellis perennis*; Compositae; Bellisoside.
* Corresponding author. Tel.: +81 3 57916239; fax: +81 3 34446192; e-mail: asaday@pharm.kitasato-u.ac.jp

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.026



	1	2	3	4	5	6	7
R_1	Glc	Glc	Rha	Rha	Rha	Rha	Glc
R_2	Ac	Ac	A	B	C	C	H
R_3	Ac	H	H	H	H	Ac	H
R_4	H	Ac	H	H	H	H	H

Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl; Ac, acetyl.

Figure 1. Structures of triterpenoid saponins **1**–**7**.

an authentic sample. On acid hydrolysis, **1** also afforded D-glucose, D-xylose, D-fucose and L-rhamnose in the ratio of 1:1:1:2 as component sugars, which were identified by HPLC analysis following their conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives.[5] The ^{1}H NMR spectrum showed five anomeric proton signals at δ 5.17 (d, J=7.5 Hz, Glc-H-1), 5.17 (d, J=7.5 Hz, Xyl-H-1), 6.03 (d, J=8.0 Hz, Fuc-H-1), 6.08 (d, J=1.0 Hz, iRha-H-1), and 6.15 (d, J=1.0 Hz, tRha-H-1). All protons signals due to sugars were then assigned by careful analysis of the ^{1}H-^{1}H COSY and 1D HOHAHA spectra. When the ^{13}C NMR data of **2** was compared with that of authentic polygalacic acid, glycosylation shifts were observed at C-2 (−1.2 ppm), C-3 (+9.9 ppm) and C-28 (−3.8 ppm), which suggested **2** was a 3,28-bisdesmoside. The glucose was connected to C-3 of the aglycon, which was deduced from the HMBC correlations between δ_H 5.17 (Glc-H-1) and δ_C 83.5 (C-3). Since the anomeric carbon signal (δ 94.5) due to fucose showed an ester-type glycoside linkage, fucose was found to be linked at C-28 of polygalacic acid. This connection and the sequence of the inner sugar chain at C-28 were further determined by analysis of the HMBC spectrum. Namely, the HMBC correlations were observed between δ_H 6.03 (Fuc-H-1) and δ_C 176.3 (C-28), δ_H 6.08 (iRha-H-1) and δ_C 75.3 (Fuc-C-2), δ_H 5.17 (Xyl-H-1) and δ_C 83.8 (iRha-C-4), δ_H 6.15 (tRha-H-1) and δ_C 83.8 (Xyl-C-3). The β anomeric configurations for the glucopyranose, fucopyranose and xylopyranose units were determined from their large $^3J_{H1,H2}$ coupling constants (7.5–8.0 Hz). For the inner and terminal L-rhamnopyranose moieties, the small $^3J_{H1,H2}$ coupling constants (1.0 Hz) and the three bond strong HMBC correlations from the anomeric proton to C-3 and C-5 of the rhamnose, indicated that the anomeric protons were equatorial, thus possessing α configuration in the ^{1}C$_4$ form.[6] Besides the signals due to the aglycon and component sugars, the ^{1}H NMR spectrum also showed two singlet methyl signals (δ 1.98 and 2.00), which could be assigned to those of two acetyl moieties. Comparing the ^{1}H NMR spectrum of **1** with that of **7**, H-4 [δ 5.52 (d, J=3.5 Hz)] of fucose and H-2 [δ 6.01 (dd, J=3.0, 1.0 Hz)] of the inner rhamnose were observed to be shifted downfield by 1.59 and 1.21 ppm, indicating that the binding site of the acetyl moieties were at C-4 of fucose and C-2 of inner rhamnose. This conclusion was supported by observation of correlations between δ_H 5.52 (Fuc-H-4) and δ_C 171.2 (Fuc-COCH$_3$), and δ_H 6.01 (iRha-H-2) and δ_C 170.6 (iRha-COCH$_3$) in the HMBC spectrum. Based on the above results, the structure of bellisoside A (**1**) was established as 3-*O*-β-D-glucopyranosyl polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-2-*O*-acetyl-α-L-rhamnopyranosyl(1→2)-4-*O*-acetyl-β-D-fucopyranoside (Fig. 2, Tables 1–4).

Bellisoside B (**2**) was obtained as an amorphous powder. The HRFABMS spectrum of **2** suggested the molecular formula of $C_{63}H_{100}O_{29}$, which is the same as that of **1**. On acid hydrolysis, **2** afforded polygalacic acid as the aglycon, and D-glucose, D-xylose, D-fucose and L-rhamnose in the ratio of 1:1:1:2 as component sugars. The β-D-glucopyranose and α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranose were connected to C-3 and C-28 of polygalacic acid, respectively, which were established by a combination of

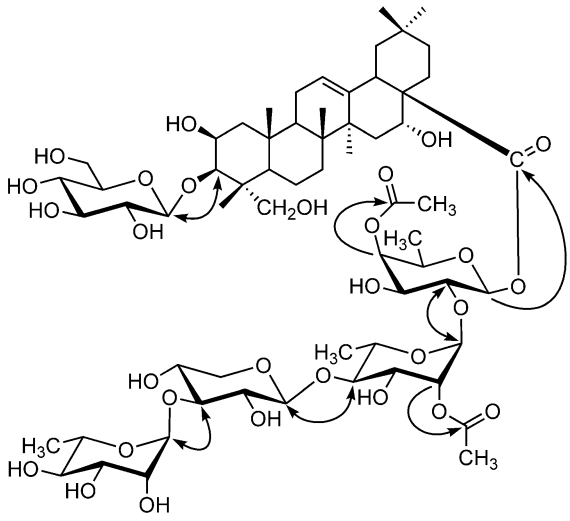


Figure 2. Key HMBC correlations for bellisoside A (**1**).

Table 1. ^{13}C NMR data for aglycones and ester moieties of **1–6**

	1	**2**	**3**	**4**	**5**	**6**
Sapogenin						
1	44.4	44.4	45.0	45.0	45.0	45.0
2	70.6	70.7	71.2	71.2	71.2	71.2
3	83.5	83.4	81.6	81.6	81.6	81.6
4	43.0	43.0	43.2	43.2	43.2	43.2
5	48.1	48.1	47.8	47.8	47.8	47.8
6	18.5	18.5	18.6	18.5	18.6	18.5
7	33.5	33.5	33.4	33.4	33.4	33.4
8	40.4	40.4	40.4	40.4	40.4	40.4
9	47.7	47.7	47.7	47.7	47.7	47.7
10	37.2	37.2	37.4	37.2	37.4	37.4
11	24.2	24.2	24.2	24.2	24.2	24.3
12	122.9	123.1	122.9	122.9	122.9	122.9
13	144.4	144.3	144.5	144.5	144.5	144.4
14	42.5	42.4	42.5	42.5	42.5	42.5
15	36.3	36.3	36.5	36.4	36.4	36.3
16	74.3	74.3	74.2	74.0	74.0	74.3
17	49.6	49.6	49.6	49.6	49.6	49.7
18	41.8	41.8	41.8	41.8	41.8	41.8
19	47.5	47.5	47.5	47.5	47.5	47.5
20	30.9	30.9	30.9	30.9	30.9	30.9
21	36.1	36.1	36.1	36.1	36.1	36.1
22	32.2	32.0	32.2	32.2	32.2	32.2
23	66.2	66.0	65.5	65.5	65.5	65.4
24	15.3	15.3	15.1	15.1	15.1	15.1
25	17.8	17.8	17.9	17.9	17.9	17.9
26	17.7	17.7	17.8	17.8	17.8	17.9
27	27.3	27.3	27.3	27.2	27.2	27.2
28	176.3	176.4	176.3	176.4	176.4	176.3
29	33.3	33.3	33.3	33.3	33.3	33.3
30	24.7	24.8	24.7	24.7	24.7	24.7
Ester moiety						
Fuc-$\underline{CO}CH_3$	171.2	171.2				
Fuc-$CO\underline{CH_3}$	20.9	20.9				
Rha-$\underline{CO}CH_3$	170.6	171.1				170.6
Rha-$CO\underline{CH_3}$	21.1	21.5				21.1
			A	B	C	C
1			172.5	170.9	170.8	170.8
2			45.1	40.8	40.7	40.7
3			65.0	67.6	68.1	68.1
4			24.1	19.9	19.9	20.0
1′				170.2	170.1	170.1
2′				21.3	41.2	41.1
3′					68.0	68.0
4′					20.0	20.0
1″					169.8	169.8
2″					41.2	41.1
3″					67.8	67.8
4″					20.1	20.0
1‴					171.6	171.6
2‴					45.3	45.3
3‴					64.1	64.6
4‴					24.1	24.1

Fuc, β-D-fucopyranosyl; Rha, α-L-rhamnopyranosyl.

the DEPT, ^1H–^1H COSY, 1D HOHAHA, HMQC, HMBC and phase-sensitive NOESY experiments, similarly as carried out on **1**. Besides the signals due to the aglycon and component sugars, the ^1H- and ^{13}C NMR spectra also showed the presence of two sets of acetyl moieties. Comparing the ^1H NMR spectrum of **2** with that of **7**, H-4 [δ 5.48 (d, J=3.5 Hz)] of fucose and H-3 [δ 5.89 (dd, J= 9.0, 3.0 Hz)] of the inner rhamnose in **2** were observed to be shifted downfield by 1.55 and 1.21 ppm, respectively, which indicated that the acetyl moieties in **2** were located at C-4 of fucose and C-3 of the inner rhamnose. This conclusion was supported by the HMBC correlations between δ_H 5.48 (Fuc-H-4) and δ_C 171.2 (Fuc-$\underline{CO}CH_3$), and δ_H 5.89 (iRha-H-3) and δ_C 171.1 (iRha-$\underline{CO}CH_3$) in the HMBC spectrum. Based on the above results, the structure of bellisoside B (**2**) was established as 3-*O*-β-D-glucopyranosyl polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-3-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-β-D-fucopyranoside.

Bellisoside C (**3**) was obtained as an amorphous powder. It revealed an $[M+Na]^+$ ion peak at *m/z* 1329 in the positive FABMS. The molecular formula of $C_{63}H_{102}O_{28}$ was confirmed by the HRFABMS spectrum. On acid hydrolysis, **3** afforded polygalacic acid as the aglycon, and D-xylose, D-fucose and L-rhamnose in the ratio of 1:1:3 as component

Table 2. ^{13}C NMR data for sugar moieties of **1–6**

	1	2	3	4	5	6
C-3 sugar	(Glc)	(Glc)	(Rha)	(Rha)	(Rha)	(Rha)
1	105.8	105.8	104.4	104.3	104.3	104.4
2	75.6	75.7	72.7	72.7	72.7	72.7
3	78.7	78.7	72.8	72.9	72.8	73.0
4	71.7	71.7	74.2	74.1	74.2	74.2
5	78.4	78.4	70.5	70.5	70.5	70.5
6	62.8	62.8	18.8	18.8	18.8	18.8
C-28 sugars						
Fuc-1	94.5	94.4	94.6	94.6	94.6	94.5
Fuc-2	75.3	77.1	74.7	74.7	74.7	75.0
Fuc-3	74.2	74.2	74.2	74.2	74.2	74.2
Fuc-4	74.6	75.0	75.2	75.0	75.1	74.9
Fuc-5	70.6	70.4	70.7	70.5	70.5	70.5
Fuc-6	16.6	16.6	16.7	16.6	16.6	16.6
Inner						
Rha-1	99.0	102.9	102.1	102.0	102.0	98.8
Rha-2	73.4	70.0	71.9	71.9	72.0	73.4
Rha-3	70.4	75.7	72.7	72.8	72.7	70.2
Rha-4	83.8	78.1	84.9	84.9	84.9	83.9
Rha-5	68.7	69.1	68.8	68.8	68.8	68.7
Rha-6	18.7	19.4	18.9	18.8	18.8	18.7
Xyl-1	106.8	106.0	107.3	107.3	107.3	106.9
Xyl-2	76.3	75.1	76.6	76.6	76.6	76.4
Xyl-3	83.8	83.8	83.6	83.6	83.6	83.5
Xyl-4	69.5	69.7	69.4	69.4	69.4	69.4
Xyl-5	67.4	67.3	67.5	67.5	67.5	67.5
Terminal						
Rha-1	102.8	103.0	102.7	102.7	102.7	102.8
Rha-2	72.6	72.7	72.7	72.7	72.7	72.6
Rha-3	72.7	72.8	73.0	72.9	73.0	72.8
Rha-4	74.2	74.3	74.2	74.1	74.2	74.2
Rha-5	70.2	70.1	70.1	70.1	70.1	70.1
Rha-6	18.8	18.8	18.8	18.8	18.8	18.8

Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl; Xyl, β-D-xylpyranosyl; Fuc, β-D-fucopyranosyl.

sugars. Careful comparison of the NMR data of **3** and **2** suggested it is also a bisdesmosidic glycoside, and possesses the same sugar chain as that of **2** at C-28. The α-L-rhamnopyranose at C-3 was deduced from the HMBC correlations between δ_H 5.78 (3-Rham-H-1) and δ_C 81.6 (C-3), and δ_H 4.41 (H-3) and δ_C 104.4 (3-Rham-C-1). Besides the signals due to the aglycon and component sugars, the ^1H- and ^{13}C-NMR spectra also showed the signals due to a secondary methyl [δ_H 1.37 (d, J=6.0 Hz), δ_C 24.1], a methylene [δ_H 2.63 (dd, J=14.0, 5.0 Hz), 2.73 (d, J=14.0, 8.0 Hz), δ_C 45.1], an oxymethylene [δ_H 4.57 (ddd, J=8.0, 6.0, 5.0 Hz), δ_C 65.0], and an ester carbonyl (δ_C 172.5), suggesting the presence of a 3-hydroxybutyric acid (HBA) moiety. The linkage of the HBA moiety to C-4 of fucose was determined by observation of the HMBC correlations between δ_H 5.57 (Fuc-H-4) and δ_C 172.5 (HBA-C-1). The absolute configuration of the asymmetric center of HBA was determined to be 3-*S* by the following chemical correlation. Alkaline methanolysis of **3** with sodium methoxide in dry methanol gave HBA methyl ester (**8**). **8** was treated with (*S*)-α-methoxy-α-trifuoromethyl-phenyl acetate chloride (MTPA-Cl) to give its 3-*O*-MTPA ester (**9**), which allows HPLC analysis with **9** and its diastereoisomer (**10**) prepared from authentic 3-*S*- and 3-*R*-HBA methyl ester, respectively (Scheme 1). Comparing the methyl, methylene and carbomethoxy protons in **9** and **10**, the methyl proton in **9** was observed at 0.1 ppm downfield shift, but the methylene and carbomethoxy protons were observed at 0.04 and 0.09 ppm upfield shift. The difference in the chemical shift in **9** and **10** is explained by the anisotropic effect of the phenyl group in the MTPA ester (Fig. 3). Based on the above results, the structure of bellisoside C (**3**) was determined as 3-*O*-α-L- rhamnopyranosyl polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-4-*O*-3-(*S*)-3-hydroxy-1-oxobutyl-β-D-fucopyranoside.

Bellisoside D (**4**) was obtained as an amorphous powder. In the positive FABMS, it revealed an $[M+Na]^+$ ion peak at *m/z* 1371 which is larger by 42 mass units than that of **3**. The molecular formula of $C_{65}H_{104}O_{29}$ was confirmed by HRFABMS. On acid hydrolysis, **4** afforded polygalacic acid as the aglycon, and D-xylose, D-fucose and L-rhamnose in the ratio of 1:1:3 as component sugars. In comparing the ^1H and ^{13}C NMR spectra of **4** and **3**, all of the aglycon and sugar signals of **4** were almost superimposable on those of **3**, while some differences were observed for the signals due to the acyl moiety, indicating that **4** has the same aglycon and sugar linkage and the same linkage position of the acylated moiety as **3**. Further analysis on the signals due to the acyl moiety, suggested the presence of an HBA moiety [δ_H 1.26 (3H, d, J=6.0 Hz), 2.64 (1H, dd, J=15.5, 5.5 Hz), 2.73 (d, J=15.5, 7.5 Hz) and 5.49 (1H, ddd, J=7.5, 6.0, 5.5 Hz); δ_C 19.9, 40.8, 67.6 and 170.9] and an acetic acid moiety [δ_H 2.01 (3H, s); δ_C 21.3 and 170.2]. The linkage of the HBA moiety to C-4 of fucose and the acetyl moiety to C-3 of the HBA moiety was determined by observation of the HMBC correlations between δ_H 5.52 (Fuc-H-4) and δ_C 170.9 (HBA-C-1), and δ_H 5.49 (HBA-H-3) and δ_C 170.2 ($\underline{CO}CH_3$). Thus, the structure of bellisoside D (**4**) was determined as

Table 3. ^{1}H NMR data for aglycones and ester moieties of **1–6**

	1	**2**	**3**	**4**	**5**	**6**
Sapogenin						
2	4.81 ddd (3.5, 3.5, 3.5)	4.83 ddd (3.5, 3.5, 3.5)	4.75 ddd (3.5, 3.5, 3.5)	4.75 ddd (3.5, 3.5, 3.0)	4.75 ddd (3.5, 3.5, 3.5)	4.76 m
3	4.35 d (3.5)	4.37 d (3.5)	4.41 d (3.5)	4.41 d (3.5)	4.40 d (3.5)	4.40 d (3.5)
12	5.63 dd (3.0, 3.0)	5.63 dd (3.0, 3.0)	5.66 dd (3.0, 3.0)	5.68 dd (3.5, 3.5)	5.68 dd (3.0, 3.0)	5.66 dd (3.0, 3.0)
16	5.15 br. s	5.18 br. s	5.20 br. S	5.23 br. S	5.22 br. S	5.16 br. S
18	3.40 dd (13.5, 3.5)	3.41 dd (13.5, 3.5)	3.42 dd (13.5, 4.0)	3.43 dd (13.0, 4.0)	3.43 dd (13.5, 3.5)	3.41 dd (13.5, 4.0)
19	2.74 dd (13.5, 13.5)	2.73 dd (13.5, 13.5)	2.75 dd (13.5, 13.5)	2.76 dd (13.0, 13.0)	2.76 dd (13.5, 13.5)	2.75 dd (13.5, 13.5)
23	4.35 d (11.0) 3.74 d (11.0)	4.34 d (10.0) 3.76 d (10.0)	3.72 s	3.72 br.s	3.72 s	3.71 br.s
24 (CH_3)	1.39 s	1.42 s	1.23 s	1.23 s	1.23 s	1.24 s
25 (CH_3)	1.58 s	1.54 s	1.58 s	1.58 s	1.58 s	1.59 s
26 CH_3)	1.18 s	1.16 s	1.20 s	1.21 s	1.20 s	1.19 s
27 (CH_3)	1.78 s	1.77 s	1.76 s	1.77 s	1.76 s	1.76 s
29 (CH_3)	0.93 s	0.92 s	0.93 s	0.93 s	0.93 s	0.93 s
30 (CH_3)	1.04 s	1.05 s	1.02 s	1.04 s	1.04 s	1.04 s
Ester moiety						
Fuc-CO$\underline{CH_3}$	2.00 s	2.00 s				
Rha-CO$\underline{CH_3}$	1.98 s	2.04 s				2.00 s
			A	B	C	C
2			2.63 dd (14.0, 5.0) 2.73 dd (14.0, 8.0)	2.64 dd (15.5, 5.5) 2.73 dd (15.5, 7.5)	2.61∼2.74* 2.75∼2.84*	2.63 dd (15.5, 5.5)[a] 2.77 dd (15.5, 7.5)[b]
3			4.57 ddd (8.0, 6.0, 5.0)	5.49 ddd (7.5, 6.0, 5.5)	5.48∼5.60*	5.47∼5.62*
4 (CH_3)			1.37 d (6.0)	1.26 d (6.0)	1.30 d (6.0)	1.33 d (6.0)
2′ (CH_3)				2.01 s	2.61∼2.74* 2.75∼2.84*	2.63 dd (15.0, 5.5)[b] 2.79 dd (15.0, 7.5)[a]
3′					5.48∼5.60*	5.47∼5.62*
4′ (CH_3)					1.32 d (6.0)	1.33d (6.0)
2″					2.61∼2.74* 2.75∼2.84*	2.64 dd (15.5, 5.5)[a] 2.82 dd (15.5, 7.5)[b]
3″					5.48∼5.60*	5.47∼5.62*
4″ (CH_3)					1.34 d (6.0)	1.33 d (6.0)
2‴					2.61∼2.74* 2.75∼2.84*	2.64 dd (15.5, 5.5)[a] 2.82 dd (15.5, 7.5)[b]
3‴					4.57 m	4.56 m
4‴ (CH_3)					1.40 d (6.0)	1.40 d (6.0)

Fuc, β-D-fucopyranosyl; Rha, α-L-rhamnopyranosyl.
*Overlapped signals.
[a,b]Signals maybe exchangable.

3-*O*-α-L-rhamnopyranosyl polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-4-*O*-3-(*S*)-3-acetoxy-1-oxobutyl-β-D-fucopyranoside.

Bellisoside E (**5**) was obtained as an amorphous powder. It revealed an $[M+Na]^+$ ion peak at *m/z* 1587 in the positive FABMS. The molecular formula of $C_{63}H_{102}O_{28}$ was confirmed by HRFABMS. The superimposable signals due to the aglycon and component sugars in ^{1}H and ^{13}C NMR spectra between **5** and **4**, together with the results of acid hydrolysis of **5**, suggested **5** is only different by the acyl moiety from **4**. Although, except the four secondary methyl signals observed at δ_H 1.30, 1.32, 1.34 and 1.40, the proton signals due to the acyl moiety were very complicated overlapping in the ^{1}H NMR spectrum, the ^{13}C NMR spectrum suggested the acyl moiety in **6** was constructed by four sets of the HBA moiety, which was also supported by the FABMS data. Assignment of the carbon of the acyl moiety was deduced from a combinational analysis of 1D HOHAHA, ^{1}H–^{1}H COSY, HMQC and HMBC spectra. The absolute configuration of the asymmetric center of HBA in **5** was determined to be 3-*S* by the same method as that of **3**. Thus, the structure of bellisoside E (**5**) was determined as 3-*O*-α-L-rhamnopyranosyl polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)4-*O*-3-(*S*)-3-{3-(*S*)-3-[3-(*S*)-3-(3-(*S*)-3-hydroxy-1-oxobutoxy)-1-oxobutoxy]-1-oxobuty}-1-oxobutyl-β-D-fucopyranoside.

Bellisoside F (**6**) was obtained as an amorphous powder. It revealed an $[M+Na]^+$ ion peak at *m/z* 1629 in the positive FABMS. The molecular formula of $C_{77}H_{122}O_{35}$ was confirmed by HRFABMS. Comparing the ^{1}H, ^{13}C NMR and FAB-MS data of **6** with those of **5**, **6** had one more acetyl group than **5**. The position of the acetyl group in **6** was suggested to be located at C-2 of the inner rhamnose from a clear downfield shift of H-2 of the inner rhamnose at δ_H 5.98 by 1.20 ppm, comparing with the ^{1}H NMR data of **5** and **6**. Furthermore, in comparing the ^{13}C NMR spectra of **5** and **6**, acylation shifts were observed for the signals due to C-1 (−3.2 ppm), C-2 (+1.4 ppm) and C-3 (−2.5 ppm) of

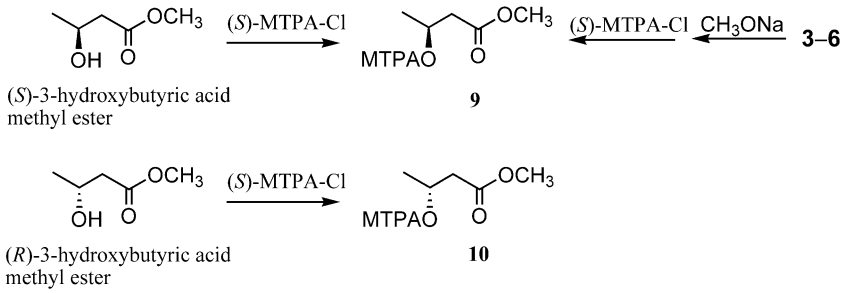
Table 4. ^{1}H NMR data for sugars of **1–6**

	1	2	3	4	5	6
C-3 sugar	(Glc)	(Glc)	(Rha)	(Rha)	(Rha)	(Rham)
1	5.17 d (7.5)	5.17 d (7.5)	5.78 d (1.0)	5.79 d (1.0)	5.78 d (1.0)	5.78 d (1.0)
2	4.03 dd (8.5, 7.5)	4.13 dd (8.5, 7.5)	4.72 dd (3.0, 1.0)	4.73 dd (3.5, 1.0)	4.72 dd (3.5, 1.0)	4.73 dd (3.5, 1.0)
3	4.19 dd (8.5, 8.0)	4.18 dd (8.5, 8.0)	4.60 dd (9.0, 3.0)	4.60 dd (9.0, 3.5)	4.60 dd (9.0, 3.5)	4.59 dd (9.0, 3.5)
4	4.24 dd (9.0, 8.0)	4.24 dd (8.5, 8.0)	4.30 dd (9.0, 9.0)	4.31 dd (9.0, 9.0)	4.30 dd (9.0, 9.0)	4.30 dd (9.0, 9.0)
5	3.92 ddd (9.0, 5.0, 2.0)	3.91 dd (8.5, 4.0)	4.94 dq (9.0, 6.0)	4.61 dq (9.0, 6.0)	4.61 dq (9.0, 6.0)	4.94 dq (9.0, 6.0)
6	4.33 dd (11.5, 5.0) 4.48 dd (11.5, 2.0)	4.36 dd (11.5, 4.0) 4.49 br.d (11.5)	1.62 d (6.0)	1.62 d (6.0)	1.62 d (6.0)	1.62 d (6.0)
C-28 sugars						
Fuc-1	6.03 d (8.0)	6.08 d (8.0)	6.06 d (8.0)	6.06 d (8.0)	6.06 d (8.0)	6.03 d (8.0)
Fuc-2	4.50 dd (9.0, 8.0)	4.47 dd (8.0, 8.0)	4.54 dd (9.0, 8.0)	4.53 dd (9.0, 8.0)	4.53 dd (9.0, 8.0)	4.49 dd (9.0, 8.0)
Fuc-3	4.28 dd (9.0, 3.5)	4.32 dd (8.0, 3.5)	4.32 dd (9.0, 3.5)	4.33 dd (9.0, 3.5)	4.33 dd (9.0, 3.5)	4.31 dd (9.0, 3.5)
Fuc-4	5.52 d (3.5)	5.48 d (3.5)	5.57 d (3.5)	5.52 d (3.5)	5.51 d (3.5)	5.54 d (3.5)
Fuc-5	4.02*	4.00 q (6.0)	4.05 q (6.0)	4.02 q (6.5)	4.03 q (6.5)	4.03 q (6.5)
Fuc-6	1.25 d (6.0)	1.25 d (6.0)	1.32 d (6.0)	1.25 d (6.5)	1.25 d (6.5)	1.26 d (6.5)
Inner						
Rha-1	6.08 d (1.0)	6.08 d (1.0)	6.23 d (1.0)	6.27 d (1.5)	6.26 d (1.5)	6.10 d (1.5)
Rha-2	6.01 dd (3.0, 1.0)	5.02 dd (3.0, 1.0)	4.80 dd (3.0, 1.0)	4.79 dd (3.0, 1.5)	4.78 dd (3.0, 1.5)	5.98 dd (3.5, 1.5)
Rha-3	4.80 dd (9.0, 3.0)	5.89 dd (9.0, 3.0)	4.66 dd (9.0, 3.0)	4.66 dd (9.0, 3.0)	4.66 dd (9.0, 3.0)	4.78 dd (9.0, 3.5)
Rha-4	4.20 dd (9.0, 9.0)	4.57 dd (9.0, 9.0)	4.34 dd (9.0, 9.0)	4.36 dd (9.0, 9.0)	4.36 dd (9.0, 9.0)	4.25 dd (9.0, 9.0)
Rha-5	4.48 dq (9.0, 6.0)	4.48*	4.47 dq (9.0, 6.0)	4.52 dq (9.0, 6.0)	4.53 dd (9.0, 6.0)	4.48 dd (9.0, 6.0)
Rha-6	1.76 d (6.0)	1.76 d (6.0)	1.73 d (6.0)	1.77 d (6.0)	1.76 d (6.0)	1.78 d (6.0)
Xyl-1	5.17 d (7.5)	4.98 d (7.5)	5.03 d (8.0)	5.04 d (7.8)	5.04 d (7.8)	5.16 d (7.5)
Xyl-2	4.06 dd (8.0, 7.5)	3.93 dd (8.0, 7.5)	4.08 dd (8.0, 8.0)	4.09 dd (8.0, 8.0)	4.09 dd (8.0, 8.0)	4.06 dd (8.0, 8.0)
Xyl-3	4.21 dd (8.0, 8.0)	4.23 dd (8.0, 8.0)	4.23 dd (8.0, 8.0)	4.24 dd (9.0, 8.0)	4.23 dd (9.0, 8.0)	4.22 dd (9.0, 8.0)
Xyl-4	4.12 ddd (10.5, 8.0, 4.5)	4.09 ddd (10.0, 8.0, 5.0)	4.10 ddd (10.5, 8.0, 5.0)	4.09 ddd (10.0, 9.0, 5.0)	4.09 ddd (10.0, 9.0, 5.5)	4.13 ddd (10.0, 9.0, 5.0)
Xyl-5	3.52 dd (10.5, 10.5) 4.21 dd (10.5, 4.5)	3.54dd (10.5, 10.0) 4.10 dd (10.5, 5.0)	3.46 dd (10.5, 10.5) 4.16 dd (10.5, 5.0)	3.46 dd (10.5, 10.0) 4.16 dd (10.5, 5.0)	3.46 dd (10.5, 10.0) 4.16 dd (10.5, 5.5)	3.53 dd (11.0, 10.0) 4.22 dd (11.0, 5.0)
Terminal						
Rha-1	6.15 d (1.0)	6.20 br.s	6.23 d (1.0)	6.24 d (1.0)	6.23 d (1.5)	6.18 d (1.0)
Rha-2	4.76 dd (3.0, 1.0)	4.82 br.d (3.5)	4.80 dd (3.0, 1.0)	4.81 dd (3.5, 1.0)	4.80 dd (3.5, 1.5)	4.76 dd (3.5, 1.0)
Rha-3	4.59 dd (9.0, 3.0)	4.62 dd (9.0, 3.5)	4.60 dd (9.0, 3.0)	4.61 dd, 9.0, 3.0)	4.60 dd (9.0, 3.5)	4.61 dd (9.0, 3.5)
Rha-4	4.31 dd (9.0, 9.0)	4.33 dd (9.0, 9.0)	4.32 dd (9.0, 9.0)	4.34 dd (9.0, 9.0)	4.33 dd (9.0, 9.0)	4.32 dd (9.0, 9.0)
Rha-5	4.93 dq (9.0, 6.0)	4.98 dq (9.0, 6.0)	4.50 dq (9.0, 6.0)	4.96 dq (9.0, 6.0)	4.95 dq (9.0, 6.0)	4.60 dq (9.0, 6.0)
Rha-6	1.66 d (6.0)	1.69 d (6.0)	1.67 d (6.0)	1.67 d (6.0)	1.67 d (6.0)	1.66 d (6.0)

Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl; Xyl, β-D-xylpyranosyl; Fuc, β-D-fucopyranosyl.
*Overlapped signals.

the inner rhamnose. This ester linkage was also supported by the HMBC correlations between δ_H 5.98 (Inner-Rham-H-2) and δ_C 170.6 ($\underline{CO}CH_3$). Thus, the structure of bellisoside F was determined as 3-*O*-α-L-rhamnopyranosyl polygalacic acid 28-*O*-α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-3-acetoxyl-α-L-rhamnopyranosyl(1→2)-4-*O*-3-(*S*)-3-{3-(*S*)-3-[3-(*S*)-3-(3-(*S*)-3-hydroxy-1-oxobutoxy)-1-oxobutoxy]-1-oxobuty}-1-oxobutyl-β-D-fucopyranoside.

Bellisosides C–F (**3–6**) are newly described triterpenoid saponins bearing an acyl side chain containing the HBA moiety. A triterpenoid saponin with an acyl side chain containing the HBA moiety is rare in nature, which has only been reported from *Solidago virga-aurea*.[7] To our knowledge, however, bellisosides E (**5**) and F (**6**) F are the first example of the triterpenoid saponin with the acyl side chain constructed by four sets of the HBA moiety. Further, the absolute stereochemistry of the HBA moiety in the triterpenoid saponins was firstly determined. A wide range of bacteria synthesize (*R*)-3-hydroxybutiric acid as an intracellular storage material, and the poly[(*R*)-3-hydroxybutiric acid] (PHB) accumulates as granules within the cytoplasm.[8] PHB is synthesized from acetyl-coenzyme A



Scheme 1. Stereochemistry of the acyl moieties in **3–6**.

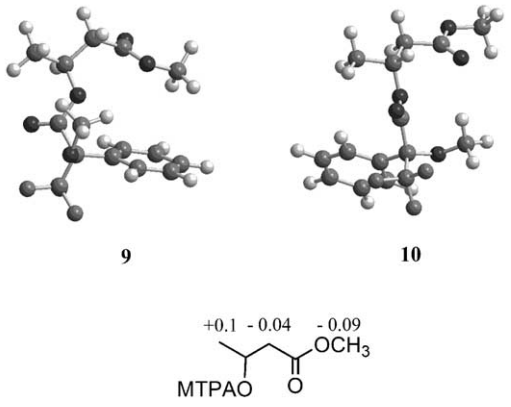


Figure 3. Computer-generated 3D structures and $\Delta\delta$ values [$\Delta\delta$ (in ppm)$=\delta_{9}-\delta_{10}$] of **9** and **10**.

(CoA) by a sequence of three enzymatic reactions. 3-Ketothiolase catalyzes the reversible condensation reaction of two acetyl-CoA molecules into acetoacetyl-CoA; the intermediate is reduced to (*R*)-3-hydroxybutyryl-CoA by NADPH-linked acetoacetyl-CoA reductase, and PHB is then produced by polymerization of (*R*)-3-hydroxybutyryl-CoA with PHB synthase. It has been shown that at least two different pathways exist for the synthesis of (*R*)-3-hydroxybutyryl-CoA. One of these is the pathway found in *Rhodospirillum rubrum*[9] in which acetoacetyl-CoA is first reduced to (*S*)-3-hydroxybutyryl-CoA by an NAD-dependent dehydrogenase. This intermediate is then converted to (*R*)-3-hydroxybutyryl-CoA. The other pathway for PHA synthesis is that found in *Azotobacter beijerinckii*,[10] and *Alcaligenes eutrophus*in[11] in which acetoacetyl-CoA is converted directly into (*R*)-3-hydroxybutyryl-CoA by NADPH-linked acetoacetyl-CoA reductase. A possible biogenetic pathway of **3–6** is proposed as shown in Figure 4.

The isolated saponins **1–7** were evaluated for their cytotoxic activities against HL-60 human promyelocytic leukemia cells. The cells were continuously treated with each sample for 72 h, and the cell growth was measured by an MTT reduction assay procedure (Table 5).[12] The bellisosides E (**5**) and F (**6**) with a long chain acyl group, which contain four molecular HBA moieties, showed strong cytotoxic activity against HL-60 cells, with respective IC_{50} values of

Table 5. Cytotoxic activity of **1–7** and cisplatine against HL-60 cells

Compound	IC_{50} (μM)
1	26.0±0.5
2	14.0±5.7
3	3.9±1.0
4	20.0±2.8
5	1.4±0.5
6	0.5±0.1
7	4.6±1.9
Cisplatine	1.8±0.06

The data shown represent the mean±SEM of two independent experiments with three determinations in each.

1.4 and 0.5 μM, while cytotoxic activity of the other saponins was moderate (IC_{50} 3.9–26.0 μM) compared with that of cisplatine used as a positive control (IC_{50} 1.8 μM). The cytotoxic activity in **1–7** may be related to the size of the acyl groups constructed from HBA attached at C-4 of fucose.

3. Experimental

3.1. General

The IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. The optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm length cell. The FABMS and HRFABMS were taken on a JEOL JMS-700 MStation. The ^{1}H and ^{13}C NMR were measured with a Varian XL-400 spectrometer in pyridine-d_5 solution and chemical shifts are expressed in δ (ppm) referring to TMS. For HPLC, a Waters model 510 HPLC system, equipped with a Shimadzu SPD-6A and Waters Differential Refractometer R401 detector was used for preparation.

Isolation. *B. perennis* L. (Compositae) was grown in the cultivation garden in Narita, Japan. Its roots (536.04 g) were extracted with MeOH (10 L×5. Evaporation of the solvent under reduced pressure from the combined extract gave the MeOH extract (85.34 g). The extract was then partitioned between EtOAc and H_2O. The H_2O phase was evaporated under reduced pressure below 40 °C to remove the EtOAc, and then subjected to a Diaion HP-20 column and further washed by MeOH to give the crude saponin fraction (BPS,

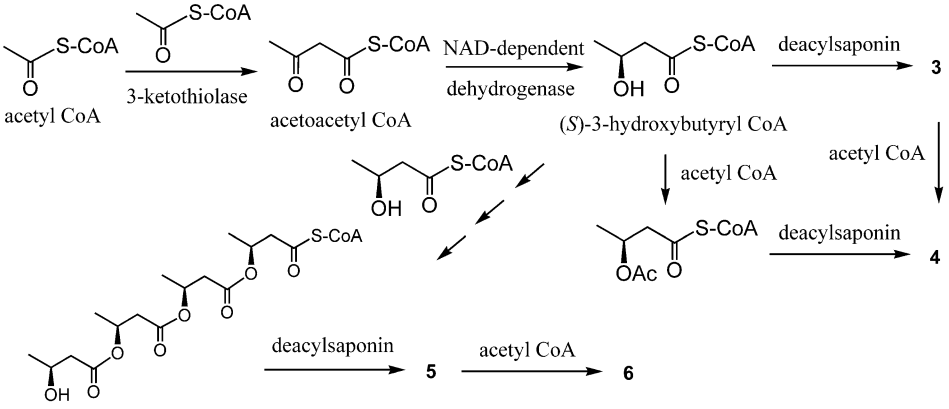


Figure 4. Hypothetical biogenetic pathway for **3–6**.

19.90 g). BPS (10.00 g) was subjected to ODS chromatography and eluted by aqueous MeOH with the ratios from 0 to 100% to give four fractions A–D. Further purification of fractions B (1.6 g) was achieved by repeated RP-HPLC (μ Bondasper 5 μm C_{18}-300 Å, 19 mm×150 mm) with solvent as 70% MeOH and NP-HPLC (Senshu Pak. Aquasil SS-5151, 19 mm×150 mm) with solvent as $CHCl_3$–MeOH–H_2O (60:18:2) to give **1** (18.5 mg), **2** (15.5 mg), **3** (21.5 mg), **4** (12.3 mg), **5** (54.6 mg), **6** (26.9 mg) and **7** (21.5 mg).

3.1.1. Bellisoside A (1). Powder, $[\alpha]_D$ −19.6° (*c* 0.45, MeOH, 24 °C). IR ν_{max} cm^{-1}: 3423, 2934, 1733, 1637, 1379, 1251, 1048. FABMS (positive) *m/z*: 1343 $[M+Na]^+$. HRFABMS (positive): observed 1343.6182, calcd for $C_{63}H_{100}O_{29}Na$ $[M+Na]^+$, 1343.6248. ^1H NMR (400 MHz, pyridine-d_5), see Tables 1 and 2. ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 3 and 4.

3.1.2. Bellisoside B (2). Powder, $[\alpha]_D$ −22.1° (*c* 0.51, MeOH, 24 °C). IR ν_{max} cm^{-1}: 3443, 2924, 1735, 1637, 1458, 1378, 1255, 1049. FABMS (positive) *m/z*: 1343 $[M+Na]^+$. HRFABMS (positive): observed 1343.6217, calcd for $C_{63}H_{100}O_{29}Na$ $[M+Na]^+$, 1343.6248. ^1H NMR (400 MHz, pyridine-d_5), see Tables 1 and 2. ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 3 and 4.

3.1.3. Bellisoside C (3). Powder, $[\alpha]_D$ −27.8° (*c* 0.47, MeOH, 24 °C). IR ν_{max} cm^{-1}: 3442, 2933, 1733, 1637, 1456, 1384, 1241, 1048. FABMS (positive) *m/z*: 1329 $[M+Na]^+$. HRFABMS (positive): observed 1329.6438, calcd for $C_{63}H_{102}O_{28}Na$ $[M+Na]^+$, 1329.6455. ^1H NMR (400 MHz, pyridine-d_5), see Tables 1 and 2. ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 3 and 4.

3.1.4. Bellisoside D (4). Powder, $[\alpha]_D$ −39.0° (*c* 0.44, MeOH, 24 °C). IR ν_{max} cm^{-1}: 3423, 2933, 1733, 1637, 1456, 1383, 1262, 1049. FABMS (positive) *m/z*: 1371 $[M+Na]^+$. HRFABMS (positive): observed 1371.6475, calcd for $C_{65}H_{104}O_{29}Na$ $[M+Na]^+$, 1371.6561. ^1H NMR (400 MHz, pyridine-d_5), see Tables 1 and 2. ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 3 and 4.

3.1.5. Bellisoside E (5). Powder, $[\alpha]_D$ −32.0° (*c* 0.85, MeOH, 24 °C). IR ν_{max} cm^{-1}: 3441, 2936, 1736, 1453, 1384, 1260, 1054. FABMS (positive) *m/z*: 1587 $[M+Na]^+$. HRFABMS (positive): observed 1587.7582, calcd for $C_{75}H_{120}O_{34}Na$ $[M+Na]^+$, 1587.7559. ^1H NMR (400 MHz, pyridine-d_5), see Tables 1 and 2. ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 3 and 4.

3.1.6. Bellisoside F (6). Powder, $[\alpha]_D$ −27.9° (*c* 0.55, MeOH, 24 °C). IR ν_{max} cm^{-1}: 3443, 2931, 1734, 1637, 1457, 1382, 1258, 1050. FABMS (positive) *m/z*: 1629 $[M+Na]^+$. HRFABMS (positive): observed 1629.7677, calcd for $C_{77}H_{122}O_{35}Na$ $[M+Na]^+$, 1629.7664. ^1H NMR (400 MHz, pyridine-d_5), see Tables 1 and 2. ^{13}C NMR (100 MHz, pyridine-d_5), see Tables 3 and 4.

3.1.7. Sterochemistry of sugars of 1–6. Each solution of **1–6** (each 1.0 mg), in 1 M HCl (dioxane–H_2O, 1:1, 200 μL) was heated at 100 °C for 1 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (1 mL×3) to remove the aglycone. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column, concentrated under reduced pressure to dryness, to give a sugar fraction. The sugar fraction was dissolved in 1 mL H_2O, to which (−)-α-methylbenzylamine (5 mg) and $NaBH_3CN$ (3 mg) in EtOH (1 mL) was added. After being set aside at 40 °C for 4 h followed by addition of glacial acetate (0.2 mL) and evaporated to dryness, the resulting solid was acetylated with acetic anhydride (0.3 mL) in pyridine (0.3 mL) for 24 h at room temperature. The reaction mixture was evaporated 5 times by adding H_2O to remove pyridine, and then passed through a Sep-Pak C_{18} cartridge (Waters) with 20% and 50% CH_3CN (each 10 ml) as solvents. The 50% CH_3CN eluate, which is the mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives of the monosaccharides, was then analyzed by HPLC under the following conditions: column, Inertsil ODS-3 (4.6×250 mm); solvent: 40% CH_3CN; flow rate, 0.8 mL/min; column temperature, 40 °C; detection, UV 230 nm. The derivatives of D-xylose, D-galactose, D-fucose, D-glucose and L-rhamnose were detected as follows: t_R (min) 21.1 (derivative of D-xylose), 23.6 (derivative of D-galactose), 24.6 (derivative of D-fucose), 27.7 (derivative of D-glucose), and 31.6 (derivative of L-rhamnose).

3.1.8. Stereochemistry of 3-hydroxybutric acid moiety. Each compound of **3–6** (each 1.0 mg) was treated with 0.28% $NaOCH_3$ in dry MeOH (100 μL) for 2 h at room temperature. The solution was neutralized with Dowex 50W-X8 (H^+) resin, and then filtered through a filter paper to remove the resin. The filtrate was evaporated. A part of the residue was trimethylsilylated with 1-trimethylsilylimidazole, analyzed by GLC–MS analysis to confirm the product of HBA methyl ester. GLC conditions: capillary column, EQUITY™-1 (30 m×0.25 mm ×0.25 μm, Supelco), oven temperature program, an initial temperature of 60 °C for 5 min, to 240 °C at 3 °C/min, hold for 5 min; injection temperature, 250 °C; carrier N_2 gas. 3-[(Trimethylsilyl)oxy]butanoic acid methyl ester was detected at 9.67 min. The residue obtained from methanolysis was treated with (*S*)-MTPA-Cl and then subjected to HPLC analysis under the following conditions: column, YMC ODS-A (4.6×150 mm); solvent: 40% CH_3CN; flow rate, 0.8 mL/min; column temperature, 40 °C; detection, UV 230 nm. The peak was detected at 8.86 min, which was identified as 3-(*S*)-3-[2-(*R*)-2-methoxy-2-phenyl-2-trifluoromethyl]-acetoxy butyric acid methyl ester (**9**), while under the same conditions, synthetic 3-(*R*)-3-[2-(*R*)-2-methoxy-2-phenyl-2-trifluoromethyl]-acetoxy butyric acid methyl ester (**10**) was detected at 9.55 min. 3-(*S*)-3-[2-(*R*)-2-methoxy-2-phenyl-2-trifluoromethyl]-acetoxy butyric acid methyl ester (**9**): ^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (3H, d, J=6.4 Hz, HBA-H_3-4), 2.62 (1H, dd, J=16.2, 8.0 Hz, HBA-H-2), 2.66 (1H, dd, J=16.2, 5.1 Hz, HBA-H-2), 3.56 (3H, s, OCH_3), 3.50 (3H, s, MTPA-OCH_3), 5.52 (1H, m, HBA-H-3), 7.40–7.42 (3H, m, MTPA-Ph), 7.49 (2H, dd, J=8.0, 2.0 Hz, MTPA-Ph). 3-(*R*)-3-[2-(*R*)-2-methoxy-2-phenyl-2-trifluoromethyl]-acetoxy butyric acid methyl ester (**10**): ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, d, J=6.2 Hz, HBA-H_3-4), 2.66 (1H, dd, J=16.5, 8.5 Hz, HBA-H-2), 2.70 (1H, dd, J=16.5, 5.2 Hz, HBA-H-2), 3.65 (3H, s, OCH_3), 3.49 (3H, s, MTPA-OCH_3), 5.51 (1H, m,

HBA-H-3), 7.40–7.42 (3H, m, MTPA-Ph), 7.49 (2H, dd, $J=8.0$, 2.0 Hz, MTPA-Ph).

3.2. Cytotoxic assay

HL-60 leukemia cells, which were obtained from RIKEN Cell Bank (Tsukuba, Japan), were maintained in RPMI 1640 medium (GIBCO RBL Co., Grand Island, NY, USA) containing heat-inactivated 10% fetal bovine serum (Bio-Whittaker, Walkersville, MD) supplemented with L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin (Meiji-Seika, Tokyo, Japan). The leukemia cells were washed and resuspended in the above medium to 3×10^4 cells/mL, and 180 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate (Iwaki Glass, Chiba, Japan). The cells were incubated in 5% CO_2/air for 24 h at 37 °C. After incubation, 20 μL of EtOH–H_2O (1:9) solution containing the sample was added to give the final concentrations of 0.1–100 μM/mL; 20 μL of EtOH–H_2O (1:9) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure. At the end of incubation, 10 μL of 5 mg/mL MTT (Sigma, St. Louis, MO, USA) in phosphate-buffered saline was added to every well, and the plate was further incubated in 5% CO_2/air for 4 h at 37 °C. The plate was then centrifuged at 1500*g* for 5 min to precipitate cells and formazan. An aliquot of 150 μL of the supernatant was removed from every well, and 175 μL of DMSO was added to dissolve the MTT formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader (Spectra Classic, Tecan, Salzburg, Austria) at 550 nm. *T*/*C* (%) score was calculated by the formulae as described below and the graph on the concentration of the samples and *T*/*C* (%) was made. The value of concentration of samples that crossed the *T*/*C* (50%) was measured as the IC_{50} values. Data are mean values of two experiments performed in triplicate. $T/C\ (\%)=(T-S)/(C-S)\times100$. *T*: OD_{550} values of the cell with samples after 3 days incubation. *C*: OD_{550} values of the cell without samples. *S*: OD_{550} values of the cell before samples were added. The IC_{50} value was defined as the concentration of sample necessary to inhibit the growth to 50% of the control.

References and notes

1. Hansel, R.; Keller, K.; Rimpler, H.; Schneider, G. *Hagers Handbuch der Pharmazeutischen Praxis*; Springer: Berlin, 1992.
2. Kroeber, L. *Pharmazeutische Zentralhalle fuer Deutschland* **1931**, *72*, 745.
3. (a) Hiller, K.; Schoepke, T.; Wray, V.; Schulten, H. R. *Pharmazie* **1988**, *43*, 850–852. (b) Schoepke, T.; Wray, V.; Kunath, A.; Hiller, K. *Pharmazie* **1990**, *45*, 870–871. (c) Schoepke, T.; Wray, V.; Rzazewska, B.; Hiller, K. *Phytochemistry* **1991**, *30*, 627–631. (d) Schoepke, T.; Wray, V.; Kunath, A.; Hiller, K. *Phytochemistry* **1992**, *31*, 2555–2557. (e) Schoepke, T.; Hiller, K. *Sci. Pharm.* **1996**, *64*, 663–668. (f) Glensk, M.; Wray, V.; Nimtz, M.; Schoepke, T. *Sci. Pharm.* **2001**, *69*, 69–73.
4. Asada, Y.; Ueoka, T.; Furuya, T. *Chem. Pharm. Bull.* **1989**, *37*, 2139–2146.
5. Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501–506.
6. Jia, Z.; Koike, K.; Nikaido, T. *J. Nat. Prod.* **1998**, *61*, 1368–1373.
7. (a) Inose, Y.; Miyase, T.; Ueno, A. *Chem. Pharm. Bull.* **1991**, *39*, 2037–2042. (b) Inose, Y.; Miyase, T.; Ueno, A. *Chem. Pharm. Bull.* **1992**, *40*, 946–953. (c) Inose, Y.; Miyase, T.; Ueno, A. *Chem. Pharm. Bull.* **1994**, *42*, 617–624.
8. Anderson, A. J.; Qawes, E. A. *Microbiol. Rev.* **1990**, *54*, 450–472.
9. Moskowitz, M. J.; Merrick, J. M. *Biochemistry* **1969**, *8*, 2748–2755.
10. (a) Ritchie, G. F. A.; Senior, P. J.; Dawes, E. A. *Biochem. J.* **1971**, *121*, 309–316. (b) Senior, P. J.; Dawes, E. A. *Biochem. J.* **1973**, *134*, 225–238.
11. (a) Haywood, G. W.; Anderson, A. J.; Chu, L.; Dawes, E. A. *FEMS Microbiol. Lett.* **1988**, *52*, 259–264. (b) Oeding, V.; Schlegel, H. G. *Biochem. J.* **1973**, *134*, 239–248.
12. Sargent, J. M.; Taylor, C. G. *Br. J. Cancer* **1989**, *60*, 206–210.

Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron
Tetrahedron 61 (2005) 2931–2939

An improved Ullmann–Ukita–Buchwald–Li conditions for CuI-catalyzed coupling reaction of 2-pyridones with aryl halides

Po-Shih Wang,[a,b] Chih-Kai Liang[a,b] and Man-kit Leung[a,b,*]

[a]*Department of Chemistry, National Taiwan University, Taipei 106, Taiwan ROC*
[b]*Institute of Polymer Science and Engineering, National Taiwan University, Taipei 106, Taiwan ROC*

Received 20 October 2004; revised 31 December 2004; accepted 5 January 2005

Abstract—An effective CuI-*trans*-*N*,*N*′-dimethylcyclohexane-1,2-diamine (DMCDA)-K_2CO_3-catalyzed coupling reaction of 2-pyridones with aryl halides is described. Under our conditions, DMCDA was found to be an effective catalyst that facilitates the coupling reactions even in toluene, a common industrial solvent. In addition, 3-bromopyridine could also be coupled effectively under these conditions, indicating that the catalytic reactivity of this system is high. The reaction could be applied for polymer modification and iterative oligo-pyridone synthesis.

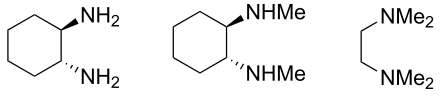
1. Introduction

N-Substituted pyrid-2-ones have attracted synthetic organic chemists because of their chemical reactivity, photochemical behavior, as well as their biological activities.[1,2] Direct C–N bond formation is one of the most convenient synthetic approaches for *N*-alkylpyridones.[3] However, synthetic examples for *N*-arylpyridones are rare.[4] Traditional copper-catalyzed Ullmann type C–N bond formation often requires the use of high temperatures as well as stoichiometric amounts of copper reagents.[5] Recently, palladium catalyzed *N*-arylations have been extensively explored.[6] In addition, Ullmann-type copper-catalyzed processes for C–N bond formation such as *N*-arylation of amines,[7] anilines,[8] amides,[9] imidazole,[10] indoles,[9] and hydrazines[11] have been reported. The reactivity of the copper catalyst strongly relies on the ligand system used in the reaction. *N*,*N*′-Dimethylethylene-diamines and derivatives are known effective ligands to promote the reactions.

Ukita has recently reported the copper-catalyzed Ullmann *N*-arylation of 2-hydroxypyridine.[4a] Copper halides, oxides, or copper powder facilitates the reaction. However, high temperatures of 120–150 °C in DMF are usually required. The reaction is sensitive to steric hindrance. *Ortho*-substituted aryl halides are ineffective toward the coupling reaction.

Li has recently reported a modification of the Ukita conditions, using CuI–$MeNHCH_2CH_2NHMe$–K_3PO_4 in dioxane to promote the coupling reaction.[4b] In his work, detail electronic effects on pyridones as well as on the aryl halides have been evaluated.

In this report, we noted that CuI could facilitate the coupling of aryl halides with 2-hydroxypyridines under milder conditions in the presence of *N*,*N*′-dimethylcyclohexane-1,2-diamine (DMCDA) as the ligand. The reaction could proceed in toluene instead of dioxane or DMF.[12] Toluene is a common solvent that could be used for industrial applications.



CHDA DMCDA TMEDA

Recently, we have attempted the synthesis of 2-amino-pyridines.[13] In one experiment, we accidentally observed that 2 equiv of 2-bromopyridine (**1**) would undergo self-condensation in the presence of CuI/DMCDA/K_2CO_3 as the catalyst to give *N*-(pyrid-2-yl)pyrid-2-one (**2**) as the major product. Although we could not conclusively differentiate the *N*-arylated or *O*-arylated structure on the basis of the NMR spectrum, X-ray crystallographic analysis of the self-condensation product (**4**) unambiguously shows that the pyridyl group is attached to the pyridone nitrogen atom (Fig. 1, Scheme 1).

Keywords: 2-Pyridones; Copper reagents; C–N coupling; Toluene.
* Corresponding author.; e-mail: mkleung@ntu.edu.tw

0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.01.063

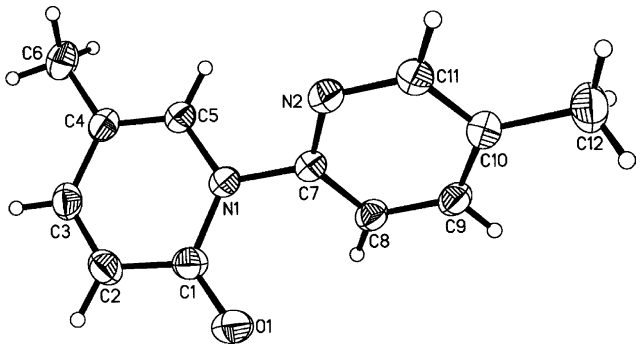
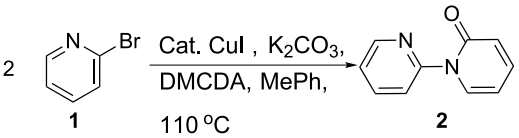


Figure 1. The ORTEP of **4**.



Scheme 1.

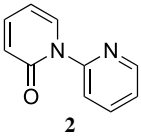
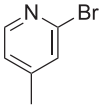
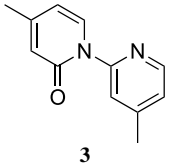
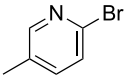
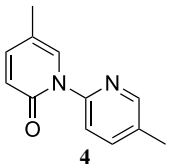
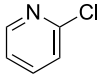
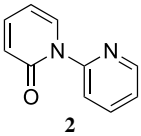
It is noteworthy that unsubstituted or tetrasubstituted 1,2-diamines such as *trans*-cyclohexane-1,2-diamine(CHDA) or tetramethylethylene-1,2-diamine (TMEDA) do not promote the self-condensation reaction, indicating that DMCDA is essential for the reaction. When CHDA was applied, no reaction occurred. On the other hand, bromo–iodo exchange reaction occurred to give 2-iodopyridine (20%) in the presence of TMEDA. The self-condensation reaction could be applied to other 2-halopyridines (Table 1).

However, 2-chloropyridine was found to be sluggish in the self-condensation reaction. Although no direct evidence was obtained for the reaction mechanism, we suspect that hydrolysis of 2-bromopyridine (**1**) under the reaction conditions would occur to give 2-hydroxypyridine. The newly generated 2-hydroxypyridine would then be further coupled with 2-bromopyridine to give (**2**). Since we could not isolate any 2-hydroxypyridine from the reaction mixture, we proposed that the coupling of 2-hydroxypyridine with 2-bromopyridine is relatively fast in comparison to the 2-hydroxypyridine formation. These observations suggested that cross coupling of 2-hydroxypyridine with other 2-aryl halides might be feasible under similar reaction conditions (Table 2).

In our initial screening experiments, 2-hydroxypyridine and bromobenzene were used as the prototype substrates for searching the reaction conditions. In contrast to Ukita's reaction conditions[4a] in which no reaction would occur in mesitylene, our reaction proceeds smoothly in toluene at reflux temperature. More surprisingly, the reaction is very specific to 2-hydroxypyridine. Other phenolic derivatives such as 3- and 4-hydroxypyridines, 2-hydroxypyrimidine, and phenol or phthalimide do not couple with bromobenzene under the above conditions. The choice of the ligand strongly affects the reactivity of the cross-coupling reaction. The use of CHDA under similar conditions gave *N*-phenylpyrid-2-one (**5**) in low yield (3%). TMEDA does not promote the reaction.

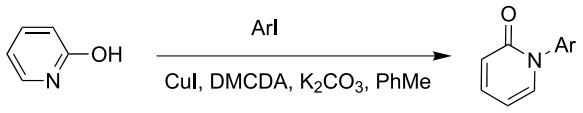
Both KOAc and K_2CO_3 were found to be effective as bases.

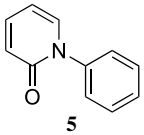
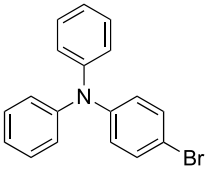
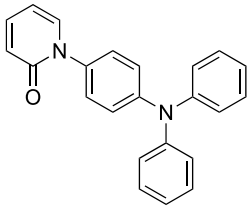
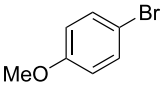
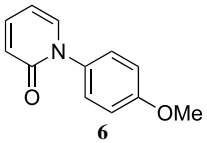
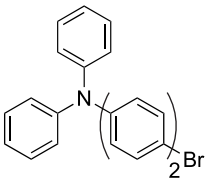
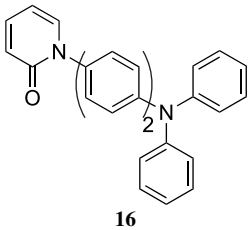
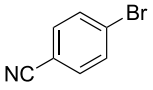
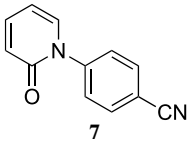
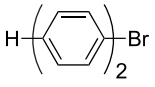
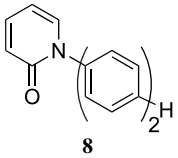
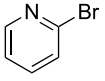
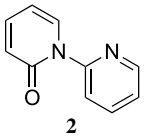
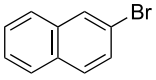
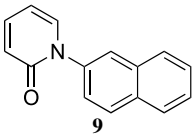
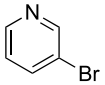
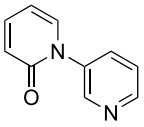
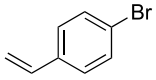
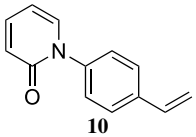
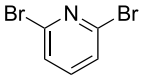
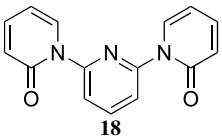
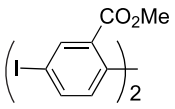
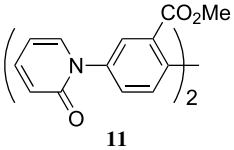
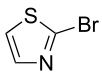
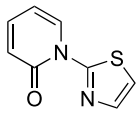
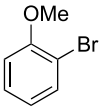
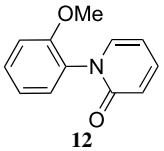
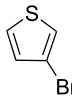
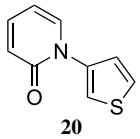
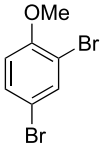
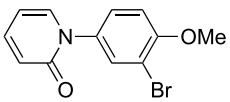
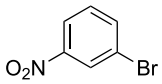
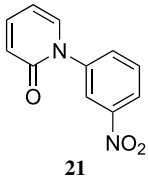
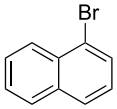
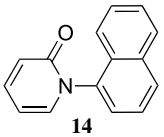
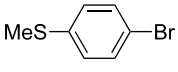
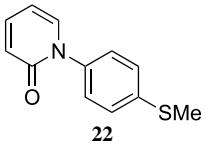
Table 1. Self-condensation of 2-halopyridines

Substrate	CuI, L (equiv)	Product (yield %)	
	0.2, 0.2	 **2**	X=Br (56) X=I (44)[a]
	0.2, 0.2	 **3**	(57)[b]
	0.2, 0.2	 **4**	(36)[b]
	0.2, 1	 **2**	(7)[c]

L: *trans*-*N*,*N*′-Dimethyl-cyclohexane-1,2-diamine.
[a] 16 h.
[b] 20 h.
[c] 24 h.

Table 2. Cross coupling of 2-hydroxypyridine with various aryl halides



Entry	Aryl hailde	Product (%)		Entry	Aryl halide	Product (%)	
1		 **5**	X=Br (91) X=1 (84)	11		 **15**	(59)
2		 **6**	(89)	12		 **16**	(79)
3		 **7**	(70)				
4		 **8**	(38)	13		 **2**	(68)
5		 **9**	(82)	14		 **17**	(76)
6		 **10**	(61)	15		 **18**	(65)
7		 **11**	(81)	16		 **19**	(33)
8		 **12**	(2)[a]	17		 **20**	(74)
9		 **13**	(37)	18		 **21**	(62)
10		 **14**	(<1)	19		 **22**	(80)

[a] In DMF.

However, KO^{t}Bu will attack 2-bromopyridine to give 2-*tert*-butoxypyridine.[14] The reaction will also proceed in other solvents such as DMF or dioxane.

However, the use of toluene gave the best results. The optimized reaction conditions of 20 mol% CuI, 20 mol% DMCDA, and 2 equiv K_2CO_3 were applied to the *N*-arylation of 2-hydroxypyridine with a number of aryl halides (Table 2). Functional groups that are compatible with this CuI-catalyzed *N*-arylation protocol include ether, thioether, triarylamine, nitrile, nitro group, styrene, and ester. No significant electronic effects on the aryl halides were observed in the reaction. As can be seen, the electron-deficient aryl bromides such as 3-$NO_2C_6H_4Br$ and 4-CNC_6H_4Br, as well as the electron-rich aryl bromides such as 4-$MeOC_6H_4Br$ or $Ph_2NC_6H_4Br$ are effectively coupled in the reaction.

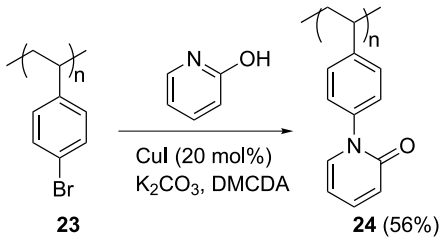
Electronic effects on 2-hydroxypyridine are significant, however. Electron-withdrawing substituents that would reduce the nucleophilicity of 2-hydroxypyridine do retard its reactivity toward the coupling reaction. Thus, the reaction of 2-hydroxy-5-nitropyridine is very sluggish. Similar results were reported in Li's conditions.

The reaction could also be applied to heterocyclic aryl halides such as pyridine, thiophene, and thiazole. In contrast to the results of Ukita's and Li's conditions, our conditions could also be applied to 3-bromopyridine to give the corresponding pyridone **17** in high yield.

The reaction is very sensitive to the steric environment of the aryl halides. Usually, *o*-substituted aryl bromides do not show enough reactivity in the reaction. Thus, regioselective coupling would occur at the 4-position of 2,4-dibromoanisole to give the *para* substituted product (entry 9, Table 2).

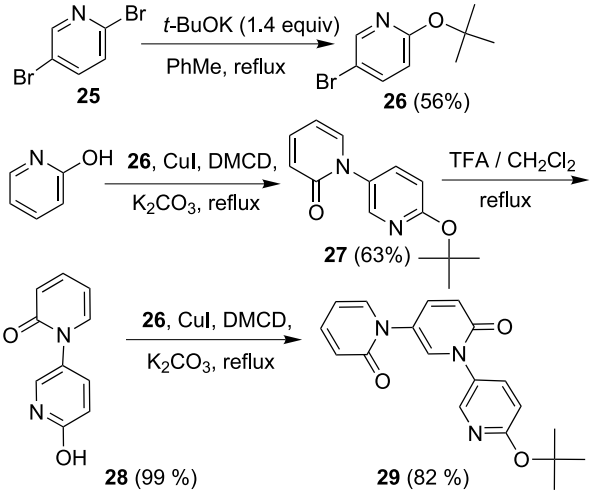
Similarly, although 2-bromonaphthalene could react smoothly to give the desired pyridone, as mentioned in Ukita's paper, 1-bromonaphthalene does not react under these conditions (compare entries 5 and 10, Table 2). We have applied this method for purifying 1-bromonaphthalene. Commercially available 1-bromonaphthalene is usually contaminated by a few percent of 2-bromonaphthalene. Since they have similar polarity and boiling point, they are difficult to separate. When the contaminated 1-bromonaphthalene was treated with 2-hydroxypyridine in the presence of the copper catalyst, the 2-bromo impurity was selectively converted to the more polar pyridone and removed by liquid chromatography. No bromo–iodo exchange was observed under these reaction conditions.

The reactivity of the reaction is so high that it could be successfully applied to polymer modification. Treatment of the commercially available poly(4-bromostyrene) (**23**) with 2-hydroxypyridine under the same conditions affords the target polypyridone (**24**) in reasonable yield. The structural assignment was supported by IR and NMR spectroscopy. The final polymer shows characteristic IR absorption bands of pyridone at 1663 and 1590 cm^{-1}, along with the disappearance of the characteristic IR absorption band of poly(4-bromostyrene) at 1073 cm^{-1}. In addition, five aromatic ^1H NMR signals at δ 6.14 (1H), 6.42 (1H), 6.81 (2H), 7.23 (2H), and 7.41 (2H), and nine sets of sp^2 ^{13}C NMR signals at δ 105.2, 120.3, 125.9, 127.6, 138.2, 138.7, 140.0, 144.9 and 160.8 suggested the polypyridone formation (Scheme 2).



Scheme 2.

The reaction could be extended to iterative oligo-pyridone synthesis (Scheme 3). As mentioned before, the bromo group of 2-bromopyridine could be replaced by *tert*-butoxide in the presence of KO^{t}Bu. Thus, reaction of **25** with KO^{t}Bu afforded **26**. CuI-catalyzed condensation of 2-hydroxypyridine with **26** gave **27** in moderated yield.



Scheme 3.

Deprotection of **27** by removal of the *tert*-butyl group in TFA afforded **28** in high yield. Compound **28** is slightly hygroscopic and has to be dried under vacuum at 110 °C before use in order to obtain the third generation of oligo-pyridone **29** in high yield. This iterative approach proved to be effective. Further application of this approach to the synthesis of tailored oligo-pyridones is ongoing (Scheme 3).

2. Experimental

2.1. The CuI-catalyzed self-condensation of 2-bromopyridine

trans-*N*,*N*′-Dimethylcyclohexane-1,2-diamine (DMCDA)

was prepared according to the supporting information in literature.[9c]

2.2. General procedure

To an oven-dried double-necked flask containing a stir-bar was charged CuI (20 mol%) and K_2CO_3 (2 equiv). The flask was evacuated and backfilled with nitrogen. A solution of 2-bromopyridine in toluene (1 M, 1 equiv) and DMCDA (20 mol%) were injected under nitrogen. The reaction mixture was stirred and heated at reflux temperature for the time specified. The resulting mixture was cooled to room temperature, diluted with dichloromethane, and filtered. The filtrate was washed with water. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified by flash chromatography on silica gel (ethyl acetate/dichloromethane).

2.2.1. 1-(2-Pyridyl)-1*H*-pyridin-2-one (2).[15] Reaction time: 16 h, white solid (56%). Mp 53–54 °C (from hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, J=5.2 Hz, 1H), 7.91 (d, J=6.8 Hz, 1H), 7.79–7.84 (m, 2H), 7.36 (ddd, J=9.2, 6.8, 2.4 Hz, 1H), 7.29 (ddd, J=6.8, 5.2, 1.2 Hz, 1H), 6.61 (d, J=9.2 Hz, 1H), 6.27 (td, J=6.8, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 151.5, 148.6, 140.0, 137.5, 135.8, 123.0, 121.8, 121.2, 106.1; IR (KBr): 3058, 2991, 1673 (C=O), 1612, 1540 cm^{-1}; FAB (NBA) 173.1 (M^++H); HRMS calcd for $C_{10}H_9N_2O$ 173.0715, found 173.0712 (M^++H), calcd for $C_{10}H_8N_2O$ 172.0637, found 172.0639 (M^+). Anal. calcd for $C_{10}H_8N_2O$ C, 69.76; H, 4.68; N, 16.27. Found C, 69.73; H, 4.70; N, 16.26.

2.2.2. 1-(4-Methylpyrid-2-yl)-4-methyl-1*H*-pyridin-2-one (3).[15] Reaction time: 20 h, colorless solid (57%). Mp 79–81 °C (from hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, J=5.2 Hz, 1H), 7.70–7.72 (m, 2H), 7.09 (ddd, J=5.2, 1.2, 0.8 Hz, 1H), 6.41 (bs, 1H), 6.10 (dd, J=7.2, 2 Hz, 1H), 2.40 (s, 3H), 2.20 (d, J=0.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 151.8, 151.7, 149.1, 148.2, 134.9, 124.0, 121.9, 119.9, 108.8, 21.4, 21.3; IR (CH_2Cl_2): 3044, 2922, 1670 (C=O), 1614, 1599, 1537, 1406 cm^{-1}; FAB (NBA) 201.1 (M^++H), 200.1 (M^+); HRMS calcd for $C_{12}H_{13}N_2O$ 201.1028, found 201.1031 (M^++H), calcd for $C_{12}H_{12}N_2O$ 200.0950, found 200.0951 (M^+). Anal. calcd for $C_{12}H_{12}N_2O$ C, 71.98; H, 6.04; N, 13.99. Found C, 71.89; H, 6.09; N, 13.98.

2.2.3. 1-(5-Methylpyrid-2-yl)-5-methyl-1*H*-pyridin-2-one (4).[16] Reaction time: 24 h, colorless solid (36%). Mp 106–107 °C (from hexane); ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.36 (bs, 1H), 7.72 (d, J=8 Hz, 1H), 7.63 (dd, J=8, 2 Hz, 1H), 7.58 (dd, J=2.4, 0.8 Hz, 1H), 7.25 (dd, J=9.2, 2.4 Hz, 1H), 6.48 (d, J=9.6 Hz, 1H), 2.38 (s, 3H), 2.11 (d, J=1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 149.7, 148.7, 142.7, 138.1, 133.1, 132.7, 121.4, 120.7, 115.0, 18.1, 17.3; IR (CH_2Cl_2): 3047, 2926, 1681 (C=O), 1614, 1593, 1530, 1498 cm^{-1}; FAB (NBA) 201.1 (M^++H), 200.1 (M^+); HRMS calcd for $C_{12}H_{13}N_2O$ 201.1028, found 201.1026 (M^++H), calcd for $C_{12}H_{12}N_2O$ 200.0950, found 200.0946 (M^+). Anal. calcd for $C_{12}H_{12}N_2O$ C, 71.98; H, 6.04; N, 13.99. Found C, 71.97; H, 6.07; N, 13.98.

2.3. General procedure for the CuI-catalyzed *N*-arylation of 2-hydroxypyridine

To an oven-dried double-necked flask containing a stir-bar was charged 2-hydroxypyridine (1 equiv), CuI (20 mol%), and K_2CO_3 (2 equiv). The flask was evacuated and back-filled with nitrogen. A solution of arylbromide in toluene (1 M, 1 equiv) and DMCDA (20 mol%) were injected under nitrogen. The reaction mixture was stirred and heated at reflux temperature for the time specified. The resulting mixture was cooled to room temperature, diluted with dichloromethane, and filtered. The filtrate was washed with water. The organic phase was collected, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified by flash chromatography on silica gel (ethyl acetate/dichloromethane).

2.3.1. 1-Phenyl-1*H*-pyridin-2-one (5).[17] Reaction time: 18 h, colourless solid (91%). Mp 153–154 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.62 (dd, J=7.2, 1.6 Hz, 1H), 7.47–7.52 (m, 3H), 7.44 (d, J=7.2 Hz, 1H), 7.33–7.41 (m, 2H), 6.47 (d, J=8.8 Hz, 1H), 6.30 (td, J=6.8, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1, 140.7, 139.6, 137.8, 129.1, 128.3, 126.3, 121.7, 105.8; IR (KBr): 3038, 1671(C=O), 1611, 1585, 1499, 1447 cm^{-1}; FAB (NBA) 172.1 (M^++H), HRMS calcd for $C_{11}H_{10}NO$ 171.0762, found 172.0759 (M^++H), calcd for $C_{11}H_9NO$ 171.0684, found 171.0686 (M^+). Anal. calcd for $C_{11}H_9NO$: C, 77.17; H, 5.30; N, 8.18. Found C, 77.30; H, 5.12; N, 8.11.

2.3.2. 1-(4-Methoxyphenyl)-1*H*-pyrid-2-one (6). Reaction time: 20 h, white solid (89%). Mp 110–111 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, d_6-acetone) δ 7.5 (ddd, J=7, 2.2, 0.8 Hz, 1H), 7.43 (ddd, J=9.2, 6.7, 2.2 Hz, 1H), 7.32 (dt, J=9.2, 2.8 Hz, 2H), 7.02 (dt, J=9.2, 2.8 Hz, 2H), 6.42 (ddd,, J=9.2, 1.2, 0.8 Hz, 1H), 6.24 (td, J=6.7, 1.6 Hz, 1H), 3.85 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 159.1, 139.5, 138.1, 133.6, 127.4, 121.6, 114.4, 105.6, 55.5; IR (KBr): 3024, 2363, 2325, 1661 (C=O), 1599, 1499, 1435 cm^{-1}; FAB (NBA) 202.1 (M^++H); HRMS calcd for $C_{12}H_{12}NO_2$ 202.0868, found 202.0865 (M^++H), calcd for $C_{12}H_{11}NO_2$ 201.0790, found 201.0797 (M^+). Anal. calcd for $C_{12}H_{11}NO_2$: C, 71.63; H, 5.51; N, 6.96. Found C, 71.93; H, 5.29; N, 6.87.

2.3.3. 1-(4-Cyanophenyl)-1*H*-pyridin-2-one (7).[18] Reaction time: 12 h, white solid (70%). Mp 165–166 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, J=8.4 Hz, 2H), 7.51 (d, J=8.4 Hz, 2H), 7.39 (ddd, J=9.2, 6.8, 2.2 Hz, 1H), 7.28 (dd, J=7, 2.2 Hz, 1H), 6.61 (d, J=9.2 Hz, 1H), 6.26 (t, J=6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.5, 144.1, 140.1, 136.5, 133.0, 127.3, 121.9, 117.7, 112.1, 106.6; IR (KBr): 3100, 3038, 2362, 2325, 1673 (C=O), 1599, 1523, 1499 cm^{-1}; FAB (NBA) 197.0 (M^++H); HRMS calcd for $C_{12}H_9N_2O$ 197.0715, found 197.0711 (M^++H), calcd for $C_{12}H_8N_2O$ 196.0637, found 196.0643 (M^+). Anal. calcd for $C_{12}H_8N_2O$: C, 73.46; H, 4.11; N, 14.28. Found C, 73.28; H, 3.98; N, 14.16.

2.3.4. 1-(Biphenyl-4-yl)-1*H*-pyridin-2-one (8).[19] Reaction time: 14 h, white solid (38%). Mp 226–227 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.68 (dd, J=6.6, 1.8 Hz, 2H), 7.58 (d, J=9.2 Hz, 2H), 7.34–7.46 (m, 7H), 6.68 (d, J=9.2 Hz, 1H), 6.29 (td, J=6.6, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2, 141.4, 139.9, 139.8, 139.7, 137.7, 128.7, 127.9, 127.6, 127.1, 126.6, 121.7, 106.0; IR (KBr): 3062, 3024, 2363, 2337, 1661 (C=O), 1585, 1535, 1485 cm^{-1}; FAB (NBA) 248.1 (M^++H); HRMS calcd for $C_{17}H_{14}NO$ 248.1075, found 248.1074 (M^++H), calcd for $C_{17}H_{13}NO$ 247.0997, found 247.0993 (M^+). Anal. calcd for $C_{17}H_{13}NO$: C, 82.57; H, 5.30; N, 5.66. Found C, 82.26; H,5.17; N, 5.46.

2.3.5. 1-(Naphthalen-2-yl)-1*H*-pyridin-2-one (9). Reaction time: 18 h, white solid, (82%). Mp 160–161 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, J=8.8 Hz, 1H), 7.81–7.89 (m, 2H), 7.79 (d, J=2 Hz, 1H), 7.48–7.54 (m, 3H), 7.37–7.43 (m, 2H), 6.68 (dd, J=9.8, 1.4 Hz, 1H), 6.25 (td, J=6.8, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 139.7, 138.5, 138.0, 133.2, 132.6, 129.0, 127.9, 127.6, 126.7, 126.6, 124.7, 124.4, 121.8, 105.9; IR (KBr): 3024, 2362, 2337, 1661 (C=O), 1585, 1535, 1499, 1461 cm^{-1}; FAB (NBA) 222.1 (M^++H),221.1 (M^+); HRMS calcd for $C_{15}H_{12}NO$ 222.0919, found 222.0921 (M^++H), calcd for $C_{15}H_{11}NO$ 221.0841, found 221.0841 (M^+). Anal. calcd for $C_{15}H_{11}NO$: C, 81.43; H, 5.01; N, 6.33. Found C, 81.16; H, 5.11; N, 6.31.

2.3.6. 1-(4-Vinylphenyl)-1*H*-pyridin-2-one (10). Reaction time: 9 h, white solid (61%). Mp 115–116 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dt, J=8.4, 2 Hz, 2H), 7.36 (ddd, J=9.2, 6.8, 2.4 Hz, 1H), 7.28–7.34 (m, 3H), 6.72 (dd, J=17.6, 10.8 Hz, 1H), 6.63 (d, J=9.2 Hz, 1H), 6.21 (td, J=6.8, 1.6 Hz, 1H), 5.77 (dd, J=17.6, 0.8 Hz, 1H), 5.30 (dd, J=10.8, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) only 10 sets of ^{13}C signals was observed δ 162.1, 140.0, 139.6, 137.6, 135.5, 126.9, 126.4, 121.8, 115.2, 105.8; IR (KBr): 3029, 1668 (C=O), 1582, 1530, 1490, 1455 cm^{-1}; FAB (NBA) 198.1 (M^++H),197.1 (M^+); HRMS calcd for $C_{13}H_{12}NO$ 198.0919, found 198.0918 (M^++H), calcd for $C_{13}H_{11}NO$ 197.0841, found 197.0839 (M^+). Anal. calcd for $C_{13}H_{11}NO$ C, 79.17; H, 5.62; N, 7.10. Found C, 79.33; H, 5.64; N, 7.28.

2.3.7. 4,4′-Bis(2-oxo-2*H*-pyridin-1-yl)biphenyl-2,2′-dicarboxylic acid dimethyl ester (11). Reaction time: 16 h, white solid (81%). Mp 218–219 °C (from CH_3CN); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J=2.4 Hz, 2H), 7.63 (dd, J=8, 2 Hz, 2H), 7.40–7.46 (m, 4H), 7.37 (d, J=8.4 Hz, 2H), 6.68 (d, J=9.2 Hz, 2H), 6.29 (t, J=6.8 Hz, 2H), 3.66 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 162.0, 142.3, 139.9, 139.7, 137.4, 131.0, 130.0, 129.6, 127.8, 121.8, 106.2, 52.1; IR (KBr): 3626, 3450, 3062, 3038, 2951, 2363, 2336, 1737 (C=O), 1661 (C=O), 1585, 1523, 1473 cm^{-1}; FAB (NBA) 457.1 (M^++H), 456.1 (M^+); HRMS calcd for $C_{26}H_{21}N_2O_6$ 457.1400, found 457.1408 (M^++H), calcd for $C_{26}H_{20}N_2O_6$ 456.1321, found 456.1326 (M^+). Anal. calcd for $C_{26}H_{20}N_2O_6$ C, 68.42; H, 4.42; N, 6.14. Found C, 68.17; H, 4.34; N, 6.17.

2.3.8. 1-(2-Methoxyphenyl)-1*H*-pyridin-2-one (12).[20] Reaction time: 16 h (in DMF), white solid (2%). Mp 86–87 °C (from Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.41 (m, 2H), 7.25 (dd, J=7.6, 1.6 Hz, 1H), 7.18 (dd, J=7.6, 1.6 Hz, 1H), 7.01–7.05 (m, 2H), 6.64 (d, J=9.2 Hz, 1H), 6.18 (td, J=6.4, 1.2 Hz, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2, 154.0, 139.7, 138.8, 130.1, 129.5, 128.4, 121.7, 120.8, 112.3, 105.3, 55.9; IR (neat): 3062, 2989, 1661 (C=O), 1599, 1523, 1485 cm^{-1}; FAB (NBA) 202.0 (M^++H); HRMS calcd for $C_{12}H_{12}NO_2$ 202.0868, found 202.0868 (M^++H), calcd for $C_{12}H_{11}NO_2$ 201.0790, found 201.0784 (M^+).

2.3.9. 1-(3-Bromo-4-methoxyphenyl)pyridin-2-one (13). Reaction time: 14 h, white solid (37%). Mp 146–147 °C (from THF); ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.65 (d, J=2.8 Hz, 1H), 7.61 (dd, J=6.8, 1.8 Hz, 1H), 7.48 (td, J=9.2, 6.8, 2.4 Hz, 1H), 7.38 (dd, J=8.8, 2.8 Hz, 1H), 7.21 (d, J=8.8 Hz, 1H), 6.45 (d, J=9.2 Hz, 1H), 6.28 (td, J=6.8, 1.2 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1, 155.5, 139.7, 137.6, 133.9, 131.1, 126.5, 121.5, 111.6, 111.5, 105.8, 56.4; IR (KBr): 3100, 3062, 3024, 2988, 2939, 2363, 2337, 1661 (C=O), 1585, 1535, 1485 cm^{-1}; FAB (NBA) 280.0 (M^++H); HRMS calcd for $C_{12}H_{11}^{79}BrNO_2$ 279.9973, found 279.9971 (M^++H), calcd for $C_{12}H_{10}^{79}BrNO_2$ 278.9895, found 278.9896 (M^+). Anal. calcd for $C_{12}H_{10}BrNO_2$ C, 51.45; H, 3.60; N, 5.00. Found C, 51.19; H, 3.58; N, 5.03.

2.3.10. 1-(4-(Diphenylamino)phenyl)pyrid-2-one (15). Reaction time: 24 h, white solid (59%). Mp 220–221 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, d_6-acetone) δ 7.56 (ddd, J=6.4, 2.4, 0.8 Hz, 1H), 7.44 (ddd, J=9.2, 6.7, 2.4 Hz, 1H), 7.29–7.36 (m, 6H), 7.05–7.13 (m, 8H), 6.44 (dd, J=9.2, 0.8 Hz, 1H), 6.26 (td, J=6.7, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 147.7, 147.0, 139.5, 138.0, 134.3, 129.2, 126.9, 124.8, 123.4, 122.7, 121.6, 105.9; IR (KBr): 3076, 3038, 2363, 2337, 1661 (C=O), 1585, 1485, 1473 cm^{-1}; FAB (NBA) 339.1 (M^++H),338.1 (M^+); HRMS calcd for $C_{23}H_{19}N_2O$ 339.1497, found 339.1493 (M^++H), calcd for $C_{23}H_{18}N_2O$ 338.1419, found 338.1417 (M^+). Anal. calcd for $C_{23}H_{18}N_2O$: C, 81.63; H, 5.36; N, 8.28. Found C, 81.48; H, 5.26; N, 8.41.

2.3.11. 1-(4′-(Diphenylamino)biphenyl-4-yl)-1*H*-pyridin-2-one (16). Reaction time: 20 h, white solid (79%). Mp 232–234 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.74 (dt, J=8.8, 2.2 Hz, 2H), 7.68 (dd, J=7, 1.4 Hz, 1H), 7.64 (dt, J=8.8, 2.2 Hz, 2H), 7.51 (ddd, J=8.8, 6.7, 2.2 Hz, 1H), 7.45 (dt, J=8.8, 2.2 Hz, 2H), 7.33 (t, J=9.2 Hz, 4H), 7.02–7.09 (m, 8H), 6.48 (d, J=8.8 Hz, 1H), 6.32 (td, J=6.7, 0.8 Hz, 1H), 6.32 (td, J=6.7, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3, 147.4, 147.3, 140.8, 139.7, 139.3, 137.8, 133.5, 129.2, 127.7, 127.3, 126.6, 124.5, 123.4, 123.0, 121.8, 105.9; IR (KBr): 3076, 3038, 2363, 2337, 1661 (C=O), 1585, 1485, 1473 cm^{-1}; FAB (NBA) 415.1 (M^++H); HRMS calcd for $C_{29}H_{22}N_2O$ 414.1732, found 414.1735 (M^+). Anal. calcd for $C_{29}H_{22}N_2O$: C, 84.03; H, 5.35; N, 6.76. Found C, 83.74; H, 5.22; N, 7.08.

2.3.12. 1-(Pyridin-3-yl)-1*H*-pyridinin-2-one (17).[21] Reaction time: 15 h, tint brown solid (76%). Mp 144–145 °C (from CH_2Cl_2/hexane=1:10); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.61–8.63 (m, 2H), 7.91 (d, J=8.8 Hz, 1H), 7.71 (dt, J=6.8, 0.8 Hz, 1H), 7.50–7.57 (m, 2H), 6.51 (d, J=8.8 Hz, 1H), 6.35 (t, J=6.8 Hz, 1H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 160.8, 148.7, 147.2, 140.8, 138.5, 137.0,

134.4, 123.6, 120.3, 105.8; IR (KBr): 3063, 3012, 2363, 2337, 1661 (C=O), 1573, 1535 cm^{-1}; FAB (NBA) 173.1 ($M^+ + H$); HRMS calcd for $C_{10}H_9N_2O$ 173.0715, found 173.0712 ($M^+ + H$). Anal. calcd for $C_{10}H_8N_2O$: C, 69.76; H, 4.68; N, 16.27. Found C, 69.63; H, 4.60; N, 15.97.

2.3.13. 1-(6-(2-Oxopyridin-1(2*H*)-yl)pyridin-2-yl)pyridin-2(1*H*)-one (18). Reaction time: 4 h, white solid (65%). Mp 176–177 °C (from CH_2Cl_2/hexane=1:10); ^1H NMR (400 MHz, $CDCl_3$) δ 7.89–7.97 (m, 3H), 7.79 (dd, J=6.8, 2 Hz, 2H), 7.36 (ddd, J=9.2, 6.8, 2 Hz, 2H), 6.60 (d, J=9.2 Hz, 2H), 6.25 (t, J=6.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 150.6, 140.1, 139.3, 135.6, 121.9, 120.4, 106.3; IR (KBr): 3100, 3051, 2363, 2337, 1673 (C=O), 1599, 1573, 1535 cm^{-1}; FAB (NBA) 266.0 ($M^+ + H$); HRMS calcd for $C_{15}H_{12}N_3O_2$ 266.0930, found 266.0922 ($M^+ + H$), calcd for $C_{15}H_{11}N_3O_2$ 265.0851, found 265.0848 (M^+). Anal. calcd for $C_{15}H_{11}N_3O_2$: C, 67.92; H, 4.18; N, 15.84. Found C, 67.66; H, 4.09; N, 15.68.

2.3.14. 1-(Thiazol-2-yl)-1*H*-pyridin-2-one (19). Reaction time: 14 h, tint yellow solid (33%). Mp 85.5–86 °C (from CH_2Cl_2/hexane=1:15); ^1H NMR (400 MHz, d_6-DMSO) δ 8.76 (ddd, J=7.6, 2, 0.8 Hz, 1H), 7.77 (d, J=3.2 Hz, 1H), 7.59–7.64 (m, 2H), 6.73 (dd, J=8, 0.8 Hz, 1H), 6.58 (td, J=7.6, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1, 155.7, 139.3, 137.5, 131.6, 121.2, 118.4, 107.2; IR (KBr): 3114, 3068, 2357, 2335, 1670 (C=O), 1599, 1541, 1495 cm^{-1}; FAB (NBA) 179.0 ($M^+ + H$); HRMS calcd for $C_8H_7N_2OS$ 179.0279, found 179.0282 ($M^+ + H$), calcd for $C_8H_6N_2OS$ 178.0201, found 178.0204 (M^+). Anal. calcd for $C_8H_6N_2OS$: C, 53.92; H, 3.39; N, 15.72; S, 17.99. Found C, 53.97; H, 3.40; N, 15.59; S, 17.67.

2.3.15. 1-(Thiophen-3-yl)-1*H*-pyridin-2-one (20).[17] Reaction time: 22 h, brown solid (74%). Mp 116–117 °C (from CH_2Cl_2/hexane=1:15); ^1H NMR (400 MHz, d_6-DMSO) δ 7.75 (dd, J=3.2, 1.4 Hz, 1H), 7.70 (dd, J=6.8, 2.4 Hz, 1H), 7.62 (dd, J=5.2, 3.2 Hz, 1H), 7.47 (ddd, J=9.2, 6.8, 2.4 Hz, 1H), 7.28 (dd, J=5, 1.4 Hz, 1H), 6.47 (d, J=9.2 Hz, 1H), 6.29 (td, J=6.8, 1.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 139.4, 138.6, 137.4, 125.4, 124.8, 121.8, 119.6, 106.0; IR (KBr): 3112, 3066, 2361, 2333, 1661 (C=O), 1583, 1515 cm^{-1}; FAB (NBA) 178.0 ($M^+ + H$); HRMS calcd for C_9H_8NOS 178.0327, found 178.0326 ($M^+ + H$). Anal. calcd for C_9H_7NOS: C, 60.99; H, 3.98; N, 7.90; S, 18.09. Found C, 61.00; H, 3.95; N, 7.83; S, 18.15.

2.3.16. 1-(3-Nitrophenyl)-1*H*-pyridin-2-one (21).[22] Reaction time: 15 h, tint yellow solid (62%). Mp 185–186 °C (from CH_2Cl_2/hexane=1:10); ^1H NMR (400 MHz, $CDCl_3$) δ 8.27–8.27 (m, 2H), 7.79 (dt, J=8, 1.6 Hz, 1H), 7.67 (t, J=8 Hz, 1H), 7.43 (ddd, J=9.2, 6.6, 2.4 Hz, 1H), 7.33 (dd, J=6.6, 1.6 Hz, 1H), 6.67 (d, J=9.2 Hz, 1H), 6.30 (t, J=6.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 148.4, 141.4, 140.3, 136.7, 132.8, 130.0, 123.2, 122.1, 121.9, 106.7; IR (KBr): 3080, 3023, 2361, 2333, 1671 (C=O), 1593, 1525 cm^{-1}; FAB (NBA) 217.0 ($M^+ + H$); HRMS calcd for $C_{11}H_9N_2O_3$ 217.0613, found 217.0612 ($M^+ + H$), calcd for $C_{11}H_8N_2O_3$ 216.0535, found 216.0534 (M^+). Anal. calcd for $C_{11}H_8N_2O_3$: C, 61.11; H, 3.73; N, 12.96. Found C, 61.30; H, 3.79; N, 12.88.

2.3.17. 1-(4-(Methylthio)phenyl)-1*H*-pyridin-2-one (22). Reaction time: 5 h, white solid (80%). Mp 141–142 °C (from CH_2Cl_2/hexane=1:10); ^1H NMR (400 MHz, d_6-DMSO) δ 7.60 (dd, J=6.6, 2 Hz, 1H), 7.48 (ddd, J=9.2, 6.6, 2 Hz, 1H), 7.30–7.38 (m, 4H), 6.45 (d, J=9.2 Hz, 1H), 6.29 (td, J=6.6, 1.2 Hz, 1H), 2.5 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 139.6, 139.1, 137.6, 137.4, 126.6, 126.5, 121.5, 105.8, 15.7; IR (KBr): 3046, 2980, 2922, 2361, 2342, 1660 (C=O), 1591, 1525, 1487 cm^{-1}; FAB (NBA) 218.0 ($M^+ + H$); HRMS calcd for $C_{12}H_{12}NOS$ 218.0640, found 218.0642 ($M^+ + H$), calcd for $C_{12}H_{11}NOS$ 217.0561, found 217.0554 (M^+). Anal. calcd for $C_{12}H_{11}NOS$: C, 66.33; H, 5.10; N, 6.45; S, 14.76. Found C, 66.36; H, 4.96; N, 6.40; S, 14.91.

2.3.18. 5-Nitro-1-phenyl-1*H*-pyridin-2-one. Reaction time: 16 h, tint yellow solid (3%). Mp 169.5–170 °C (from CH_2Cl_2/hexane=1:10); ^1H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, J=3.2 Hz, 1H), 8.14 (dd, J=10.4, 3.2 Hz, 1H), 7.47–7.56 (m, 3H), 7.36–7.39 (m, 2H), 6.64 (d, J=10.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 139.6, 139.0, 133.3, 130.8, 129.64, 129.61, 126.1, 120.3; IR (KBr): 3085, 2361, 2333, 1667 (C=O), 1621, 1553, 1497, 1450 cm^{-1}; FAB (NBA) 217.0 ($M^+ + H$); HRMS calcd for $C_{11}H_9N_2O_3$ 217.0613, found 217.0613 ($M^+ + H$), calcd for $C_{11}H_8N_2O_3$ 216.0535, found 216.0530 (M^+). Anal. calcd for $C_{11}H_8N_2O_3$ C, 61.11; H, 3.73; N, 12.96. Found C, 60.91; H, 3.76; N, 12.62.

2.3.19. 2-*tert*-Butoxy-5-bromopyridine (26). To an oven-dried 100 mL double-necked flask was charged a solution of 2,5-dibromopyridine (4.90 g, 21 mmol) and sodium *tert*-butoxide (3.25 g, 30 mmol) in toluene (30 ml). The reaction mixture was stirred and heated at reflux temperature for 2.5 h. The resulting suspension was cooled to room temperature and filtered through celite. The collected organic phase was concentrated, and purified by flash chromatography on silica gel (hexane/dichloromethane) to give colorless liquid (2.65 g, 56%). Note that the compound is a slightly hygroscopic liquid and should be distilled before use. Bp 70 °C (0.15 mmHg). ^1H NMR (400 MHz, d_6-DMSO) δ 8.20 (d, J=2.4 Hz, 1H), 7.80 (dd, J=8.8, 2.8 Hz, 1H), 6.66 (d, J=8.8 Hz, 1H), 1.50 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 146.8, 140.5, 114.9, 111.2, 80.2, 28.6; IR (neat): 2980, 2933, 2353, 2343, 1573, 1545, 1459, 1363 cm^{-1}; HRMS (EI, 70 eV) calcd for $C_9H_{12}^{79}BrNO$ 229.0102, found 229.0097 (M^+). Anal. calcd for C_9H_{12}-BrNOC, 46.98; H, 5.26; N, 6.09. Found C, 46.93; H, 5.41; N, 5.93.

2.3.20. 1-(6-*tert*-Butoxypyridin-3-yl)-1*H*-pyridin-2-one (27). To an oven-dried 25 mL double-necked flask was charged 2-hydroxypyridine (1.26 g, 13.2 mmol), CuI (20 mol%), and K_2CO_3 (2 equiv). The flask was evacuated and backfilled with nitrogen. A solution of 2-*tert*-butoxy-5-bromopyridine (3.03 g, 13.2 mmol) in toluene (13.2 mL) and DMCDA (20 mol%) were injected under nitrogen. The reaction mixture was stirred and heated at reflux temperature for 11 h. The resulting mixture was cooled to room temperature, diluted with dichloromethane, and filtered. The filtrate was washed with water. The collected organic phase was dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified by flash chromatography on

silica gel (ethyl acetate/dichloromethane) to give white solid (2.03 g, 63%). Mp 188–189 °C (from CH_2Cl_2/hexane = 1:10); ^1H NMR (400 MHz, d_6-acetone) δ 8.14 (d, J = 2.8 Hz, 1H), 7.71 (dd, J = 9.2, 2.8 Hz, 1H), 7.57 (ddd, J = 6.8, 2, 0.8 Hz, 1H), 7.47 (ddd, J = 9.2, 6.8, 2 Hz, 1H), 6.75 (d, J = 9.2 Hz, 1H), 6.45 (dt, J = 9.2, 0.8 Hz, 1H), 6.28 (td, J = 6.8, 1.2 Hz, 1H), 1.60 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.1, 162.2, 143.1, 139.9, 137.8, 136.5, 130.6, 121.5, 113.1, 106.0, 80.3, 28.6; IR (KBr): 3047, 2971, 2923, 2361, 2333, 1667 (C=O), 1583, 1535, 1477 cm^{-1}; FAB (NBA) 245.1 (M^+ + H); HRMS calcd for $C_{14}H_{17}N_2O_2$ 245.1290, found 245.1289 (M^+ + H). Anal. calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found C, 68.77; H, 6.82; N, 11.40.

2.3.21. 1-(6-Hydroxypyridin-3-yl)-1*H*-pyridin-2-one (28). To an oven-dried 25 mL flask was charged 1-(6-*tert*-butoxypyridin-3-yl)pyridin-2(1*H*)-one (0.75 g, 3.06 mmol), CH_2Cl_2 (15 mL), and TFA (5%). The reaction mixture was stirred and heated at reflux temperature for 1 h. The resulting solution was concentrated and washed with hexane to give white solid. Note that this compound is hygroscopic and soluble in water. Normal extraction workup procedure is inappropriate in this case. The white solid was dried under vacuum at 110 °C for overnight to afford 0.57 g, 99% yield of **28**. Mp 284–285 °C (from CH_2Cl_2/hexane = 1:5); ^1H NMR (400 MHz, d_6-DMSO) δ 12.07 (bs, 1H), 7.60–7.63 (m, 2H), 7.44–7.51 (m, 2H), 6.44 (d, J = 9.6 Hz, 1H), 6.40 (d, J = 10 Hz, 1H), 6.26 (td, J = 6.8, 1.2 Hz, 1H); ^{13}C NMR (100 MHz, d_6-DMSO) δ 161.3, 161.2, 140.6, 140.5, 139.4, 133.7, 121.7, 121.0, 118.6, 105.5; IR (KBr): 3446, 3140, 3065, 2799, 2353, 1677 (C=O), 1621, 1583, 1525, 1469 cm^{-1}; FAB (NBA) 189.1 (M^+ + H); HRMS calcd for $C_{10}H_9N_2O_2$ 189.0664, found 189.0667 (M^+ + H), calcd for $C_{10}H_8N_2O_2$ 188.0586, found 188.0587 (M^+). Anal. calcd for $C_{10}H_8N_2O_2$: C, 63.82; H, 4.28; N, 14.89. Found C, 63.41; H, 4.57; N, 15.01.

2.3.22. 1-(1-(6-*tert*-Butoxypyridin-3-yl)-1,6-dihydro-6-oxopyridin-3-yl)-1*H*-pyridin-2-one (29). To an oven-dried 10 mL double-necked flask containing a stir-bar was charged with 1-(6-hydroxypyridin-3-yl)-1*H*-pyridin-2-one (0.19 g, 1.01 mmol), CuI (20 mol%), and K_2CO_3 (2 equiv). The flask was evacuated and backfilled with nitrogen. A solution of 2-*tert*-butoxy-5-bromopyridine (0.28 g, 1.21 mmol) in toluene (1 mL) and DMCDA (20 mol%) were injected under nitrogen. The reaction mixture was stirred and heated at reflux temperature for 12 h. The resulting mixture was cooled to room temperature, diluted with dichloromethane, and filtered. The filtrate was washed with water. The collected organic phase was dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified by flash chromatography on silica gel (ethyl acetate/dichloromethane/MeOH) to give white solid (0.28 g, 82%). Mp 216–217 °C (from CH_2Cl_2/hexane = 1:10); ^1H NMR (400 MHz, d_6-DMSO) δ 8.22 (d, J = 2.8 Hz, 1H), 8.00 (d, J = 2.8 Hz, 1H), 7.78 (dd, J = 8.8, 2.8 Hz, 1H), 7.73 (dd, J = 7.2, 2 Hz, 1H), 7.62 (dd, J = 10, 2.8 Hz, 1H), 7.49 (ddd, J = 9.2, 6.8, 2 Hz, 1H), 6.80 (d, J = 8.8 Hz, 1H), 6.53 (d, J = 10 Hz, 1H), 6.46 (d, J = 9.2 Hz, 1H), 6.29 (td, J = 6.8, 1.2 Hz, 1H), 1.60 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 162.1, 161.0, 143.2, 140.3, 139.1, 137.3, 136.4, 135.5, 130.0, 122.0, 121.7, 121.5, 113.3, 106.5, 80.6, 28.6; IR (KBr): 3055, 2970, 2361, 2333, 1679 (C=O), 1661 (C=O), 1619, 1573, 1525, 1463 cm^{-1}; FAB (NBA) 338.2 (M^+ + H); HRMS calcd for $C_{19}H_{20}N_3O_3$ 338.1505, found 338.1503 (M^+ + H), calcd for $C_{19}H_{19}N_3O_3$ 337.1426, found 337.1439 (M^+). Anal. calcd for $C_{19}H_{19}N_3O_3$ C, 67.64; H, 5.68; N, 12.45. Found C, 67.40; H, 5.63; N, 12.59.

Acknowledgements

We thank the National Science Council of Republic of China for the financial support (NSC 93-2113-M-002-008).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.01.063

References and notes

1. For reviews, see (a) Sieburth, S. McN. In *The Inter- and Intramolecular [4+4] Photocycloaddition of 2-Pyridones and Its Application to Natural Product Synthesis*; Harmata, M., Ed.; Advances in Cycloaddition; JAI: Greenwich, CT, 1999; Vol. 5, p 85. (b) Mehta, G.; Singh, V. *Chem. Rev.* **1999**, *99*, 881–930. (c) Joule, J. A.; Mills, K.; Smith, G. F. *Heterocyclic Chemistry*, 3rd ed.; Chapman and Hall: London, 1995; pp 87–93. (d) Kuzuya, M.; Noguchi, A. *Trends Org. Chem.* **1991**, *2*, 73.
2. (a) Kohmoto, S.; Noguchi, T.; Masu, H.; Kishikawa, K.; Yamamoto, M.; Yamaguchi, K. *Org. Lett.* **2004**, *6*, 683–685. (b) Tanaka, K.; Fujiwara, T.; Urbanczyk-Lipkowska, Z. *Org. Lett.* **2002**, *4*, 3255–3257. (c) Zhu, M.; Qiu, Z.; Hiel, G. P.; Sieburth, S.; McN *J. Org. Chem.* **2002**, *67*, 3487–3493. (d) Ader, T. A.; Champey, C. A.; Kuznetsova, L. V.; Li, T.; Lim, Y.-H.; Rucando, D.; Sieburth, S. McN. *Org. Lett.* **2001**, *3*, 2165–2167. (e) Lee, Y.-g.; McGee, K. F., Jr.; Chen, J.; Rucando, D.; Sieburth, S. McN. *J. Org. Chem.* **2000**, *65*, 6676–6681. (f) Sieburth, S. McN. ; McGee, K. F., Jr.; Zhang, F.; Chen, Y. J. *Org. Chem.* **2000**, *65*, 1972–1977. (g) Bach, T.; Bergmann, H.; Harms, K. *Org. Lett.* **2001**, *3*, 601–603. (h) Bossharth, E.; Desbordes, P.; Monteiro, N.; Balme, G. *Org. Lett.* **2003**, *5*, 2441–2444.Margolin, S. B. US patents: 5,518,729 1996, 5,716,632, 1998; 5,962,478 1999, 6,090,822 2000; 6,114,353 2000.
3. (a) Itami, K.; Yamazaki, D.; Yoshida, J.-i. *Org. Lett.* **2003**, *5*, 2161–2164. (b) Yamamoto, Y.; Takagishi, H.; Itoh, K. *Org. Lett.* **2001**, *3*, 2117–2119. (c) O'Neill, B. T.; Yohannes, D.; Bundesmann, M. W.; Arnold, E. P. *Org. Lett.* **2000**, *2*, 4201–4204. (d) Moreno-Mañas, M.; Pleixats, R.; Villarroya, M. *Tetrahedron* **1993**, *49*, 1457–1464. (e) Katritzky, A. R.; Sengupta, S. *Tetrahedron Lett.* **1987**, *28*, 5419–5422. (f) Sugahara, M.; Moritani, Y.; Kuroda, T.; Kondo, K.; Shimadzu, H.; Ukita, T. *Chem. Pharm. Bull.* **2000**, *48*, 589–591.
4. (a) Sugahara, M.; Ukita, T. *Chem. Pharm. Bull.* **1997**, *45*,

719–721. (b) Li, C. S.; Dixon, D. D. *Tetrahedron Lett.* **2004**, *45*, 4257–4260. (c) Renger, B. *Synthesis* **1985**, 856–860. (d) Lam, P. Y. S.; Vincent, G.; Bonne, D.; Clark, C. G. *Tetrahedron Lett.* **2002**, *43*, 3091–3094.

5. Early copper catalyzed C–N bond coupling was reported by Gilman, H.; Moore, L. O. *J. Am. Chem. Soc.* **1957**, *79*, 3485–3487.
6. (a) Wolfe, J. P.; Wagaw, S.; Marcoux, J.-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805–818. (b) Hartwig, J. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046–2067.
7. (a) Kwong, F. Y.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 793–796. (b) Kwong, F. Y.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2002**, *4*, 581–584.
8. (a) Gujadhur, R.; Venkataraman, D.; Kintigh, J. T. *Tetrahedron Lett.* **2001**, *42*, 4791–4793. (b) Gujadhur, R. K.; Bates, C. G.; Venkataraman, D. *Org. Lett.* **2001**, *3*, 4315–4317. (c) Goodbrand, H. B.; Hu, N.-X. *J. Org. Chem.* **1999**, *64*, 670–674. (d) Kelkar, A. A.; Patil, N. M.; Chaudhari, R. V. *Tetrahedron Lett.* **2002**, *43*, 7143–7146. (e) Lang, F.; Zewge, D.; Houpis, I. N.; Volante, R. P. *Tetrahedron Lett.* **2001**, *42*, 3251–3254.
9. (a) Klapars, A.; Antilla, J. C.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 7727–7729. (b) Klapars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421–7428. (c) Antilla, J. C.; Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 11684–11688. (d) Kang, S.-K.; Kim, D.-H.; Park, J.-N. *Synlett* **2002**, 427–430.
10. Kiyomori, A.; Marcoux, J.-F.; Buchwald, S. L. *Tetrahedron Lett.* **1999**, *40*, 2657–2660.
11. Wolter, M.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2001**, *3*, 3803–3805.
12. While DMF is a teratogen, dioxane is a carcinogen suspect agent.
13. (a) Leung, M.-k.; Mandal, A. B.; Wang, C.-C.; Lee, G.-H.; Peng, S.-M.; Cheng, H.-L.; Her, G.-R.; Chao, I.; Lu, H.-F.; Sun, Y.-C.; Shiao, M.-Y.; Chou, P.-T. *J. Am. Chem. Soc.* **2002**, *124*, 4287–4297. (b) Chien, C.-H.; Leung, M.-k.; Su, J.-K.; Li, G.-H.; Liu, Y.-H.; Wang, Y. *J. Org. Chem.* **2004**, *69*, 1866–1871.
14. Substitution of the bromo group by sterically less hindered alkoxides has been previous reported by several teams. For reference, see Tomioka, K.; Shinmi, Y.; Shiina, K.; Nakajima, M.; Koga, K. *Chem. Pharm. Bull.* **1990**, *38*, 2133–2135.
15. Taylor, E. C.; Harrison, K. A.; Rampal, J. B. *J. Org. Chem.* **1986**, *51*, 101–102.
16. Bain, B. M.; Saxton, J. E. *J. Chem. Soc.* **1961**, 5216–5223.
17. Sugahara, M.; Ukita, T. *Chem. Pharm. Bull.* **1997**, *45*, 719–721.
18. Werner, D. D. M.; Christos, T.; Bertram, C.; Johannes, G.; Christopher, B. *Eur. Pattern* **2002**, *57*, 236.
19. Masakatsu, M.; Nobuko, W. *JP Pattern* **2003**, *81*, 977.
20. Jane, D. S.; Hutchinson, J. H.; Shaw, A. W.; Graham, S. L.; Ciccarone, T. M. *Eur. Pattern* **1999**, *17*, 777.
21. Kane, V. V.; Harvey, W.; Levine, S. D. *J. Heterocyclic Chem.* **1976**, *13*, 673–674.
22. Alicja, R.; Alicja, S.; Halina, B.-D. *Acta Polon. Pharm.* **1963**, *20*, 155–167.